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Short Communication

Crystalloid Lumps from the Skin of a Patient with *Lupus Erythematosus Panniculitis*: Chemical Analysis

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Summary. Analysis of the lumps mentioned in the title revealed calcium, phosphate, and carbonate besides minor quantities of other inorganic ions. Moreover, saturated, unsaturated, and odd numbered fatty acids as well as extractable proteins of about 29, 45, and 66 kD were identified.

Keywords. *Lupus erythematosus panniculitis*; Crystalloid lumps; Fatty acids; Proteins.

Kristalline Ablagerungen in der Haut eines Patienten mit *Lupus erythematosus panniculitis*: Chemische Analyse (Kurze Mitt.)

Zusammenfassung. Die Analyse der im Titel genannten Ablagerungen ergab Calcium, Carbonat und Phosphat neben geringen Mengen anderer anorganischer Ionen. Außerdem wurden gesättigte, ungesättigte und ungeradzahlige Fettsäuren neben extrahierbaren Proteinen von *ca.* 29, 45 und 66 kD nachgewiesen.

Introduction

In very few cases *lupus erythematosus panniculitis* leads to calcinosis, characterized by crystallization of calcium salts in cutis and subcutis of the human skin [1]. Some crystalline lumps (2–5 mm in diameter) from a patient (male, multi morbid, born 1944, *lupus erythematosus panniculitis* diagnosed more than 20 years ago; Ca^{2+}, albumin, total protein, antibodies (as far as determined) within normal ranges) were analyzed by chemical methods.

Results and Discussion

Because the patient mentioned above is suffering *inter alia* from gout, the lumps were checked for uric acid. Negative results of the murexide reaction [2] and of

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mass spectrometrical measurements exclude this supposition. Elementary analysis of the crystals which had been washed with petroleum ether and dried resulted in 11.29% C, 2.30% H, 2.98% N, 17.08% Ca, 0.71% Na, and 0.21% K. The amount of residue after evaporation of the petroleum ether extract was only 2–8% of the original weight, depending on the sample.

Qualitative analysis after treatment of washed lumps with 35% nitric acid at room temperature (even so, they could not be dissolved completely) revealed Ca^{2+}, CO_3^{2-}, and PO_4^{3-} ions. After disintegration of the lumps by molten Na_2CO_3/K_2CO_3 (1:1 by weight) [3] we found 25.5% PO_4^{3-} [4]. Because the lumps turned black when being heated on a platinum sheet due to carbonization, it became evident that the carbon content could not be due to CO_3^{2-} only. The *Liebermann-Burchard* reaction [5] as well as mass spectrometry excluded Δ^5 unsaturated steroids. Fatty acids extracted from the lumps (about 3.3%) were subjected to positive ion GC-EIMS, revealing small amounts of tetradecanoic acid ($C_{14}H_{28}O_2$), 9-hexadecenoic acid ($C_{16}H_{30}O_2$), and traces of pentadecanoic acid ($C_{15}H_{30}O_2$) (see below). The most intense signal of the GC run corresponds to hexadecanoic acid ($C_{16}H_{32}O_2$), followed by a substantial amount of 9-octadecenoic acid ($C_{18}H_{34}O_2$) and some octadecanoic acid ($C_{18}H_{36}O_2$). These acids were identified by comparison of their mass spectra with those of authentic compounds [6].

The patient had been treated with an ointment containing paraffins as a basis. Although paraffins are generally regarded to be metabolically inert [7], *Tulliez et al*. [8] report on metabolization of heptadecane to heptadecanoic acid. According to the special information of the producer, the ointment basis mentioned above is

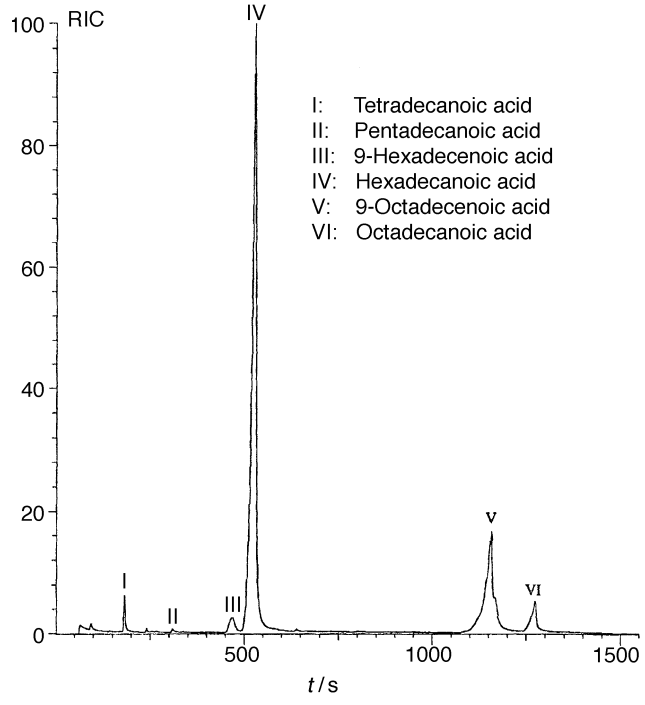

Fig. 1. GC of fatty acids

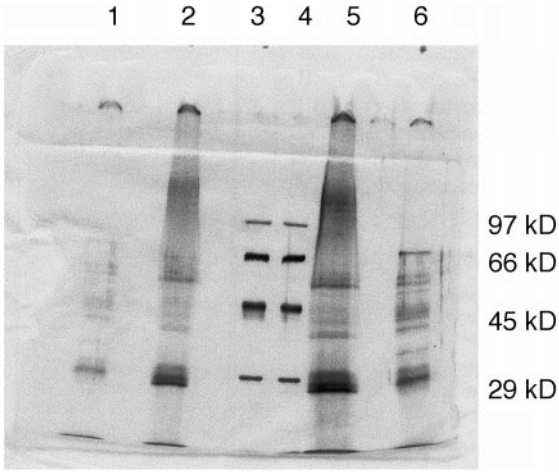

Fig. 2. Separation of proteins from crystalloid lumps, SDS-PAGE (for explanation see text); columns 1 and 6: 1st extr., 10 and 20 µl, resp., columns 2 and 5: 2nd extr., 10 and 20 µl, resp.; columns 3 and 4: standards (97 kD: phosphorylase a; 66 kD: BSA; 45 kD: ovalbumin; 29 kD: carboanhydrase)

free of pentadecane and pentadecanoic acid. Crystals from skin, not treated with this ointment, were not available.

The data mentioned cannot explain the content of 3% N in the lumps (no NH_4^+ could be detected). Therefore, lumps were freed from fatty acids and inorganic salts; the remaining material, not soluble in 1 *N* HCl, amounts to 30% of the weight of the lumps and was insoluble in all usual solvents but swelled in dimethylformamide or dimethylsulfoxide. This fraction contained 40.91% C, 6.70% H, and 13.41% N, thus pointing to proteins. Hydrolysis by 6 *N* HCl as usual, followed by TLC, revealed numerous substances showing a positive ninhydrin reaction [9].

Therefore, lumps as obtained from the patient were directly extracted by sonication in Triton X 100/Tris-HCl buffer [10, 11]. After centrifugation, the supernatant and the pellet were analyzed according to *Laemmli* [12] by SDS-PAGE (*S*odium *D*odecyl *S*ulfate/*P*oly*A*crylamide *G*el *E*lectrophoresis) revealing many protein bands (Fig. 2; supernatant: tracks 1 and 6; pellet: tracks 2 and 5; silver staining [13]). Dominant bands were found near 29, 45, and 66 kD. According to these results, the lumps may contain about 1% of extractable proteins.

Due to shortage of material, no further experiments were performed.

Experimental

Crystalloid lumps were defatted in a Soxhlet device with petroleum ether (40–60 °C). Phosphate: Determination by anion chromatography. Device: Biotronic IC 5000; room temp.; integrator: Shimadzu C-R4 AX; eluent: 1.0 mmol Na_2CO_3, 2.5 mmol $NaHCO_3$; flux: 1.5 ml/min; detection: conductivity; column: BTI AN, Biotronik; injection volume: 100 µl. Fatty acids: 60 mg of pulverized and defatted lumps were suspended in 0.5 ml of 2 *N* HCl and extracted three times with freshly distilled diethyl ether. After evaporation and drying at the oil pump, the fatty acids obtained (2 mg) were subjected to GC-EIMS; column: fused silica SGE BP 5, 25 m, 0.25 mm diameter; El-MS: 70 eV, Finnigan MAT 95. Results: see Fig. 1. Proteins: a) A suspension of 240 mg of defatted lumps in 3 ml of 1 *N* HCl was extracted with diethyl ether (3×3 ml). After 7 days at 4 °C, the mixture was filtered, the aqueous phase was evaporated *in vacuo* (inorganic salts, 205 mg, free of C and N), and

the suspended material, not soluble in 1 *N* HCl, was collected: 73 mg (30% of the lumps), insoluble in all usual organic solvents, but soluble in 2 *N* HCl or 2 *N* NaOH with slight warming. This fraction was hydrolyzed by 6 *N* HCl (sealed tube, 100 °C, 24 h), followed by TLC (silica; CH_3CN/0.1 mol sodium acetate 60:40); detection: ninhydrin.

b) 5 mg of crystalloid material were suspended in 0.5 ml of 1% Triton X 100/25 m*M* Tris-HCl, $pH = 8$ [10, 11], whirlmixed, and repeatedly sonicated for 10 s under ice cooling. After centrifugation for 15 min at 11000 g, 100 µl of the supernatant were diluted with 100 µl of sample buffer (3% SDS, 5% mercaptoethenol, 100 m*M* Tris-HCl, pH 6.8) [12], and 20 µl of this solution were analyzed by SDS-PAGE [12]. The pellet of the first centrifugation was suspended in 150 µl of sample buffer, heated to 95 °C for 10 min, and centrifuged for 3 min (see above). 100 µl of the supernatant were diluted with 100 µl of water, heated to 95°C for 3 min, and 10 µl of the solution obtained were analyzed by SDS-PAGE. Results: see Fig. 2.

Note added in proof

Prof. *N. Korber*, Institute of Inorganic Chemistry, University of Regensburg, has established by powder X-ray diffractometry that the calcium phosphate component of these crystals consists of hydroxylapatite.

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