	Experiment title: Lipid organization and orientation in mixtures prepared with synthetic ceramides	**Experiment number:** **2602260**
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Report: We performed measurements during a 3-days session in april 2005. The beam conditions (beam intensity and beam alignment) were excellent. We were able to perform all the scheduled experiments, This also includes measurements, in which the lipid phase behaviour was studied as function of temperature.

The natural function of the skin is it's barrier function. The barrier is located in the intercellular regions in the outermost layer of the skin, the horny layer. The major lipid classes are ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA). In SC the lipids are organized in two lamellar phases with periodicities (d) of 6 and 13 nm (1-2). The 13 nm phase is considered to be of crucial importance for the skin barrier function. In stratum corneum at least 6 ceramides are present referred to as CER1 to CER6. The ceramides differ from each other by the headgroup architecture. The fatty acid chain linked to either a sphingosine (CER1, CER2, CER4, CER5) or phytosphingosine base (CER3, CER6 and CER9 (see below)) has a long chain length up to C26. CER1 has an exceptional structure, namely an ω-hydroxy fatty acid of approximately 33 C atoms towards which a linoleate chain is chemically linked (referred to as CER1-oleate), the fatty acid chain is linked to a sphingosine chain. However, in vivo also small fractions of CER1-oleate and CER1-stearate are present and in human stratum corneum also CER9 is present, which is the counterpart of CER1 with a phytosphingsine base.

The main goal in our research is to understand the individual role of the ceramide in the formation of the 13 nm phase, the orthorhombic and the liquid phase and to study the effect of substances that either increase the permeability or decrease the permeability of the skin.

1. The composition of the synthetic CER mixture was varied. Studies were performed to examine whether ceramides with a spingosine moiety and a phytosphingosine moiety are both present to form the 13 nm phase. Therefore the composition of the CER mixture was systematically varied. In one group of studies the number of CER based on the sphingosine base was gradually reduced. CER1 was replaced by CER9 and was kept at a fixed level as from previous studies it is obvious that the presence of acyl ceramides are very crucial for the formation of the 13 nm phase. These studies revealed that the number of CER with a sphingsine base can be reduced, but that CER with a sphingosine moiety cannot be completely removed from the CER mixtures without the formation of additional phases. The same trend is observed in another group of studies in which the number

of CER based on a phytosphingosine base is reduced. CER based on a phytosphingosine moiety are also requited to avoid the formation of additional phases. In future these studies will be continued with Fourier Transformed Infrared spectroscopy, which provides information on the formation of hydrogen bondings in the head group region. All studies have been carried out once and need to be repeated.

2. The effect of exogenous substances on the lipid organization in stratum corneum and in lipid mixtures (prepared with ceramides) was studied. The experiments have been carried out successfully. The exogenous substances almost do not affect the lipid phase behaviour in the stratum corneum.

3. A first series of studies were performed in onder to replace CER- linoleate by Br-substituted CER1-linoleate. These studies were performed in order to be able to change the electron density profile within the 13 nm lamellar phase. This will enable us to calculate the electron density profile within the 13 nm phase. The experiments have been carried out successfully. However, additional studies have to be carried out as some conflicting results have been obtained.

4. In another series of experiments lipids were sprayed on a porous membrane using the airbrush method in order to orientate the lipid lamellae (similarly as in stratum corneum) parallel to the surface. The studies haven been carried out very successfully. The lipids can be indeed be oriented similarly as in stratum corneum, while the formation of the 6 and 13 nm phase still occurs. However, this is only possible in the absence of hydration.