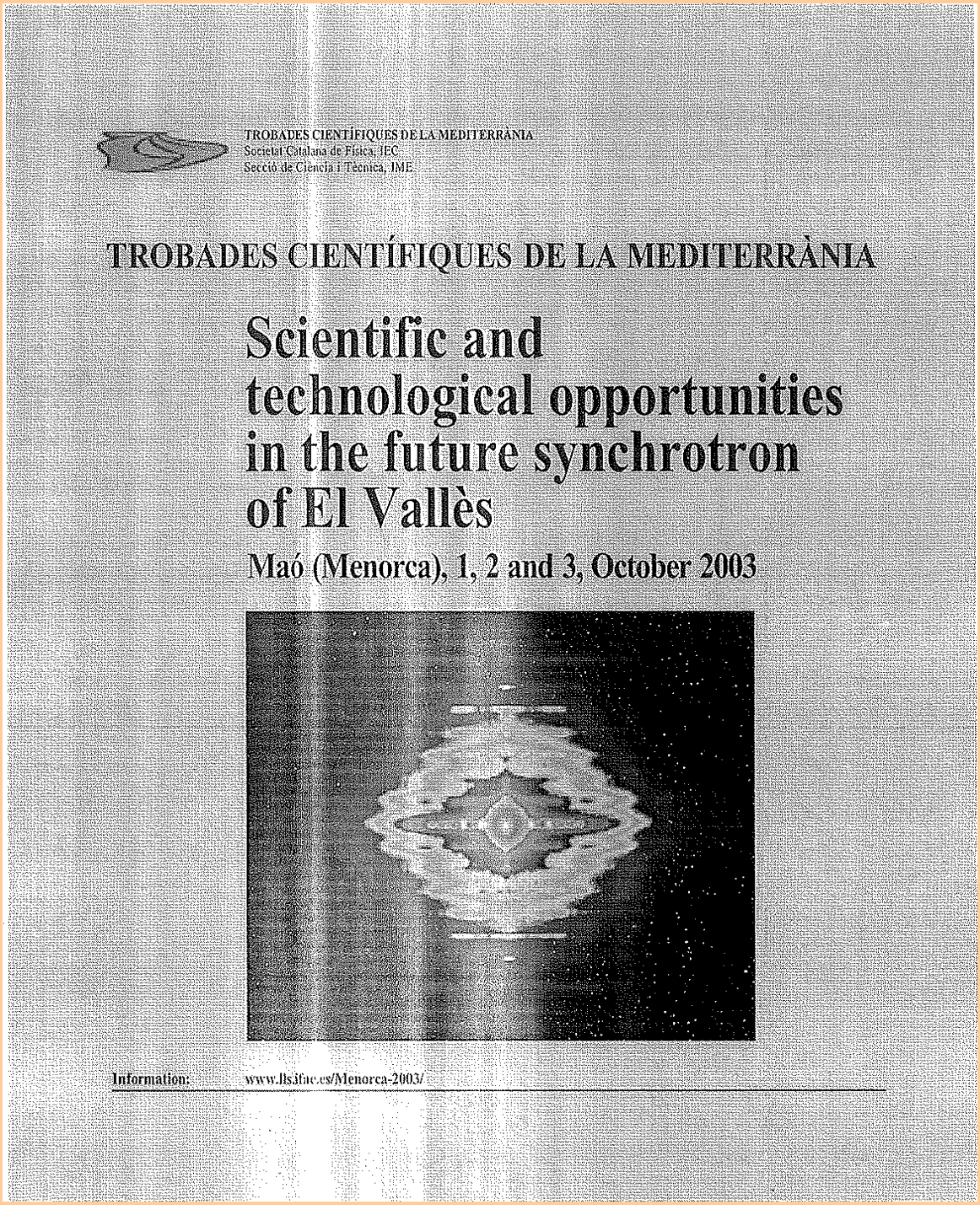
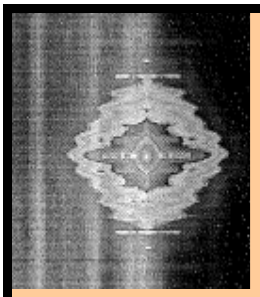
XIX TROBADES CIENTÍFIQUES DE LA MEDITERRANIA



MAO (MENORCA) 1th-3th October 2003



XIX Trobades Científiques de la Mediterrània

«Scientific and technological opportunities in the future synchrotron of El Vallès»

Maó (Menorca), 1-3 Octubre 2003

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LIPID ANALYSIS AND PROTEIN DISTRIBUTION OF THE EXTRACELLULAR SURFACE OF THE CORNIFIED ENVELOPE

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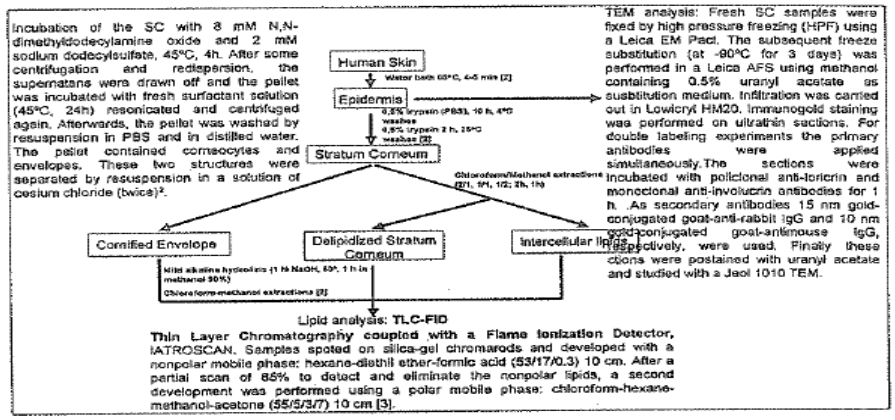
OBJECTIVE: Study of the presence of FFA in the CE and elucidation of the disposition of the loricrin in the CE

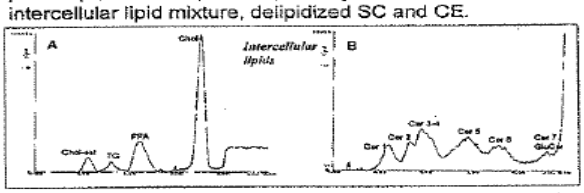
INTRODUCTION: The cornified envelope (CE) of the stratum corneum (SC) consists of a layer of highly crosslinked insoluble proteins covalently bound to a layer of lipids. These covalent bonds have been described as ester linkages in which the carboxylic group of the repetitively arrayed glutamic (Glu) of involucrin (protein rich in Glu) is bound to the hydroxyl group of the ceramides (Cer)[1]. However, some studies have pointed to the involvement in these covalent bonds of not only Glu and Cer, but also free fatty acids (FFA) and serine residue (Ser) of loricrin (protein rich in Ser residue and the most abundant in the CE) [2].The problem is that the FFA analyzed, which were presumably linked to the Ser, could also come from other membrane proteins. In addition, this FFA-Ser bond would involve an superficial arrangement of the loricrin.



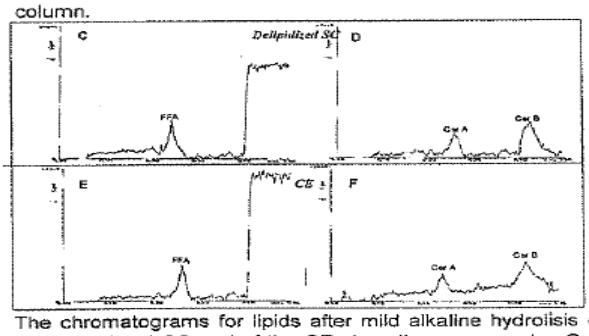
RESULTS

Lipid Analysis

TLC-FID chromatograms of the lipids developed with the nonpolar mobile phase (A, C and E) and with the polar mobile phase (B, D and F) corresponding with the extractions from intercellular lipid mixture, delipidized SC and CE.

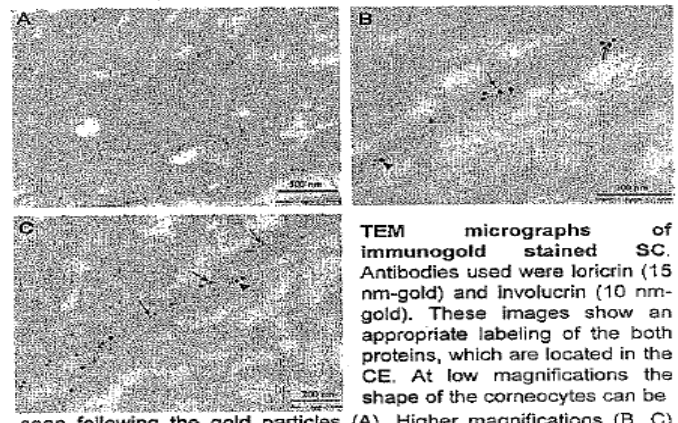


In the nonpolar fraction, cholesteryl esters (Chol-est), triglicerides (TG), free fatty acids (FFA) and cholesterol were detected (A). The polar lipids were mainly ceramides (Cer), the cholesteryl sulfate was probably retained at the botton of the column.



The chromatograms for lipids after mild alkaline hydrolisis of the delipidized SC and of the CE show the same peaks. One of them corresponding with the FFA (chromatogram C and E) and two peaks more with mobilities similar to Cer 2 and Cer 6. These peaks could be associated with the omega hydroxil Cer covalently bonded to the CE, i.e. Cer A and Cer B[4].

TEM observations



TEM micrographs of immunogold stained SC. Antibodies used were loricrin (15 nm-gold) and involucrin (10 nm-gold). These images show an appropriate labeling of the both proteins, which are located in the CE. At low magnifications the shape of the corneocytes can be seen following the gold particles (A). Higher magnifications (B, C) show the preferent external disposition of the involucrin (arrow). The loricrin could arrange just below the involucrin (arrowhead)[5]. However, some loricrin (open arrows) was also detected on the outermost part of the protein layer of the CE indicating the external disposition of some loricrin domains that could be accessible to form linkages with the FFA.

CONCLUSIONS

FFA were found in the isolated CE and in the delipidized SC after mild alkaline hydrolisis. This fact indicated the presence of FFA in the CE.

Immunogold electron microscopy shows a possible disposition of the loricrin in the extracellular side of the CE, in a similar way as the involucrin.

The linkage FFA-loricrin could be an ester bond between the hydroxyl group of the serine residue (Ser) of the loricrin and the carboxylic group of the FFA. Thus, a the new arrangement of the CE in which not only Cer-Glu bonds but also FFA-Ser bonds could be involved.

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