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Structure of human stratum corneum as a function of temperature and hydration: A wide-angle X-ray diffraction study

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Summary

Wide-angle X-ray diffraction measurements were performed as a function of temperature and hydration on human stratum corneum. Reflections located close to the beam stop ($d > 1$ nm) could be explained on the basis of two lamellar structures. These findings confirmed results obtained with small-angle X-ray diffraction (Bouwstra et al., *J. Invest. Dermatol.*, 97 (1991b) 1005–1012), in which the several complicated diffraction peaks could be ascribed to two lamellar structures with repeat distances of 6.4 and 13.4 nm. In the wide-angle region of the diffraction pattern, we found two diffraction lines, that are characteristic of orthorhombic and hexagonal lateral packing of the lipids. Moreover, the strongest reflections of polycrystalline cholesterol were detected. Upon heating stratum corneum, a phase transition from an orthorhombic to a hexagonal phase in the lateral packing of the lipids at 40°C was observed. Between 60 and 75°C disordering of the lamellar structure occurred, while the hexagonal lateral packing was still present. Between 75 and 95°C, the hexagonal lateral packing disappeared. No changes were found in the reflection lines characteristic of protein. After recrystallisation of the lipids from 120 or 95°C, several higher diffraction orders of the lamellar structure with a repeat distance of 13.4 nm were found. Variation in the hydration level between 6 and 40% w/w did not lead to a shift in the position of the reflections characteristic of lateral packing. In addition, in the recent paper cited above, swelling of the bilayers was not observed, indicating that no water is absorbed between the lamellar regions of human stratum corneum.

Introduction

The main barrier for diffusion of substances through the skin is the outermost layer of the skin, the stratum corneum. The stratum corneum consists of corneocytes filled with keratin filaments, while in the intercellular spaces lamellar sheets of lipids are located. Since it is assumed that transport of material mainly takes place through the intercellular route, effort has been put into the elucidation of the lipid lamellar structure and changes in this structure after treatment with penetration enhancers. Information on the lipid structure can be obtained by techniques such as thermal analysis, electron microscopy,

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wide-angle X-ray diffraction (WAXD) and small-angle X-ray diffraction (SAXD).

The first studies on the structure of stratum corneum using WAXD and SAXD were carried out by Swanbeck (1959), Swanbeck and Thyresson (1961, 1962) and Goldsmith and Baden (1970). These investigations all suggested that lipids were formed around keratin filaments. Wilkes et al. (1973) performed a detailed study on the structure of rat and human stratum corneum and observed several phase changes upon heating. Moreover, it was shown that the characteristic α-keratin reflection (0.51 nm) was not present in the diffraction pattern of human and rat stratum corneum. The 0.51 nm reflection was observed by Baden et al. (1973), but they used isolated keratin. More recently, it has become apparent that the lipids are arranged in lamellar sheets between the corneocytes. Elias et al. (1983) reported results obtained by WAXD that are in support of this theory. Friberg and Osborne (1985) performed a study on human stratum corneum using SAXD and calculated a repeat distance of 6.5 nm, which was based on a lamellar structure. A detailed study was carried out by White et al. (1988) on murine stratum corneum using a combination of SAXD and WAXD. They observed a lamellar lipid structure with a repeat distance of 13.1 nm. The lateral packing of the lipids was assumed to be orthorhombic or hexagonal. At around 40°C the lateral packing of the lipids underwent a phase transition, while the lamellar structure became more disordered at this temperature. This phase change had been detected previously by Wilkes et al. (1973) in human and rat stratum corneum, but no clear interpretation was given. In recent studies, Bouwstra et al. (1991a) determined a repeat distance of 6.5 nm in human stratum corneum, however, a value of approx. 13 nm could not be excluded. Garson et al. (1991) performed experiments at room temperature on human stratum corneum. Using WAXD they found many diffraction arcs and rings, which had not been observed before. They also excluded the presence of α-keratin in human stratum corneum and assumed deformed β-keratin to be present therein. These authors determined two lamellar repeat distances of 6.5 and 4.5 nm. In a recent study using SAXD (Bouwstra et al., 1991b), the scattering curve of human stratum corneum could be explained by two repeat distances of 13.4 and 6.4 nm, respectively. This interpretation was concluded based on a comparison of the scattering curves before and after recrystallisation of the stratum corneum lipids. The recrystallisation resulted in a lamellar structure with a repeat distance of 13.4 nm. Murine (Hou et al., 1991) and human (Bouwstra et al., 1991a) stratum corneum lamellae did not show swelling upon hydration. In this study, the structure of human stratum corneum was investigated as a function of temperature and hydration using WAXD. In addition, the structure of stratum corneum was examined after recrystallisation of its lipids. As a result of the temperature-dependent experiments, the recrystallisation procedure and the correlation with recent results obtained using SAXD (Bouwstra et al., 1991b), a more detailed interpretation of the diffraction pattern could be made.

Materials and Methods

Isolation of stratum corneum

Abdomen or mama skin obtained after surgical operation was dermatomed to a thickness of 200 μm. The stratum corneum was separated from the epidermis by incubation of a 0.1% trypsin solution in phosphate-buffered saline (PBS) on filter paper for 24 h at 37°C. The stratum corneum was subsequently treated with a 0.1% solution of trypsin inhibitor in PBS. Then the stratum corneum was dried and stored in a desiccator. Before use the stratum corneum was hydrated to give water contents varying between 6 and 40% w/w. Water contents are defined as % w/w = 100%(weight hydrated stratum corneum–weight dry stratum corneum)/weight hydrated stratum corneum. Stratum corneum of three donors was used.

Experimental set-up

The diffraction patterns were obtained with the fibre diffraction camera at station 7.2 of the Synchrotron Radiation Source in Daresbury. A 0.60 m long fused quartz mirror, positioned at a

distance of 11 m from the source, is used to focus the radiation in the vertical plane. Subsequently, the white beam is monochromated and focussed by a cylindrically bent G(111) monochromator with a symmetric cut of 10.5° resulting in a 13 : 1 compression of the asymmetric incoming beam. The Bragg angle is set at 14.25° so that the monochromated radiation has a wavelength of 0.148 nm. The distance from the source to the monochromater is 21 m. Several slit sets are used to reduce the parasitic scatter.

The cross-section of the X-ray beam in the focal plane is approx. 0.5×0.3 mm^2. In order to improve the spatial resolution of this camera, the beam cross-section was further reduced by placing a collimator, with a diameter of 0.3 mm, between the exit window of the beam line and the sample. The photon flux at the sample position was estimated to be 2×10^{10} photons/s. The temperature-controlled sample holder was placed inside an He-filled chamber in order to reduce air scatter and absorption. To avoid evaporation of water, the sample cell was sealed with mica windows. To prevent the direct beam hitting the film on which the diffraction pattern was recorded, a small beam stop was glued on the mylar exit window of the He chamber.

Patterns were recorded on CEA X-ray films. Three films were mounted simultaneously in the film holder in order to increase the dynamic range of the film material. The sample-to-film distance was 9.3 cm. The scattering angle (ϑ), defined as half of the angle between the incident beam and the diffracted X-rays, is related to the repeat distance (d) by the Bragg equation $2d \sin \vartheta = n\lambda$. n is the order of the diffraction peak and λ denotes the wavelength. The scattering angle varied between 1.4 and 15°, corresponding to repeat distances varying over the range between 0.3 and 3 nm. In this paper, the position of the reflections will be denoted by the repeat distance d.

The stratum corneum sheets were oriented in a sample cell with four mica windows. In this way diffraction patterns could be obtained with the direct beam perpendicular and parallel to the stack direction. In the first configuration, the path length through the sample was 2 mm. In the perpendicular configuration this path length was approx. 1 mm (50 sheets of stratum corneum).

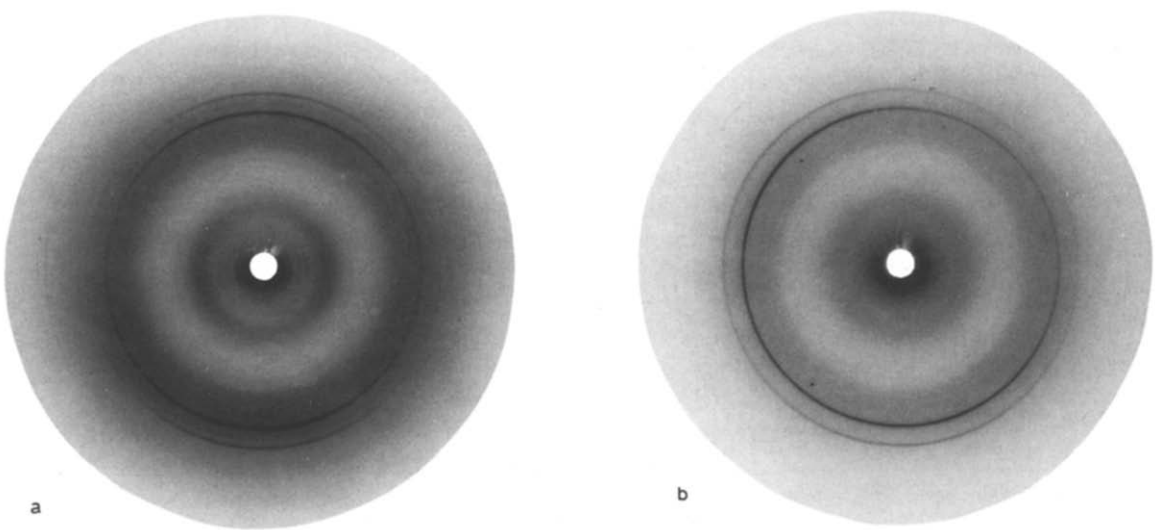

Fig. 1. (a) Diffraction pattern of human stratum corneum in the parallel configuration (donor III). (b) Diffraction pattern of human stratum corneum in the perpendicular configuration (donor III). The arcs at low angles are not present in the perpendicular configuration.

The temperature of the sample cell could be adjusted between 25 and 120°C using a wire in combination with a thermocouple. The latter was located in the sample cell.

Measurements

The following experiments were carried out.

(1) Stratum corneum hydrated to 6, 20 and 40% w/w was measured at room temperature.

(2) Stratum corneum hydrated to 20% w/w was measured at 25, 45, 60, 75, 90 and 110°C.

(3) Stratum corneum hydrated to 20% w/w was measured after recrystallisation of its lipids. Recrystallisation was obtained by heating the stratum corneum to various temperatures (60, 75, 90 and 120°C) and cooling to room temperature, after which the stratum corneum was equilibrated for at least 24 h.

(4) Stratum corneum was heated to 120°C and then allowed to cool to room temperature in order to recrystallize the lipids in the stratum corneum. After recrystallisation the stratum corneum was measured at 45, 60, 75 and 120°C.

(5) Stratum corneum was measured after chloroform/methanol extraction.

Results and Discussion

General description of the diffraction pattern

An example of the scattering pattern obtained in the parallel configuration using stratum corneum from donor III is shown in Fig. 1a. In Fig. 1b a diffraction pattern recorded in the perpendicular configuration is shown. Several reflections, i.e., rings (diffuse and sharp) and arcs, were

TABLE 1

Intensities of the reflections obtained from two different donors together with the explanations

d (nm)			Position/configuration	Intensity	Explanation
I	II	III			
n.d. [a]	n.d. [a]	2.74	// M	arc, s	2.68 : 13.4 (5)
2.28		2.35	// M	arc, s	2.13 : 6.4 (3) [b], 2.27 : 6.8 (3) [b], 2.23 : 13.4 (6) [b]
	1.98		// M	arc, vw	1.91 : 13.4 (7)
1.68	1.70	1.71	// M	arc, w	1.60 : 6.4 (4), 1.70 : 6.8 (4), 1.68 : 13.4 (8), cholesterol
	1.52	1.52	// M	arc, w	1.48 : 13.4 (9)
1.39	1.38	1.33	// M	arc, m	1.28 : 6.4 (5), 1.36 : 6.8 (5), 1.34 : 13.4 (10)
0.97	0.97	0.97	+, // M, E	diffuse ring, s	soft keratin
		0.94	// M	arc, m	
0.716	0.72	0.718	// M, E	arc, s	
	0.571	0.575	+, // M, E	ring, m	cholesterol
	0.520	0.525	+, // M, E	ring, w	cholesterol
	0.508	0.513	+, // M, E	ring, w	cholesterol
	0.485	0.488	+, // M, E	ring, w	cholesterol
		0.46	// M	arc, vw	
0.46	0.46	0.46	+, // M, E	diffuse ring, s	soft keratin
0.417	0.417	0.415	+, // M, E	ring, s	crystalline lipid/gel. lipid
0.378	0.376	0.375	+, // M, E	ring, s	crystalline lipid
0.360	0.357	0.359	// M	arc, m	
0.304			// M, E	ring, w	

[a] n.d., not determined.

[b] For example, 2.13 : 6.4 (3) = the calculated diffraction line is 2.13 nm, which is the third-order diffraction peak of the lamellar phase with a repeat distance of 6.4 nm.

Reflections of stratum corneum observed from three donors (I–III) are listed. Sample oriented: +, perpendicular; //, parallel; measurements performed at ambient temperature; reflection observed: M, on the meridional; E, on the equator; intensity: s, strong; w, weak; vw, very weak; m, medium.

observed and are listed in Table 1. The arcs were observed on the meridional axis of scattering patterns obtained with samples in the parallel configuration, implying that the oriented structures are aligned parallel to the surface of the stratum corneum. This conclusion was drawn previously by Garson et al. (1991). A control measurement of the empty sample cell with mica windows exhibited various diffraction spots, of which most are located at higher scattering angles. These diffraction spots showed considerable variability in position, intensity and number of spots. The diffraction pattern varied with time. In most experiments, only a small number of spots were observed, however, the variability in intensity and position necessitates very careful analysis of the diffraction patterns. Therefore, during our experiments the empty sample cell was measured frequently. It appeared that a part of the spots and dotted diffraction lines was observed with an empty cell, while isotropic diffraction lines and most arcs were only seen with stratum corneum in the sample cell. The exact origin of the spots and dotted rings remains to be clarified.

Reflections observed in perpendicular and parallel configuration

In Fig. 1 two very strong reflection rings located at 0.378 and 0.417 nm (in this paper the reflections will be denoted by the values obtained with stratum corneum of donor I, since those obtained for donors II and III are similar; see Table 1) have been detected. In the parallel configuration the intensities of these rings are stronger in the equatorial direction than in the meridional direction. The reflections at 0.378 and 0.417 nm are probably due to crystalline lipids arranged in an orthorhombic perpendicular sublattice (Small, 1986). The two sharp reflection rings were also obtained in murine (Elias et al., 1983; White et al., 1988), human (Wilkes et al., 1973; Garson et al., 1991) and rat (Wilkes et al., 1973) stratum corneum. According to White et al. (1988), the reflection at 0.417 nm could partly be due to a hexagonal packing of the alkyl chains, in which the alkyl chains are able to rotate a few degrees along the alkyl chain within the lattice. This packing is looser than that in the crystalline state. The positions of the reflection based on the (pseudo) hexagonal phase (0.41–0.42 nm) and on an orthorhombic perpendicular structure (0.41 nm) are very similar which renders the task of completely resolving these two reflections very difficult. After extraction with a mixture of chloroform/methanol, the diffraction line at 0.417 nm is still present, although of very low intensity. Since approx. 10% w/w of the total amount of lipids are chemically linked to the corneocyte envelope (Wertz et al., 1989), which makes extraction of these lipids with cholesterol/chloroform solution impossible, it might be that hexagonal lateral packing exists in these lipids, referred to as the corneocyte lipid envelope.

The broad diffuse diffraction ring at 0.46 nm is probably due both to hydrocarbon chains in the liquid state (mean interchain distance: 0.46 nm) and to soft keratin located in the corneocytes. That this ring is indeed partly due to soft keratin can be concluded from the patterns observed after extraction of the lipid from the stratum corneum with chloroform/methanol solution. The broad diffraction band at 0.46 nm is still present together with the broad diffraction ring at approx. 0.98 nm, which is also characteristic of soft keratin. The broad non-uniform band at 0.98 nm indicates a slight orientation. This band was not found in murine stratum corneum (White et al., 1988), indicating that murine stratum corneum does not contain soft keratin. A reflection with a rather weak intensity is present at 0.305 nm. This feature appears to be due to another crystalline lipid phase, since the diffraction line disappears at a higher temperature than the reflection at 0.378 nm. A more detailed discussion is given below.

Variations in the scattering pattern were observed in stratum corneum obtained from different donors. This has been reported earlier by Garson et al. (1991). In our experiments, one such variation was the presence of the weak diffraction lines at 0.571, 0.52, 0.508 and 0.485 nm in stratum corneum from donor II. Approximately the same diffraction lines were also present in donor III (see Table 1), while they were absent in stratum corneum from donor I. The four reflections are consistent with the strongest

reflections of the powder diffraction pattern of cholesterol (De Wolff, 1989). This led us to conclude that cholesterol is probably present in the form of small crystals in human stratum corneum. Although Garson et al. (1991) detected these reflections, they did not recognize these reflections as part of the cholesterol diffraction pattern. Their suggestion of the presence of cholesterol in stratum corneum was based on the reflections at 3.35, 1.68, 0.83 and 0.415 nm (group D in their paper). The reflections at 3.35 and 1.68 nm have indeed been tabulated (De Wolff, 1989) for anhydrous cholesterol, but might also be due to higher diffraction orders of the two lamellar structures (Bouwstra et al., 1991b). This is discussed further in the following section. Although the reflection at 0.83 nm has also been tabulated, its intensity in the powder diffraction pattern of cholesterol is very low.

Oriented structures in parallel geometry

Oriented structures were only observed in parallel geometry. Two diffraction arcs with strong intensity are located at 0.716 and 0.360 nm. These reflections were found in all three donors. Two additional diffraction arcs located at 0.94 and 0.46 nm were only observed in stratum corneum of donor III. The four reflections were previously found by Garson et al. (1991) at room temperature and ambient humidity of 45%. The reflections do not correspond with those characteristic of α-keratin (1.0, 0.51 and 0.15 nm) or of β-keratin (1.0, 0.47 and 0.15 nm). Since the diffraction lines at 0.716 and 0.360 nm were insensitive to both temperature increase (see below) and chloroform/methanol extraction, these reflections may be due to proteins rather than lipids. The reflections at 0.94 and 0.46 nm were also unaffected by temperature increase. The presence of these reflections after chloroform/methanol extraction has not been investigated.

In order to obtain a better understanding of the source of these reflections, additional experiments must be performed. The reflections at 0.94 and 0.46 nm were also observed in murine stratum corneum (White et al., 1988), while those at 0.716 and 0.36 nm were not detected. In murine stratum corneum, the reflections were insensitive to both temperature increase and lipid extraction. These reflections were attributed to the corneocyte lipid envelope.

At low diffraction angles ($d > 1.0$ nm), several diffraction arcs are located. The corresponding d values of these arcs vary between 2.28 and 1.24 nm. From SAXD it appeared that the diffraction pattern of donor I was most exceptional, exhibiting a strong diffraction peak corresponding with a repeat distance of 6.8 nm, while the repeat distance corresponding to this peak position in most cases varies between 6.2 and 6.5 nm. Moreover, the shoulder on the main diffraction peak was almost absent. This shoulder is assumed to be based on the unit cell with a repeat distance of 13.4 nm (Bouwstra, 1991b). The expected higher order reflections were calculated from the repeat distances of 6.8 nm (donor I), and 13.4 and 6.4 nm (donors II and III), respectively. Comparing the values of the observed reflections with those of the higher order reflections calculated from the lamellar structures with respective repeat distance of 6.8, 6.4 and 13.4 nm led us to conclude that the reflections at low angles can be identified as higher order diffraction peaks that are partly due both to the lamellar structure with a repeat distance of 6.4 or 6.8 nm and to a lamellar structure with a repeat distance of 13.4 nm (see Table 1), although the reflection at 1.68 nm might be partly due to cholesterol (depending on the donor). On comparison of the reflection patterns obtained from the stratum corneum of donors I–III, a more pronounced presence of the reflections ascribed to only the lamellar structure with a repeat distance of 13.4 nm in donors II and III was noted compared to donor I.

In the experimental set-up used during the SAXD studies (Bouwstra, 1991b) the smallest repeat distance which could be measured was approx. 2.0 nm, making the detection of the reflection at 2.28 nm indeed possible.

Variation in hydration level

The hydration level was varied between 6 and 40% w/w. Upon hydration from 6 to 20% w/w the reflections at 0.378 and 0.417 nm became sharper but were not shifted. This can be explained based on ordering of the lateral packing

of alkyl chains, but no lateral swelling took place. Between 20 and 40% w/w hydration no changes in the scattering pattern were observed. SAXD (Bouwstra et al., 1991b) revealed that swelling of the bilayers did not occur upon hydration. Assuming that the density does not change upon hydration leads to the conclusion that hardly any water is absorbed between the bilayers. This is in agreement with the C-H stretch vibration of the hydrocarbons being independent of the water content (Mak et al., 1991). From the present experiments, our impression is that the intensity of the diffraction arcs at 0.716 and 0.360 nm is influenced by the water content in the stratum

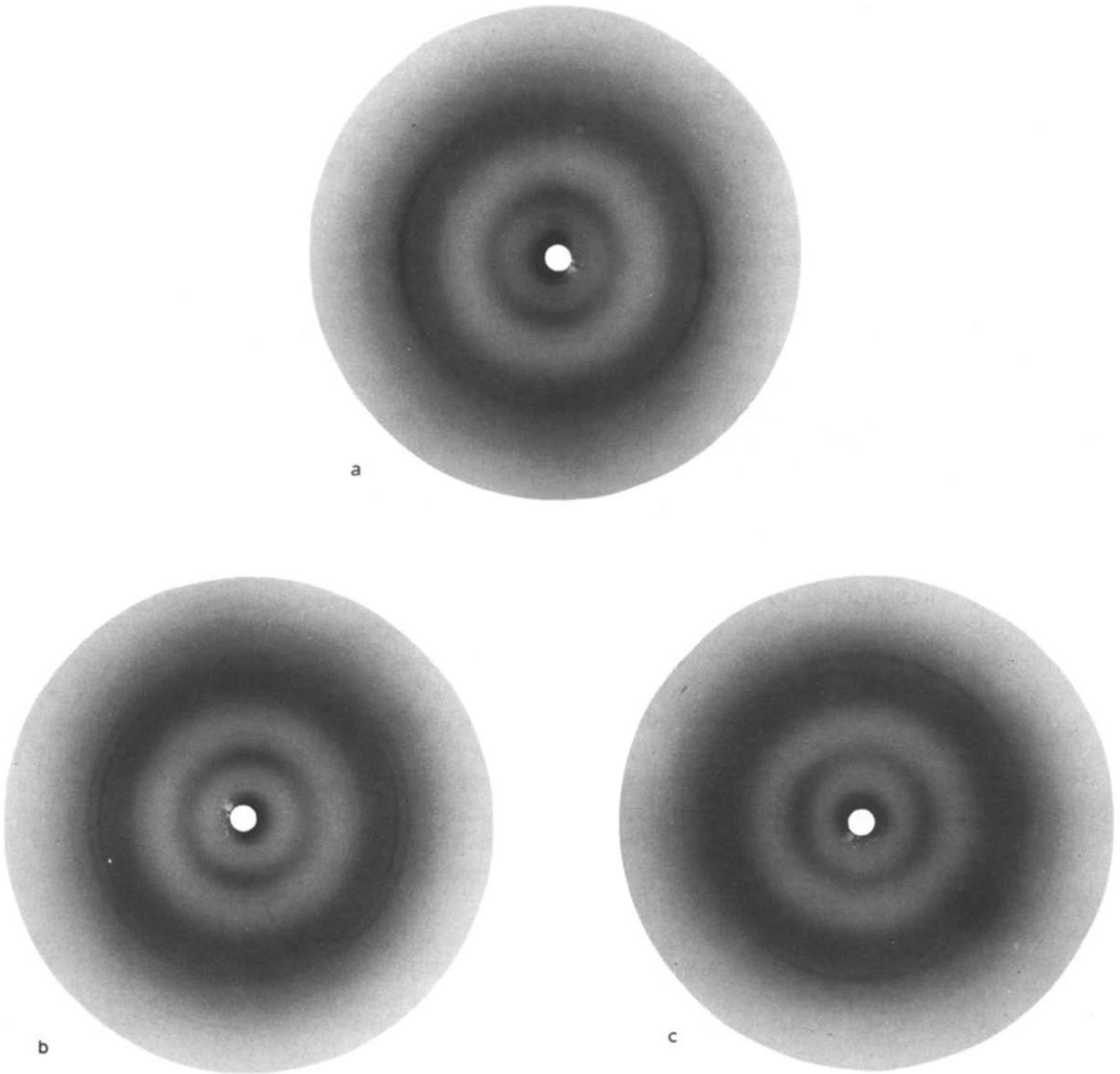

Fig. 2. (a) Diffraction pattern in the parallel configuration (donor III) at 45°C. The diffraction line corresponding to a d value of 0.378 nm has disappeared. (b) Diffraction pattern in the parallel configuration (donor III) at 75°C. The diffraction arcs close to the beam stop have disappeared. (c) Diffraction pattern in the parallel configuration (donor III) at 95°C. The diffraction ring at 0.415 nm has disappeared. Diffraction patterns at 0.36 and 0.716 nm are still present together with the diffraction bands at 0.98 and 0.46 nm.

corneum, however, further investigation is required in order to elucidate this phenomenon.

Variation in temperature

The diffraction patterns of human stratum corneum were measured at 23, 45, 60, 75, 90 and 120°C. The experiments were carried out with donor I and II. The first lipid phase transition takes place at approx. 40°C. At 45°C (see Fig. 2a) the diffraction ring at 0.378 nm, corresponding to an orthorhombic crystalline structure, has almost disappeared. This is in agreement with the observations of Wilkes et al. (1973). Since the diffraction line at 0.417 nm is still present at 45°C, the most likely explanation is a change in lateral packing from an orthorhombic to a hexagonal phase. This transition takes place without affecting the lamellar repeat distances (Bouwstra et al., 1991b), indicating that no change in the tilt angle of the hydrocarbon chains with respect to the base plane occurs. This phase change was also observed in murine stratum corneum (White et al., 1988) in the same temperature region.

At 45°C the diffraction pattern from stratum corneum of donor III exhibits an additional diffraction ring at 0.389 nm. The appearance of this diffraction line was observed only when stratum corneum of donor III was used, and was not detected in murine stratum corneum (White et al., 1988). This appears to be exceptional behaviour. The reflection might be due to the formation of an additional phase, e.g., pseudo hexagonal lateral packing of the lipids, however, the diffraction ring was still present at 120°C, which would not be expected in the case of lipids.

At 75°C (see Fig. 2b), the reflections at low diffraction angles, corresponding to the higher orders of the two lamellar structures, disappeared. These findings correspond with studies carried out using SAXD (Bouwstra et al., 1991b), in which it was shown that the long-range order (lamellar structures with repeat distances of 6.4 and 13.4 nm) completely disappeared between 60 and 75°C. This is the temperature range of the second lipid phase transition. The reflection at 0.417 nm is still present at this temperature, although its intensity appears to be reduced. It seems that although the long-range order has disappeared, the short-range order is still at least partly present.

TABLE 2

Disappearance of several reflections as a function of temperature

d (nm)	Explanation	Temperature (°C) of disappearance
2.28	lamellar	75
1.70	lamellar/cholesterol	75/95 [a]
1.39	lamellar	75
0.94	?	– [b]
0.98	soft keratin	–
0.716	?	–
0.575	cholesterol	90–95 [c]
0.525	cholesterol	90–95
0.513	cholesterol	90–95
0.488	cholesterol	90–95
0.46	soft keratin	–
0.417	hexagonal	95
0.378	orthorhombic	45
0.360	?	–

[a] Reflection disappeared at 75°C (donor I) or 95°C (cholesterol crystals, donor III).

[b] Reflections still present at 120°C.

[c] The temperature at which the wide-angle cholesterol reflections disappeared was difficult to determine due to the low intensity of the reflections at higher temperatures.

Measurements were carried out with stratum corneum from donors I and III. Reflections are denoted by the values obtained with stratum corneum of donor I, except for the cholesterol reflections.

At 90°C the short-range order was also lost, as can be concluded from the disappearance of the reflection at 0.417 nm. The most likely explanation is a change from a hexagonal to a liquid crystalline phase. The liquid crystalline phase will only result in a broad diffraction band at 4.6 nm. This band is indeed present on the photograph (see Fig. 2c), but is also a characteristic feature of soft keratin. The diffraction pattern from stratum corneum of donor III still exhibits the exceptional reflection at 0.389 nm. The cholesterol reflections disappeared at approx. 90–95°C.

At 110°C the sharp arc-shaped reflections at 0.716 and 0.360 nm and that at 0.94 nm are still present, indicative of a protein-based origin, since it would be expected that all lipids would have been transformed to a liquid crystalline structure

at this temperature. The soft keratin reflections (0.46 and 0.98 nm) are still present. It is interesting to note that no changes in the intensity and position of the remaining reflections were observed with respect to the measurements at 90°C. It appears that the denaturation of protein occurring at approx. 107°C (Golden et al., 1987; Bouwstra et al., 1989) does not influence the protein reflections.

Recrystallisation of lipids

After heating to 120 or 90°C and cooling to room temperature, the lipids in stratum corneum recrystallise. In a recent study (Bouwstra et al., 1991b) it was shown that the lipids crystallise in a single lamellar structure with a repeat distance of 13.4 nm. After recrystallisation the diffraction peaks are rather sharp which indicates a well-ordered structure. The diffraction pattern (no 13.4 nm unit cell reflections were found before thermal treatment, see Table 1) revealed several low-angle reflections. The distances of these reflections are in agreement with the higher order reflections calculated on the basis of a unit cell with a repeat distance of 13.4 nm (see Table 3) confirming the results obtained by SAXD (Bouwstra et al., 1991b) that the intercellular lipids recrystallise in a single lamellar structure. Comparison of the reflections obtained by scanning the meridional with those of untreated stratum corneum (donor I) shows more reflections in the region in which $d > 1$ nm, moreover, the diffraction pattern is similar to that of donor II, in which the lamellar structure with a repeat distance of 13.4 nm is more pronounced. In the wide-angle region, the only difference between the patterns obtained after recrystallisation from 120°C and the pattern for the case where thermal treatment was omitted is the disappearance of the weak 0.305 nm reflection. Moreover, all the reflections due to crystalline cholesterol have disappeared. The appearance of the two diffraction

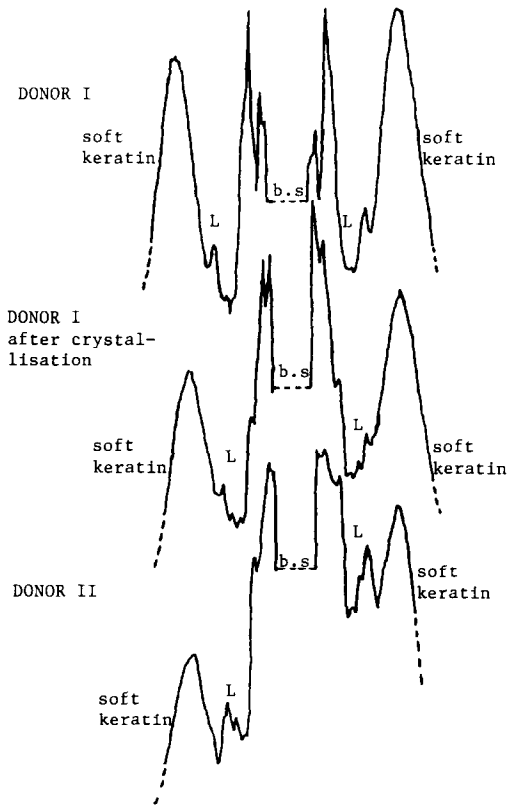

Fig. 3. Density of the diffraction pattern after recrystallisation of the stratum corneum (donor I) as compared with untreated stratum corneum (donors I and II). The scan in the meridional direction was made from the diffraction pattern obtained in the parallel orientation. Only the scans between the reflections of soft keratin ($d = 0.97$ nm) are shown. This part only covers the higher order reflections of the lamellar structures (designated L). b.s., position of the beam stop.

TABLE 3

Comparison of measured and calculated repeat distances

d (measured)	2.29	1.95	1.70	1.51	1.40	1.24
d (calculated)	2.23	1.91	1.67	1.49	1.34	1.24
Order	6	7	8	9	10	11

After recrystallisation of the lipids in stratum corneum (donor I) from 120 or 95°C several reflections could be detected. These reflections are based on a lamellar structure with a repeat distance of 13.4 nm. The reflections at wider angles ($d < 1.0$ nm) did not differ from those before the heating and recrystallisation of the lipids and have therefore been omitted. d is expressed in nm.

rings at 0.378 and 0.417 nm indicates that the lateral packing of the lipids is unchanged with respect to untreated stratum corneum. Even in extracted lipids similar lateral packing was observed (White et al., 1988). It appears that the lateral structures are less sensitive to thermal treatment than the long-range ordering of the lamellae.

Heating to 75°C and recrystallisation yielded the same results as those obtained using SAXD. Broad diffraction patterns were observed in the small-angle region. Using SAXD a shift in the main peak position was observed, indicating the preferred form of crystallisation of the lipids to be a lamellar structure with a repeat distance of 13.4 nm. After recrystallisation from 75°C the long-range ordering of the lamellae was not present.

Recrystallization of the lipids from 120°C followed by heating to several temperatures (carried out only with donor III) revealed one clear difference in the scattering pattern in the wide-angle region compared to stratum corneum without thermal pretreatment. The reflection at 0.417 nm disappeared at 75°C, while in the heating run before crystallisation this reflection disappeared at 95°C (see Fig. 4). It is unclear whether this represents a general trend or if this behaviour is exceptional due to the presence of the reflection at 0.389 nm in the diffraction pattern of donor III.

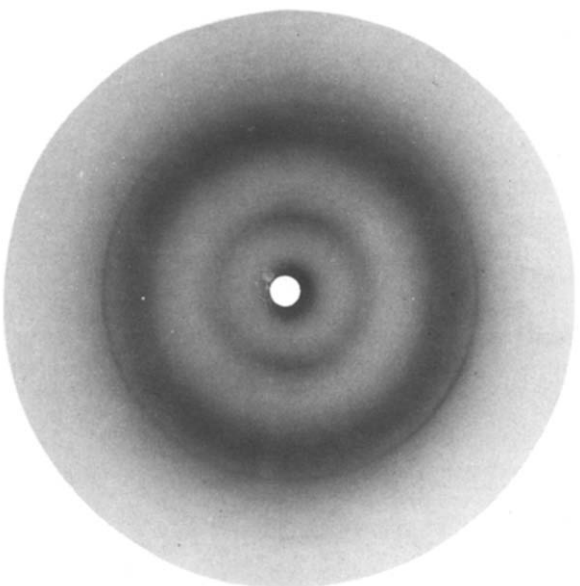

Fig. 4. Diffraction pattern of donor III in the parallel configuration after recrystallisation of the lipids from 120°C and reheating to 75°C. The diffraction line characteristic of hexagonal packing ($d = 0.375$ nm) has disappeared.

Conclusion

Comparing the structure of human stratum corneum with that of murine stratum corneum, one may conclude that the lateral packing of the lipids is very similar. In both types the lateral packing of the lipids is orthorhombic, although the arrangement of a proportion of the lipids in a hexagonal structure cannot be excluded. Moreover, a phase change from an orthorhombic to a hexagonal structure between 20 and 45°C was observed in both murine and human stratum corneum. Concerning the lamellar structure, differences were found. At room temperature murine stratum corneum lipids form a lamellar structure with a repeat distance of 13.1 nm (White et al., 1988), while human stratum corneum lipids are arranged in two lamellar structures with repeat distances of 6.4 and 13.4 nm (Bouwstra et al., 1991b). Upon heating to 45°C the scattering pattern of murine stratum corneum became diffuse, while no change was observed in the lamellar structure of human stratum corneum in this temperature range. At 70°C the lateral hexagonal packing of the lipids in murine stratum corneum was transformed to a liquid crystalline phase, while in human stratum corneum this phase change occurs between 75 and 95°C. Therefore, it appears that most of the phase changes in murine stratum corneum occur at lower temperatures than in human stratum corneum. The keratin/protein reflections observed in murine and human stratum corneum differ to a large extent. In murine stratum corneum no soft keratin reflections are observed, while these are clearly evident in human stratum corneum. In human stratum corneum we observed two reflections at 0.716 and 0.36 nm which are difficult to identify. Only in one donor were two additional reflections (0.94

and 0.46 nm) found which were also present in murine stratum corneum. White et al. (1988) attributed these reflections to the corneocyte envelope. Similar reflections were also detected in human stratum corneum by Garson (1991).

The results of WAXD experiments can be correlated with thermal analysis experiments. The thermal transition at approx. 40°C can be attributed to a phase change from an orthorhombic to a hexagonal phase. Between 60 and 75°C, corresponding to the temperature range of the second thermal transition, the reflections based on the two lamellar structures disappeared. At 75°C the hexagonal packing was still present, indicating that the lamellae had not completely disappeared. A disordering of the lamellar structure took place between 60 and 75°C. Between 75 and 90°C a phase change from hexagonal packing to a liquid phase took place. This represents the temperature range of the third thermal transition. The temperature at which this transition occurs is sensitive to protein denaturation. Such protein denaturation, which occurs at approx. 107°C, shifts the third thermal transition approx. 10–15°C to lower temperatures. Reheating stratum corneum after recrystallisation of the lipids from 120°C also resulted in a shift in the temperature range where the lipids transformed from hexagonal to liquid packing (donor III). This does not necessarily mean that the third thermal transition is not influenced by proteins, since a temperature decrease in the hexagonal to liquid phase transition can still be induced by a change in proteins at 120°C. Between 95 and 120°C no changes in the diffraction pattern were observed from which it was concluded that protein denaturation had no effect on the reflections.

Comparing the results reported by Garson et al. (1991) with those obtained in the present study leads to the conclusion that most of the continuous rings and arcs observed in the earlier study were also found in this investigation. The interpretations of the diffraction rings and arcs due to cholesterol and those which originate from the lamellar structures are more detailed in this study. Concerning the Bragg spots or dotted rings located at higher angles (small d), our impression is that at least part of these reflections are not due to the stratum corneum. In the paper of Garson et al. (1991), very similar dotted rings and discrete Bragg spots were described (group K in their paper). They ascribed these reflections to stratum corneum. These differences in observation and interpretation remain to be resolved.

In the present study, large changes in the diffraction pattern were not detected upon hydration, which is quite remarkable. It appears that although water acts as a penetration enhancer (Tiemessen, 1989; Potts and Francoeur, 1991), neither lateral swelling nor swelling of the bilayers occurs upon hydration. Moreover, penetration of drugs is assumed to take place mainly through the lipid bilayers. The clarification of these perplexing and contradictory results constitutes an interesting and important objective of future investigation.

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