Structural Investigations of Human Stratum Corneum by Small-Angle X-Ray Scattering

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The structure of human stratum corneum was investigated with small-angle X-ray scattering (SAXS). At room temperature the scattering curve was characterized by a strong intensity at low scattering vector (Q < 0.8 nm⁻¹) and two complicated diffraction peaks originating from a lamellar structure of the lipids. The lamellar lipid structure in the stratum corneum transformed to a disordered structure between 65°C and 75°C, the same temperature region at which a thermal lipid transition occurred. After cooling down to room temperature a recrystallization of at least a part of the lipids took place, after which only one unit cell with a repeat distance of 13.4 nm could be detected. Comparison of the scattering curve of the stratum corneum after crystallization with the scattering curve of the stratum corneum before recrystallization leads to the conclusion that in the original curve the lipids are divided in two unit cells with repeat distances of 6.4 nm and 13.4 nm. From model calculations it appears that the latter unit cell consists of more than one bilayer. The scattering curves of stratum corneum hydrated at various levels were measured. A change in the water content of stratum corneum between 6% w/w and 60% w/w (fully hydrated) did not result in swelling of the bilayers, but the scattering curve obtained with stratum corneum hydrated to 60% w/w differed from those at lower hydration levels: the scattering curve at 60% w/w showed only the diffraction peaks corresponding to a unit cell with a repeat distance of 6.4 nm. This observation implies that the ordering of a part of the lipids is reduced at very high water contents, which may explain the strong penetration-enhancing effects of water in the stratum corneum. J Invest Dermatol 97:1005–1012, 1991

The stratum corneum is the outermost layer of the skin, which acts as the main barrier for diffusion of substances through the skin. The stratum corneum consists of flattened cells embedded in a matrix of lipids. The lipids are arranged in a lamellar phase and at least a part of its lipid bilayers are in the gel or the crystalline state. In general a lamellar phase consists of alternating layers of water and lipid bilayers. Perpendicular to the stacking axis the size of the layers are considered to be of infinite length. Several studies have been carried out in order to gain information about the structure of lipids and proteins in stratum corneum. The reason for this is that lipid regions in stratum corneum are supposed to act as permeation channels for drugs. Studies carried out on the structure of the stratum corneum include, e.g., thermal analysis [1–5], small-angle X-ray scattering [6–9], wide-angle X-ray scattering [9,10], and Fourier transform infrared spectroscopy [11,12].

With thermal analysis several transitions could be detected [1–5]. The transitions at lower temperature originated from lipids, whereas the transitions at higher temperatures were influenced by proteins or originated from denaturation of proteins. Most of the transitions are influenced by the hydration level in the stratum corneum: an increase in water content resulted in an increase in the average ratio peak height to full width at half maximum for the thermal transitions.

Friberg et al [6] used small-angle X-ray scattering (SAXS) to study the lipid arrangement in human stratum corneum. They found a very broad diffraction peak from which they calculated a corresponding repeat distance of 6.5 nm. White et al [7] studied the lipid structure of murine stratum corneum with wide-angle X-ray scattering (WAXS) and SAXS. They observed a repeat distance of 13.1 nm. With WAXS the lipid structure of murine stratum corneum was characterized by two diffraction lines located at 0.412 nm and 0.375 nm. Increase in temperature resulted in a disordering of the lipid structure. Bouwstra et al [8] and human stratum corneum with SAXS and observed a repeat distance of 6.5 nm, but could not exclude the existence of a repeat distance of approximately 13 nm. They also studied the effect of hydration on the lipid structure in the stratum corneum. No swelling of the bilayers was observed upon varying the hydration level between 6% to 40% w/w. Until now the differences in repeat distances between murine stratum corneum and human stratum corneum have not been understood. Wilkes [10] studied the structure of human and rat stratum corneum with WAXS as a function of temperature. Depending on the orientation of the stratum corneum with respect to the primary beam they found various diffraction peaks: positioning the neonatal rat stratum corneum normal to the primary beam resulted in two lipid bands at 0.42 and 0.37 nm, whereas halos were present at 0.46 nm and at 0.98 nm when passing the X-rays parallel to the surface of the stratum corneum. These halos are common for protein structures. In the case of human stratum corneum a third lipid reflection was observed corresponding to a repeat distance of 0.46 nm.

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Abbreviations:
d: repeat distance
I: intensity
n: order of the diffraction peak
N: number of unit cells
PBS: phosphate-buffered saline
Q: scattering vector
\( \lambda \): wave length
\( \Theta \): scattering angle

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nm. At higher temperatures rat and human stratum corneum also showed a disordering of the lipid structure. In a very recent publication an extensive study has been published by J.-C. Garson et al [9]. They observed an oriented structure in human stratum corneum and could detect many diffraction peaks in the wide-angle region. One of the most important conclusions is the existence of protein that is very similar to β-keratine. They used a high-flux synchrotron radiation source. In the small-angle region they observed repeat distances of 6.5 nm and 4.5 nm and attribute these bands to lamellar structures consisting of different types of lipids.

Because detailed information of the lamellar structure in human stratum corneum is still not known, a study has been carried out with SAXS. Measurements were carried out after heating the stratum corneum to various temperatures. To investigate the reversibility of the transitions diffraction patterns were obtained after heating/cooling cycles. Because water is a very potent penetration enhancer and the organization of the lipids at various water content is of fundamental interest the lipid structure was also studied as function of the hydration level in the stratum corneum up to 60% w/w.

MATERIALS AND METHODS
Preparation of Stratum Corneum Samples
Abdomen skin obtained after surgical operation was dermatomed to a thickness of 200 μm. The stratum corneum was separated from the epidermis by digestion of an 0.1% trypsin in phosphate-buffered saline (PBS) solution (pH = 7.4) at 37 °C for 12 h. The stratum corneum was subsequently treated with a 0.2% solution of trypsin inhibitor (type 11-S from bovine pancreas, Sigma Chemicals, The Netherlands) in PBS. The stratum corneum was dried and stored in a desiccator over silica gel. Before use the stratum corneum was hydrated over salt solutions or water. The stratum corneum was hydrated to water contents varying between 6% w/w and 60% w/w. The water contents were measured by weighing the stratum corneum before [sc(hyd)] and after (sc(dry)) drying. The hydration levels were calculated as follows: [sc(hyd) − sc(dry)] / sc(hyd) × 100%.

SAXS All measurements were carried out at the Synchrotron Radiation Source at Daresbury Laboratories using station 8.2. This station has been built as part of a NWO/SERC agreement. The camera produces a highly collimated beam with a cross section of 0.4 × 4 mm² at the sample position. With the SRS operating at 200 mA and 2 GeV the X-ray intensity is approximately 4 × 10¹⁸ photons/second with λ (wavelength) = 0.15 nm at the sample position. Smearing of the diffraction pattern due to the fine size of the X-ray beam is negligible. The sample to detector distance can be set between 0.2 to 4.5 m, enabling studies of systems with repeat distances 0.4 < d < 100 nm. For data collection a multiwire quadrant detector was used. This detector can handle count rates up to 250,000 counts/second. The detector system spatial resolution is 0.5 mm. The resulting resolutions in the repeat distance depends on the scattering angle Θ. Examples of the obtained resolution expressed in d are 0.13 nm at Q = 1 nm⁻¹ (d = 6.3 nm), 0.06 nm at Q = 0.15 nm⁻¹ (d = 4.2 nm), and 0.015 nm at Q = 0.03 nm⁻¹ (d = 2.1 nm), in which Q is the scattering vector defined as 4πsinΘ / λ.

The detector definitely improves the signal-to-noise ratio at higher diffraction angle compared with the previously used linear detector [8]. For all the experiments the sample-to-detector distance was set to 2.0 m. The diffraction patterns were normalized with respect to synchrotron-beam decay and absorption of the sample. Corrections for positional inhomogeneity in the detector sensitivity were performed as well. No smoothing algorithms were applied to the data. In order to correct the curve for background scattering, the scattering curve of the empty cell was subtracted from the scattering curves obtained with the sample cell filled with stratum corneum.

Calibrations were performed with the help of a wet rat tail collagen sample with a repeat distance of 67 nm. One or two sheets of stratum corneum, the total weight being approximately 5 mg, were approximately randomly packed in a specially designed temperature-controlled sample cell. The volume of the inner compartment of the sample cell was 1 × 1.5 × 8 mm³. The sample cell was sealed by a lid using two little screws. Between the lid and the sample cell a rubber ring was located to avoid water loss during the measurement. The sample cell was placed in a sample holder. The temperature was measured and controlled by a thermocouple, which was placed in the sample holder near the sample cell. The sample was heated by an electric wire, which was located in the sample holder. The sample cell was equipped with two mica windows.

The scattering intensities are plotted as a function of the scattering vector Q. The exposure time for all measurements was 15 min. The stratum corneum sample was checked for damage that might occur by the primary beam. For that purpose the scattering data of stratum corneum were collected in 15 cycles; in each cycle data collection took place during 1 min. Comparing the various scattering curves no differences were detected. Therefore no damage of the stratum corneum was observed caused by X-rays.

In order to obtain information about structural changes as function of temperature, stratum corneum samples were measured in a temperature range between 25 °C and 120 °C.

In a second series of experiments the stratum corneum was heated to various temperatures, equilibrated for 1 h, and subsequently cooled to room temperature in order to crystallize the lipids. The stratum corneum was equilibrated for at least 48 h, after which the scattering curves were measured. Possible degradation of the lipids were checked with thin-layer chromatography.

In a third series of experiments the scattering curves of stratum corneum were measured as a function of the water content. The hydration level in the stratum corneum varied between 6% w/w and 60% w/w. All the experiments were carried out with stratum corneum from one donor, except for the curves measured at various temperatures. These measurements have been carried out with stratum corneum from another donor.

RESULTS
In Fig 1a the scattering profile of human stratum corneum hydrated to 40% w/w is shown. The scattering curve is characterized by a large intensity at Q < 0.8 nm⁻¹ and a broad diffraction peak at Q = 0.98 nm⁻¹. The broad diffraction peak clearly exhibits a shoulder on the right-hand side, indicating that it actually consists of two partially unresolved peaks. Sometimes a second very small shoulder has been detected at a higher Q value. At Q = 1.85 nm⁻¹ a weak diffraction peak could be detected. The weak diffraction peaks exhibit a shoulder on the right-hand side as well. In order to determine the source of the various peaks and the source of the strong scattering at low angle, the scattering pattern was compared with the pattern obtained with stratum corneum after extraction of the lipids with a chloroform/methanol mixture. The results are shown in Fig 1b. After extraction both diffraction peaks disappeared although the strong scattering at low angle was still present. It seems that the diffraction peaks are caused by the lipids in the stratum corneum, whereas the strong scattering at low angle is due to the proteins in the corneocytes.

A unit cell of a particular structure is the smallest three-dimensional unit from which one can generate the complete lattice in the three-dimensional space by stacking the unit cells in a regular manner. An example is shown in Fig 2. With SAXS, by which the scattering of X-rays are measured at very small scattering angle, information can be obtained about the larger units that form the structure, e.g., the arrangement of lamellae in the lamellar phase (Fig 3) or the arrangement of the tubes in an hexagonal phase. According to Bragg's law, nλ = 2πsinθ, a reciprocal relationship exists between the dimensions of the structure and the scattering angles. In the case of a lamellar structure generally only information can be obtained over dimensions perpendicular to the lamellae, which in Fig 3 will be along the Z axis.

A lamellar phase results in a scattering curve with Bragg reflections (diffraction peaks, the dark spots in Fig 3) located at equidistant positions in Q space, i.e., the reflections are located at Q₁,
Figure 1. a) The scattering curve of human stratum corneum hydrated to 40% w/w. A strong diffraction peak and a weak diffraction peak could be detected both consisting of a main position and a shoulder on the right-hand side. b) The scattering curve of human stratum corneum after extraction of the lipids.

\[ Q_n = 2\pi n/d \]

where \( Q_n \) is the position of the nth order diffraction peak, \( Q_1 \) is the position of the first order diffraction peak, and \( n \) is an integer. The value at which the shoulder of the weak diffraction peak is positioned. It seems that the main position of the strong diffraction peak and the shoulder of the weak diffraction peak are caused by a lamellar structure with a repeat distance of 6.4 nm. A unit cell with a repeat distance of 6.4 nm cannot be used to explain the source of the shoulder of the strong diffraction peak and the main peak of the weak diffraction doublet. These peaks should be based on another repeating unit. This point will be discussed below. Another explanation for the diffraction peaks might be the presence of a unit cell with a repeat distance of 12.8 nm. In that case the main position of the strong diffraction peak should be of second order. The main position of the weak diffraction peak should then be explained by the presence of cholesterol crystals, which exhibit a reflection at \( Q = 1.88 \text{ nm}^{-1} \) \( (d = 3.35 \text{ nm}) \). However, the third-order diffraction peak is calculated at \( Q = 1.47 \text{ nm}^{-1} \), which is not at the position of the shoulder of the strong diffraction peak located at \( Q = 1.38 \text{ nm}^{-1} \). It seems that this discrepancy is too large to justify a unit cell with a repeat distance of 12.8 nm.

Figure 2. An example of a unit cell, from which the whole lattice can be generated.

Figure 3. A lamellar structure together with its Bragg reflections in the Q space. \( d \) is the repeat distance of the lamellar structure.
However, the shoulder on the descending scattering curve is not observed after methanol/chloroform extraction. This might be caused by a disordering of the lipid envelope during extraction.

Between 75°C and 90°C, the temperature region of the third thermal transition, the shoulder of the descending scattering curve disappeared, but a small very broad peak appeared at a higher Q value (see arrow in Fig 5b). The disappearance of the shoulder confirms the suggestion that this shoulder in the scattering curve can be correlated with the third thermal transition. At 120°C, which is above the denaturation temperature of the proteins, only a descending scattering curve was observed.

**Lipid Structure in Stratum Corneum After Recrystallization** In order to find a more detailed interpretation for the scattering curve, the lipids were allowed to recrystallize after heat-

![Figure 4](image-url)  
*Figure 4.* The thermal transitions of human stratum corneum hydrated to 20% w/w.

**Structure of the Stratum Corneum as a Function of Temperature** Various studies are known in which the thermal behavior of stratum corneum have been investigated. Although it is known that the occurring transitions are due either to lipids or to proteins in the stratum corneum, it is not exactly known whether the lipid transitions are due to melting of the alkyl chains or that phase transition occurs in which the lamellar phase transforms to another phase. The thermal behavior of human stratum corneum, which is shown in Fig 4, has been published before [5]. The first and second thermal transition appeared at 37°C and 70°C. Both peaks were ascribed to reversible phase transitions of the lipids in the stratum corneum. The third transition (85°C) is probably due to lipids associated with the proteins in the stratum corneum. The transition was only reversible in the cases that heating did not exceed the denaturation temperatures. Finally, the fourth irreversible transition is due to the denaturation of the proteins in the stratum corneum.

In order to obtain more information about the origin of the thermal transitions of the lipids the scattering curves were measured as a function of temperature. The results are shown in Fig 5a,h. The scattering curve measured at 60°C does not differ significantly from that measured at 25°C indicating that no detectable disordering of the lamellae or a change in d occurred during the thermal transition. The scattering curve obtained at 67°C, which is in the middle of the temperature range of the second thermal transition, differed from those obtained at lower temperatures. Both the main diffraction peak and its shoulder were significantly reduced in intensity. At 75°C the main diffraction peak and the weak diffraction peak completely disappeared. Only a shoulder on the descending scattering curve remained at Q = 1 nm⁻¹. It seems that the system underwent a phase change in this temperature region. Because no new diffraction peaks appeared a disordering of the lamellar phase is the most likely explanation. Whether this is disorder of the first or second kind, or this is based on undulations of the bilayers, cannot be determined from the data.

The origin of the shoulder on the descending curve still present at 75°C is not fully understood, but from the thermal analysis results it could be concluded [5] that it can be caused by lipids linked to proteins in the stratum corneum. As in the case of the third thermal transition [5] the shoulder on the scattering curve is sensitive to protein denaturation. After recrystallization of the lipids from 120°C the shoulder is not present on the scattering at 75°C.

The presence of only a shoulder on the scattering curve and the absence of higher-order diffraction peaks implies that the long-range order completely disappeared. In fact, the shoulder in the diffraction curve could possibly be caused by the presence of one well-ordered lipid layer, and this could be the corneocyte lipid envelope [13]. These lipids, which are linked to the corneocyte envelope, should still be present after extraction of stratum corneum.

![Figure 5](image-url)  
*a) The scattering profile of human stratum corneum hydrated to 20% w/w at various temperatures. The temperatures have been indicated. b) The scattering profile of human stratum corneum hydrated to 20% w/w at various temperatures. The temperatures have been indicated. At 90°C the scattering curve exhibits a very poor "peak" (arrow).
on the right-hand side of the strong diffraction doublet and the source of the main position of the weak diffraction peak are not known.

First the results obtained after heating to 120°C and cooling to room temperature will be interpreted. In this curve at least five diffraction peaks were observed, of which three are quite strong. These peaks are located at equidistant positions. The exception is the distance between the position of the primary beam (located at $Q = 0$ nm$^{-1}$) and the first diffraction peak. This distance is twice the other interpeak distances. Moreover, the position of the first peak, located at $Q = 0.94$ nm$^{-1}$ has significantly shifted with respect to the position of the main diffraction peak in the original scattering curve. It was concluded that after heating to 120°C and cooling the lipids recrystallized in a lamellar structure that differed from that obtained from the original scattering curve and that the diffraction peak located at $Q = 0.94$ nm$^{-1}$ is a second-order diffraction peak. For the length of the unit cell calculated from this second-order diffraction peak [$n = 2$ in Eq. (1)], a value of 13.4 nm was found. In Fig 6b the scattering curve after heating to 120°C has been plotted at another scale. In this curve a slight change in the slope of the scattering curve can be observed at approximately $Q = 0.5$ nm$^{-1}$. It is approximately the position at which the first-order diffraction peak was expected. The change in the slope at $Q = 0.5$ nm$^{-1}$ is also observed in the original scattering curves as is shown in Fig 7b. It seems that the first-order diffraction peak is present, but the intensity of this peak is very low, which is quite remarkable. An explanation for this phenomenon is given below using a model with several bilayers in one unit cell. The steeply descending background makes it difficult to distinguish the peak from the background. After comparing the scattering curve obtained after heating to 120°C with the original scattering curve the following observations can be made. The third-order diffraction peak of the 120°C curve is located at the same position as the shoulder on the right-hand side of the main diffraction peak. In addition the fourth-order diffraction peak in the 120°C curve is located at the main position of the weak diffraction peak of the original scattering curve. From these observations it was concluded that the two peaks (doublets) in the original scattering curve are related to two unit cells, with repeat distances of 6.4 nm and 13.4 nm, respectively. The scattering at the main position of the strong diffraction peak originates from a unit cell with a repeat distance of 6.4 nm, whereas the intensity at the shoulder of the strong diffraction peak and the main position of the weak diffraction peak results from a unit cell with a repeat distance of 13.4 nm.

From the full width at half maximum the mean number of bilayers has been calculated with the Scherrer equation [14]. The average number of unit cells appeared to be 6. In the second-, third- and fourth-order diffraction peaks the full width at half maximum does not differ within the experimental error. Because broadening of the peaks caused by disorder of the second kind increases as a function of $Q$ [14] it seems that this disordering is absent after recrystallization.

In Fig 5a it is shown that the shoulder as well as the main position of the main diffraction peak decreased in intensity between 60°C and 75°C. This indicates that in both unit cells the lipid arrangement changed in the same temperature range. This directly implies that it is impossible to distinguish the lipids in the two cells with thermal analysis.

The scattering curve obtained after heating to 95°C and subsequent cooling to room temperature showed a similar scattering profile as that obtained after heating to 120°C and cooling down, although the intensities of the individual peaks of the former curve are lower. Heating to 75°C and cooling down to room temperature resulted in another profile of the scattering curve: only one diffraction peak was observed, of which the main position was already shifted towards the position of the second-order diffraction peak in the 120°C curve. This indicates that the lipids crystallized in a lattice with the same unit cell as the 120°C, but that the long-range order is lower compared to stratum corneum heated to higher temp-
Structure of Stratum Corneum as a Function of the Hydration Level

Because water is an effective penetration enhancer [15] the changes in the lipid structure induced by changes in the water content of the stratum corneum is of fundamental interest. For this reason scattering curves were obtained of stratum corneum samples hydrated between 6% w/w (almost dry) and 60% w/w (fully hydrated). The curves are shown in Fig 7a. The position of the main diffraction peak does not change between 6% w/w and 40% w/w hydration level. A small shift in the top of the peak is observed in the case of 60% w/w hydration, but this could also be due to the lower intensity of the peak, because the peak is located on a very steep descending scattering curve. This indicates that the repeat distance does not change upon hydration and that no swelling of the bilayers occurs. This is quite remarkable, because the hydration level varied between 6 and 60% w/w. It seems that water is intercalated into the bilayers for very small amounts and that most of the water is absorbed by the corneocytes in the stratum corneum. This is in accordance with recent findings of Mak et al [16], who did not observe any influence of water content in the stratum corneum in the infrared red C-H absorbance determined by FTIR. Another possibility is the forming of water-rich regions in intercellular regions in the stratum corneum (phased separation), but this is not confirmed by electron microscopy observations. The intensity of the peak increases if the hydration level increases from 6% w/w to 40% w/w, indicating a stronger ordering of the lamellae at higher water contents. If it is assumed that the main route of substances is located in the lipids in the stratum corneum, why is water such an effective penetration enhancer? A part of the answer can be given after examining the scattering curves drawn in Fig 7a, b.

It was found that the curve of 60% w/w hydrated stratum corneum does not have the shoulder on the right-hand side of the main diffraction peak (Fig 7a) and that the change in the slope of the scattering curve at $Q = 0.5 \text{ nm}^{-1}$ is not present (Fig 7b). Additionally the intensity of the main position of the weak diffraction peak decreased upon hydration from 40% w/w to 60% w/w. From these observations it was concluded that the unit cell with a repeat distance of 13.4 nm disappeared upon hydration of the stratum corneum from 40% w/w to 60% w/w. Due to the low intensity of the main diffraction peak in the 60% w/w curve it is more likely that the lipids originally arranged in the unit cell of 13.4 nm transformed to a disordered lipid structure instead of undergoing a phase transition into a lipid arrangement of the 6.4-nm unit cell. A part disordering of the lipid lamellar structure could explain the penetration enhancement of water at high hydration levels in human stratum corneum.

Model Calculations

To obtain a better understanding of the arrangement of the lipids in the unit cell, model calculations were carried out for the lipids located in a unit cell with a length of 13.4 nm, because in this case the relative intensities of six diffraction orders are known. For the calculations, the scattering curve measured after recrystallization of the lipids from 120°C was used, because the curve of untreated stratum corneum does show only partly resolved peaks. In the model calculations disorder of the first and second kind [17] were neglected. Moreover, it was assumed that the lipids are randomly orientated in the sample cell. According to Levine [18] in general the scattering intensity $I$ as a function of the scattering vector $Q$ from a stack of lamellae is given by

$$I(Q) = \left[ \int_{-\infty}^{\infty} \rho(x) \exp(-iQx) dx \right]^2 \frac{\sin^2(NQd)}{\sin^2(Qd)}.$$  

In this equation $N$ is the number of bilayers, $x$ the spatial coordinate, $\rho(x)$ the electron density of an individual bilayer, and $d$ the repeat distance. The first part of this equation is the square of the Fourier transform of the electron density of the bilayer, the so called form factor. The second part of the equation is the interference factor, which only depends on the repeat distance and the number of bilayers. The most striking characteristic of the scattering curve under
Table I. Experimental and Calculated Intensities

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*The calculated intensities are compared with the experimental data of stratum corneum after heating to 120°C and cooling to room temperature. The intensities of the second-order diffraction peak of the calculated and experimental data are set to 1 (scaling). n is the order of the diffraction peak.

consideration is the low intensity of the first-order diffraction peak. Model calculations in previous studies [7,8] already showed that a low intensity of the first-order diffraction peak can only be achieved by assuming a unit cell with more than one bilayer allowing only small differences in the electron densities of the bilayers. Therefore in this particular case the form factor in Eq. (2) is the Fourier transform of the electron density of a unit cell that might consist of more than one bilayer, which is in fact the electron density of the repeating unit perpendicular to the lamellae. In the second term in Eq. (2) the number of bilayers N is replaced by the number of unit cells, which is the repeating unit. The calculated intensities were divided by Q^2, the Lorentz factor, to make the calculated intensities directly comparable with the measured intensities. Using Q^2 it is assumed that the lamellae are randomly oriented in the sample cell. From the 120°C curve in Fig. 6a the height of the peaks were calculated by subtraction of the baseline from these peaks. The resulting experimental intensities are given in Table I. For the first-order diffraction peak we assumed a low but non-zero intensity. In the calculations the mean number of unit cells (N) was set to 6, according to the number of unit cells obtained with the Scherrer equation. The width of the calculated peaks were in good agreement with the experimentally obtained peak width. In the ideal electron density profile of the bilayers, used in the calculations, the number of unit cells does not influence the relative intensities of the diffraction peaks. By applying variations to the electron density profile the intensities of the diffraction peaks were fitted. The best fit was obtained with the electron density profile shown in Fig. 8. The calculated intensities can be found in Table I. The obtained electron density model possesses three alkyl chain regions, of which two are identical. It was impossible to fit the diffraction peaks with a model with only two identical alkyl chain regions. A similar model for the arrangements of the alkyl chains with two different hydrocarbon regions was also proposed by Swarzemandruber et al [19] as a sequence of one narrow and two broad alkyl chain regions. The widths of the two different hydrocarbon regions in our model are 1.5 nm and 3.05 nm. These differences in length agree very well with the lipid arrangement proposed by Swarzemandruber: the short alkyl chains of the ceramides (approximately 13 C atoms in the hydrocarbon region, completely extended 1.7 nm in length) are intercalated in the bilayers with a width of 1.5 nm, whereas the longer alkyl chains of the same molecules, with a mean length of approximately 26 C atoms [20] (completely extended 3.5 nm in length) are embedded in the hydrocarbon regions of adjacent bilayers with a width of 3.05 nm in width. Assuming that at least two alkyl chain layers are present in one bilayer and that the width of the hydrocarbon regions is only 3.05 nm this unequivocally shows that the alkyl chains in the lipid layers interdigitate in the bilayers.

However, the head group regions, which are characterized by a higher electron density, are very large, especially those located between the two adjacent hydrocarbon regions with equal width (see Fig. 8, 2.9 nm). It was impossible to simulate the intensities of the diffraction peaks with smaller head-group regions because that resulted in a too-high intensity of the fifth-order diffraction peak. The width of the headgroup regions cannot be caused by the lipids present in the stratum corneum, because the main components, ceramides, free fatty acids, triglycerides, and cholesterol, all consist of small head groups. The large head-group regions (1.5 and 2.9 nm) may be explained by proteins intercalated in the lipid bilayers, causing an extension of the high-electron-density regions.

**DISCUSSION**

It is reasonable that the ultrastructure of the lipids in human stratum corneum consists of domains built up of two unit cells with repeat distances of 6.4 nm and 13.4 nm, respectively. Very recently, several higher-order lamellar lipid reflections were found using WAXS, which can also be explained by the two unit cells. Whether both domains are randomly distributed in human stratum corneum or each domain is located in certain regions of the stratum corneum is not yet clear. Garson et al [9] revealed lamellar spacings of 6.5 and 4.5 nm for human stratum corneum. They did not find a repeat distance of 13.4 nm because the small first-order peak of this lamellar structure was not observed in their study, but their results showed that both unit cells are stacked perpendicular to the surface of the stratum corneum. They also stated that the 6.5- and 4.5-nm repeat distances are in accordance with the model proposed by Swarzemandruber et al [19]. In our opinion this is open to discussion, because Swarzemandruber did not propose stacks of lamellae with repeat distances of 6.5 and 4.5 nm, but the so-called Landmann units, which consist of a regular pattern of broad and narrow alkyl chain regions, in the sequence broad, narrow, broad, narrow, broad, broad, narrow, etc. This may result in longer repeat distances as shown in our model calculations in this paper.

Garson et al [9] also observed various peaks (shoulders) in the range from 4 nm to 4.5 nm. In their paper it is not clear whether all these peaks are found in one piece of stratum corneum, or that the peak varies in stratum corneum from different donors. In our experiments the intensity of this shoulder varies to a large extent between stratum corneum from different donors. Sometimes a second shoulder is detected. This second shoulder is shown in Fig. 5a on the scattering curve obtained at 23°C. These variations between different donors and the additional shoulder may explain the various peaks Garson et al obtained.

In murine stratum corneum only one unit cell with a repeat distance of 13.1 nm was observed [7]. This undoubtedly results in the conclusion that stratum corneum from different sources can possess quite different lipid structures. The unit cell in murine stratum corneum contains probably also more than one lipid bilayer [7]. In the case of murine stratum corneum an increase in temperature already leads to a diffuse pattern at 40°C. This was not observed with human stratum corneum at 45°C. The differences in lipid structure between murine and human stratum corneum cannot be detected with thermal analysis, because both unit cells undergo a phase transition in the same temperature region. Another difference between the two types of stratum corneum is the recrystallization behavior of the lipids. In murine stratum corneum the lipids recrystallized in exactly the same lattice as before the heating/cooling cycle, whereas recrystallization of the lipids in human stratum corneum resulted in a different structure as could be concluded from the diffraction pattern. The difference may originate from the fact

![Figure 8](image-url)
that human stratum corneum lipids possess the two types of unit cells, and that the lipids only recrystallize in one type. Because remarkable differences exist between murine and human stratum corneum, it should be interesting to investigate other types of stratum corneum.

Concerning the thermal transition at 40°C, it seems that this transition is only due to a change in the packing of the lipids in the lamellae. This is confirmed by investigations of Wilkes et al [10]. In their studies they observed a disappearance of the lipid band corresponding to a d of 0.37 nm. According to White et al [7] this could be a change from an orthorhombic structure to hexagonal packing.

Another interesting point is the influence of water on the lipid structure in human stratum corneum. No swelling of the bilayers occurs upon hydration, which was unexpected because water is a very effective penetration enhancer for hydrophilic as well as lipophilic drugs [15]. Water certainly has an influence on the lipid bilayer structure as is shown in Fig 7a,b: an increase in water content up to 40% w/w results in an increase in intensity of all the diffraction peaks. With thermal analysis also an increase in the ratio peak high to half-width was observed for the lipid transitions by increasing the water content to 40% w/w. With SAXS a very interesting phenomenon was observed: a further increase in water content leads to the disappearance of the unit cell with a repeat distance of 13.4 nm. Because the intensity of the remaining diffraction peak is quite low it is concluded that the unit cell is transformed to a disordered structure. This might account for the strong penetration-enhancing effects of water at very high water contents (from 40% w/w up to 50% w/w). Whether the disappearance of the 13.4-nm unit cell is a general trend or this is a characteristic of the stratum corneum used in these experiments will be investigated in the future.

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REFERENCES

16. Mak VHW, Potts RO, Guy RH: Does hydration affect intercellular lipid organization in the stratum corneum? (submitted)