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SALPETER-INDUCED DERMAL CHANGES ELECTRON-MICROSCOPICALLY INDISTINGUISHABLE FROM PSEUDOXANTHOMA ELASTICUM

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Abstract. Salpeter-induced calcium deposits in ten patients showed a close clinical and histopathological similarity to the lesions of pseudoxanthoma elasticum. By electron microscopy and selected area diffraction analyses of the calcium deposits we find the changes indistinguishable from the changes previously described in involved skin of patients suffering from PXE. Clinically our patients were compared with the fourteen patients with traumatic calcification previously described.

Key words: Pseudoxanthoma elasticum; Calcification; Salpeter; Elastic fibre; Collagen; Connective tissue

Salpeter-induced calcium deposits in human skin were originally demonstrated by Christensen (3). All cases followed skin contact with dry salpeter. The cutaneous lesions were clinically reminiscent of and histopathologically indistinguishable from pseudoxanthoma elasticum (PXE). To reveal the character of such lesions, we studied the ultrastructure and performed an electron diffraction analysis of the calcium deposits of seven patients. The ultrastructure and the findings are compared with previous electron microscopical studies of PXE.

MATERIALS AND METHODS

Ten elderly farmers from Denmark and Sweden all presented the same history: 30–50 years ago, while fertilizing, they spread Norwegian Hydrous Salpeter (Table I) with their hands. The shirt sleeves were rolled up over the elbow and the weather was damp or foggy. After spreading thus for some hours they felt a burning skin sensation and soon corrosive ulcers formed. One of the farmers developed corrosion ulcers on his legs located by the upper parts of his rubber boots.

The ulcers healed in 2–3 weeks, and, after healing, the skin lesions remained unchanged. The skin lesions were provoked only on one single occasion. None of the patients had a family history of PXE. The skin showed yellow-white slightly elevated plaques measuring 0.5–1.5 cm^2 (Fig. 1). No other evidence of PXE was observed, including angioid streaks.

Biopsies were taken of both involved and normal skin from 7 of the 10 patients. The specimens were fixed in 6% glutaraldehyde solution with 7.5% sucrose, buffered at pH 7.4 with 0.1 M cacodylate and postfixed in 1% osmic acid. After dehydration in graded alcohols, the specimens were embedded in Epon 812. Ultrathin sections were cut on an LKB ultramicrotome. For bright- and dark-field microscopy, sections were stained with uranyl acetate and lead citrate. To determine the crystal structure of mineralized deposits, selected area diffraction patterns were recorded from deposits in unstained specimens. To determine the diffraction constant of the microscope, diffraction patterns of evaporated, thin, polycrystalline gold films were exposed without altering the lens excitations (7, 12).

A Siemens Elmiskop I electron microscope was used for microscopy and diffraction, and a JEOL 100 U electron microscope for tilted beam dark-field microscopy.

RESULTS

Elastic fibres

The skin lesions contained increased amounts of elastic fibres. Most were heavily infiltrated by an electron-dense material (Fig. 2), whereas this was

Table I. *The chemical composition of hydrous salpeter*

Norwegian salpeter
$Ca(NO_3)_2$, $2H_2O$, $Ca(NO_3)_2$
5 $Ca(NO_3)_2$, NH_4NO_3, 10 H_2O
78% $Ca(NO_3)_2$
6% NH_4NO_3
15% H_2O

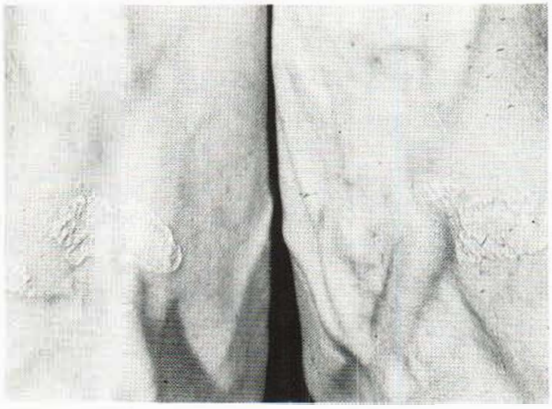

Fig. 1. Yellow-white, slightly elevated plaques in both elbow regions of one of the patients.

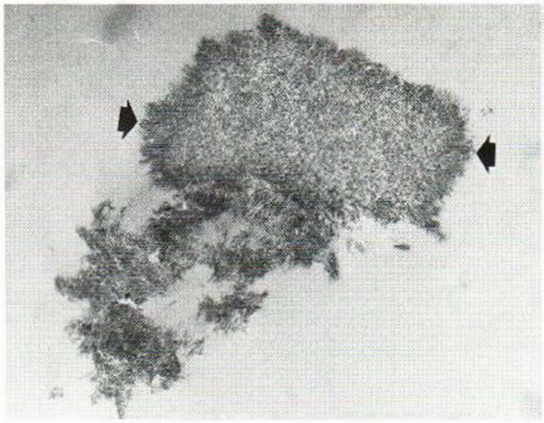

Fig. 3. Bright-field micrograph of apatite deposit in an unstained elastic fibre. Needle-like structures are seen protruding from the peripheral borderline (arrows). ×36 700.

never observed outside the elastic fibres. The deposit appeared inside the elastic matrix in two zones, viz. a central area containing coarse granules and needles, and a narrow, highly electron-dense peripheral line containing needles only (Fig. 3). Selected area diffraction analysis revealed that the deposits represented calcium apatite crystals, consisting of either calcium hydroxyapatite or calcium fluoroapatite (Fig. 4 and Table II). To establish the connection between the diffraction rings and the distribution of the crystals, tilted beam dark-field microscopy was performed. The result is shown in Fig. 5. The apatite crystals are localized exclusively to the electron-dense deposits. Uninvolved skin showed no increase of elastic tissue or deposits.

Collagen fibrils

Involved skin showed collagen fibrils irregularly arranged and with varying, mostly increased, diameters (Fig. 6). Many of the fibrils had a flower-like appearance in cross sections, with the periphery of the fibril split into subunits (Fig. 7). In longitudinal sections, the fibrils appeared as wire-like figures twisted around their axis (Fig. 8). The number of altered collagen fibrils varied from one patient to another. Two patients showed pronounced

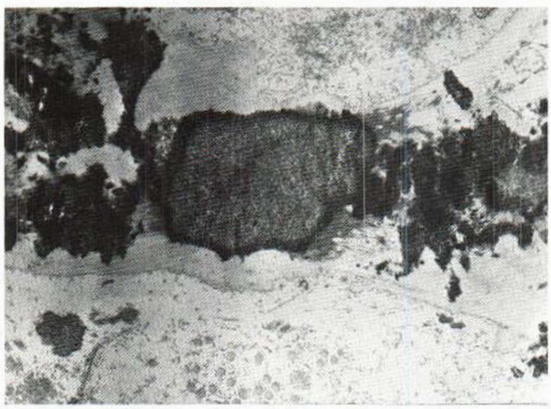

Fig. 2. Bright-field micrograph of apatite deposit in a stained elastic fibre. ×11 700.

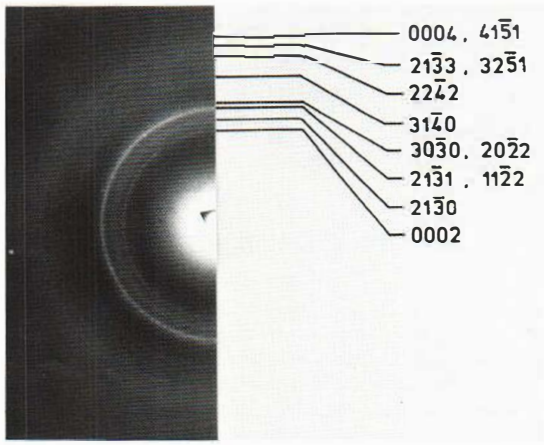

Fig. 4. Selected area diffraction pattern from an apatite deposit. The diffraction pattern, indexed according to Table II, shows that the deposit is of apatite type. The indices refer only to the intense rings of the ASTM files.

Table II. *Experimental electron diffraction data obtained from a mineralized deposit in unstained elastic fibre compared with X-ray diffraction data for calcium fluoroapatite and calcium hydroxyapatite*

ASTM data			
Miller indices[a] (*hkil*)	Fluoro-apatite[b] (*d*(*hkil*) Å)	Hydroxy-apatite[c] (*d*(*hkil*) Å)	Experi-mental data (*d*(*hkil*) Å)
$10\bar{1}0$	8.12	8.17	
$10\bar{1}1$	5.25	5.26	
$11\bar{2}0$	4.684	4.72	
$20\bar{2}0$	4.055	4.07	
$11\bar{2}1$	3.872	3.88	
$20\bar{2}1$	3.494	3.51	
0002	3.442	3.44	3.48
$10\bar{1}2$	3.167	3.17	3.21[d]
$21\bar{3}0$	3.067	3.08	3.07
$21\bar{3}1$	2.800	2.814	2.82
$11\bar{2}2$	2.772	2.778)	2.73
$30\bar{3}0$	2.702	2.720)	
$20\bar{2}2$	2.624	2.631	2.64[d]
$30\bar{3}1$	2.517	2.528	
$21\bar{3}2$	2.289	2.296)	2.27
$31\bar{4}0$	2.250	2.262)	
$22\bar{4}1$	2.218	2.228	
$31\bar{4}1$	2.140	2.148	
$30\bar{3}2$	2.128	2.134	
$11\bar{2}3$	2.061	2.065	
$40\bar{4}0$	2.028	2.040	
$20\bar{2}3$	1.997	2.000)	1.96
$22\bar{4}2$	1.937	1.943)	
$31\bar{4}2$	1.884	1.890	
$32\bar{5}0$	1.862	1.871)	1.84
$21\bar{3}3$	1.837	1.841)	
$32\bar{5}1$	1.797	1.806)	
$41\bar{5}0$	1.771	1.780)	
$40\bar{4}2$, $30\bar{3}3$	1.748	1.754)	1.75
0004, $41\bar{5}1$	1.722	1.722)	
$10\bar{1}4$	1.684	1.684	

[a] The four-index notation, commonly used for hexagonal crystal. The index i is given by $i=-(h+k)$.
[b] ASTM card No. 15–876. Calcium fluoroapatite has a hexagonal crystal structure with $a_0=9.3684$ Å and $c_0=6.8841$ Å.
[c] ASTM card No. 9–432. Calcium hydroxyapatite has a hexagonal crystal structure with $a_0=9.418$ Å and $c_0=6.884$ Å.
[d] These rings are very weak and cannot be resolved in the reproduction of fig.

changes, three moderate changes, and one few changes.

No collagen changes could be demonstrated in uninvolved skin.

Granulofilamentous material

Compact masses of a granulofilamentous material were seen in all patients. The material surrounded elastic fibres, collagen fibrils and here and there

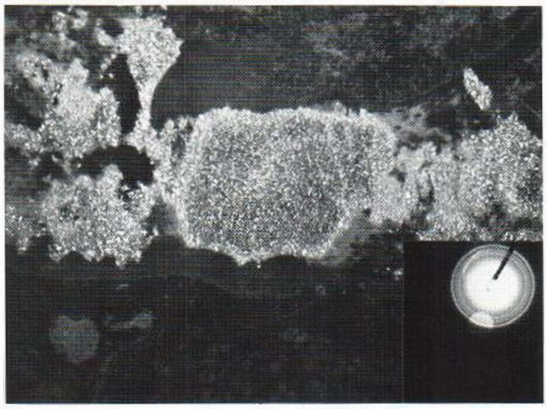

Fig. 5. Dark-field micrograph of the same apatite deposit as shown in Fig. 2. The inset shows the selected area diffraction pattern with the segments of the diffraction rings used for forming the dark-field image, as indicated by the position of the objective aperture. ×11700.

fibroblasts, and appeared either granulomatous or filamentous, but mostly as a mixture (Fig. 9).

Granulofilamentous material was never found in uninvolved skin.

Cells

The number and appearance of fibroblasts and mast cells did not differ from those of normal skin.

DISCUSSION

Histopathologically, salpeter corrosions could not be distinguished from PXE lesions (3). Electron

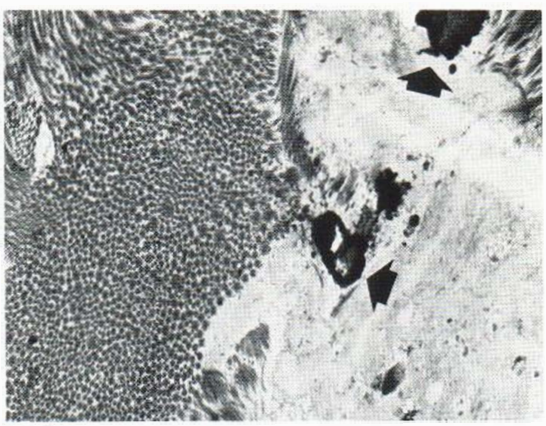

Fig. 6. Collagen fibrils irregularly arranged and with varying diameters (arrows). In the elastic fibres, apatite deposits are shown (arrow heads). ×5400.

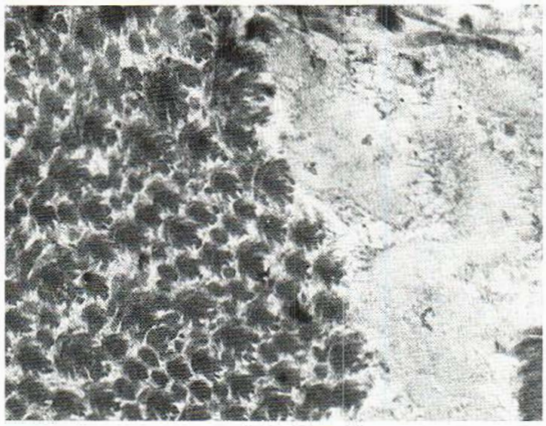

Fig. 7. Collagen fibrils in cross section, most with a flower-like appearance and the periphery split into subunits. ×10 800.

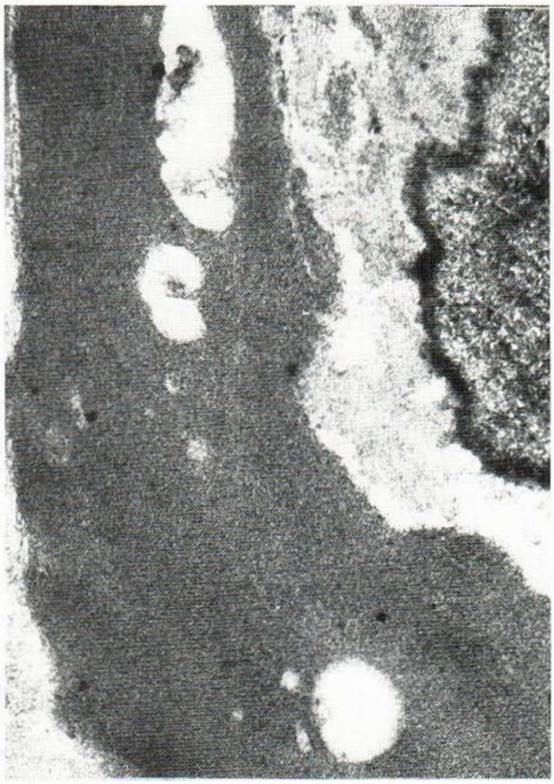

Fig. 9. A granulofilamentous material. Top right, an apatite deposit is seen. ×14 900.

microscopy of involved skin of patients with PXE revealed both collagen changes and granulofilamentous material similar to the findings in the present study (5). Calcium deposits inside elastic fibres have been considered characteristic of PXE (5, 9). Otkjær Nielsen et al. (12) demonstrated that the deposits in PXE lesions appeared in two zones, viz. a moderately electron-dense central region with coarse granules and needles, and a highly electron-dense peripheral line with needles only. Selected area diffraction analysis of PXE deposits showed that they consist mainly of apatite crystals. Their results are in agreement with our findings. Hence, our study emphasizes that the ultrastructure, including diffraction analysis, of salpeter lesions and PXE skin lesions are identical. None of our patients had a family history of PXE, nor any clinical evidence of PXE. These facts,

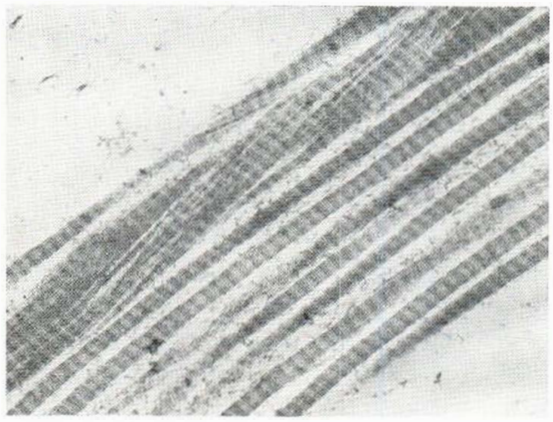

Fig. 8. Longitudinal section, a similar figure is seen with collagen fibrils twisted around their axes. ×10 800.

Table III. *Fourteen reported cases of traumatic calcium deposition in the skin*

Year	Authors	Conc. $CaCl_2$	Preceding trauma	No. of pats.
1935	Oppenheim	10%	+	1
1946	Hepplestein	40%	+	1
1955	Nash	40%	+	1
1957	Zackheim	Dry	–	2
1958	Sneddon & Archibald	3½%	+	2
1961	Botvinich et al.	Dry	–	1
1964	Clendenning & Auerbach	?	+	2
1965	Schoenfeld et al.	?	+	4

combined with the identical exposure to salpeter, makes the PXE diagnosis less credible.

A total of 14 cases of traumatic calcium deposition in skin have been encountered (Table III). Most of these lesions developed following contact with solutions of calcium chloride in various concentrations, while all our cases followed contact with dry salpeter powder. Three cases of calcium deposition in skin after exposure to dry calcium chloride powder have been reported (2, 15). Clendenning & Auerbach (4) considered two requirements for the production of calcium deposition in skin, viz. 1) minor trauma; 2) prolonged contact with calcium salts followed by or concomitant with the trauma. All our patients described dermatitis symptoms soon after the contact with the salpeter powder. The friction of salpeter powder between cloth and skin may have produced minor abrasion of the skin.

REFERENCES

1. Blümcke, S., Langnes, U., Liesegang, B., Fooke-Achterrath, M. & Thiel, H. J.: Light and electron microscopic and element analysis of Pseudoxanthoma elasticum. Beitr Pathol *152:* 179, 1974.
2. Botvinich, J., Smith, R. G. & Stein, S. C.: Calcium chloride necrosis of the skin. J Mich Med Soc *60:* 743, 1961.
3. Christensen, O. B.: An exogenous variety of Pseudoxanthoma elasticum in old farmers. Acta Dermatovener (Stockholm) *58:* 319, 1978.
4. Clendenning, W. E. & Auerbach, R.: Traumatic calcium deposition in skin. Arch Dermatol *89:* 360, 1964.
5. Danielsen, L., Kobayasi, T., Larsen, H. W., Midtgård, K. & Christensen, H. E.: Pseudoxanthoma elasticum. A clinico-pathological study. Acta Dermatovener (Stockholm) *50:* 355, 1970.
6. Edmonds, O. P.: Dermatitis from solutions of sodium and calcium chloride in the coal mining industry. Br J Ind Med *12:* 320, 1955.
7. Heidenreich, R. D.: Fundamentals of Transmission Electron Microscopy. Monographs and Text in Physics and Astronomy Series, Vol. XIII. John Wiley, Interscience, 1964.
8. Hepplestein, A. G.: Calcium necrosis of the skin. Br J Ind Med *3:* 253, 1946.
9. Huang, S., Steele, H. D., Kumor, G. & Parher, J. C.: Ultrastructural changes of elastic fibres in Pseudoxanthoma elasticum. Arch Pathol *83:* 108, 1967.
10. Nash, P. H.: Occupational calcium chloride necrosis of the skin. Br Med J *i:* 586, 1955.
11. Oppenheim, M.: Hautveränderungen durch Chlorkalzium bei der Herstellung der sogenannten Eislutcher. Wien Klin Wochenschr *48:* 207, 1935.
12. Otkjær-Nielsen, A., Johnson, E., Hentzer, B., Danielsen, L. & Carlsen, F.: Apatite crystals in Pseudoxanthoma elasticum. A combined study using electron microscopy and selected area diffraction analysis. J Invest Dermatol, in press.
13. Schoenfeld, R. J., Grehin, J. N. & Mehregan, A.: Calcium deposition in the skin. Neurology *15:* 477, 1965.
14. Sneddon, J. B. & Archibald, R. M. L.: Traumatic calcinosis of the skin. Br J Dermatol *70:* 211, 1958.
15. Zackheim, H. S. & Pinkus, H.: Calcium chloride necrosis of the skin. Arch Dermatol *76:* 244, 1957.

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