[Original] Matsumoto Shigaku, 14 : 41~48, 1988

key words: analytical electron microscopy — calcinosis universalis — ultrastructure — hydroxyapatite — mineral component

Analytical Electron Microscopic Study of Mineral Deposits in a Case of Calcinosis Universalis

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Summary

Calcinosis universalis with dermatomyositis occurred in a 58-year-old woman. Tissues removed from the patient's sublingual region were studied mainly by analytical electron microscopic methods. According to an elemental analysis using a wavelength dispersive X-ray spectroscope, most of the mineral deposits contained the elements sodium, phosphorus, calcium, rhenium, and some deposits also contained sulphur and magnesium. The elements sodium, sulphur, chlorine, and calcium were found in the stromal tissues of the material. Energy dispersive X-ray spectroscopy demonstrated that the analysis-peaks of phosphorus and calcium were higher in the mineral deposits than in the stromal tissues. The mineral deposits throughout the specimen were mainly composed of hydroxyapatite, judging from field-limited area electron diffraction and X-ray diffraction examinations.

Introduction

Calcinosis universalis is a rare complication associated with collagen diseases, particularly scleroderma, dermatomyositis, and systemic lups erythematosus, therefore few case reports are available and those consist mainly of radiographic features, a few histopathological findings, and clinical data[1~5]. We were fortunate enough to examine a case of calcinosis universalis associated with dermatomyositis occurring in a 58-year-old woman[6], and studied its ultrastructural appearance[7]. These ultrastructural features were summarized as follows: the calcification was closely related to the foci of fibrinoid degeneration and membranous structures appearing in the

(accepted for publication on February 6, 1988)

stromal matrix seemed to have a matrix vesicle-like function, causing initial calcification. The purpose of the present study was to examine, by analytical electron microscopy, the mineral composition of such deposits to obtain more information about this disorder.

Materials and Methods

The patient, a 58-year-old woman, was diagnosed as having calcinosis universalis secondary to dermatomyositis[6]. Her medical history in brief is the following: at the age of 15, she had chronic articular rheumatism. About 10 years ago, she noticed hard masses in the anterior neck region and both elbow joints; one year later, calcified materials in her abdomen were pointed out by her doctor during consultation for stomachache. About 3 years ago, she noticed rock-hard masses on the floor of her mouth which gradually enlarged and caused pain during mastication and was admitted to the Clinic of Oral and Maxillofacial Surgery, Shinshu University Hospital, for treatment. Routine clinical laboratory data (EKG, CPK, etc.) were within normal limits. Under X-ray photographs, a large number of small radiopaque particles were found widely distributed throughout her body, mainly located along the large vessels; in the head and neck area, these were mainly in the sublingual and neck regions (Figs. 1, 2). The calcified materials were removed from the sublingual region to improve tongue movement.

The removed tissues were fixed immediately in Karnovsky's solution[8] at 4°C, and then embedded in paraffin for histopathological study after being pretreated with or without demineralization in 10% formic acid with formalin. The sectioned specimens were stained with the following reagents: hematoxylin-eosin (H-E), van Gieson's, Mallory's azan, and von Kossa's stains. The stained specimens were then examined by a light microscope.

For transmission electron microscopy (TEM), the specimens fixed in Karnovsky's solution were postfixed in 1% osmium tetraoxide and embedded into Epon 812, having been pretreated with or without demineralization in 5% ethylene-diamine-tetra-acetic acid (EDTA) at 4°C. The ultrathin sections were stained with uranyl acetate and lead citrate (U-Pb), and then examined by an electron microscope (JEOL JEM 100-B). Field-limited area electron diffraction and energy dispersive X-ray analysis were carried out on the unstained specimens by an analytical electron microscope (JEOL JEM 1200 EX).

For scanning electron microscopy (SEM) and electron probe microanalysis (EPMA), the fractured specimens were processed by critical-point drying and carbon-coating methods. The Karnovsky's solution fixed specimens without demineralization were dehydrated and then embedded into Epon 812. The surface of the Epon 812 was polished and the polished surface was coated with carbon. Both groups of specimens were observed by a JEOL JCXA 733 X-ray microanalyser.

Some specimens were ground, and examined by an XD-9 X-ray diffractometer (Shimadzu, Japan).

Results

Light microscopic findings:

A large number of calcified deposits strongly stained with hematoxylin were observed in the connective tissues of undemineralized specimens (Fig. 3), they reacted positively to von Kossa's stain. In the demineralized specimens, the stainability of these deposits to hematoxylin obviously decreased, and they stained yellow with van Gieson's, and weak orange with Mallory's azan stains. All specimens showed an amorphous globular pattern in these stainings.

Transmission electron microscopic findings:

Mineral deposits were observed as uniform, electron-dense materials mainly in the areas of fibrinoid degeneration. High-power magnification revealed that they were composed of columnar crystals of varying size (Fig. 4). Field-limited area electron diffraction patterns of these crystals, identified as hydroxyapatite, were similar to the crystals in normal bone tissue (Fig. 4, insert).

Generally, these calcified materials showed variation in their electron density and, especially in large mineral deposits, an electron-dense layer was found in the periphery.

Scanning electron microscopic and electron microanalytical findings:

In the composition images, the mineral deposits were observed as light structures demonstrating irregular surfaces surrounded by a dark matrix (Figs. 5, 6). The brightness of individual the masses varied from slightly dark to very bright, with a striped and/or amorphous globular pattern.

Electron probe microanalysis of the calcified masses with an energy dispersive X-ray spectroscope (EDS), revealed that the mineral deposits mainly consisted of calcium and phosphorus (Fig. 7-a, b), also the stromal matrices contained traces of the same elements and sulphur (Fig. 7-c, d). The levels of elements detected varied according to the point under analysis.

According to the elemental analysis using a wavelength dispersive X-ray spectroscope (WDS), most of the mineral deposits contained the elements sodium, phosphorus, calcium, and rhenium; some of them sulphur and magnesium as well. The elements sodium, sulphur, chlorine, and calcium were present in the stromal tissues (Fig. 8).

X-ray diffraction examination:

According to the X-ray diffraction pattern obtained from the mineral deposits, the main peak occurred at a diffraction angle of 32°, and other peaks, at angles of 25.8°, 31.8°, 32.8°, and 49.4°. This pattern indicated that the mineral deposits were chiefly composed of hydroxyapatite.

Discussion

Calcinosis universalis is a rare complication associated with collagen diseases. The few case reports consisted mainly of radiographic findings and a few histopathological features. Nielsen et al.[9] studied the ultrastructure and the composition of the mineral elements of the lesion. However, as far as we know, there are no other studies at the electron microscopic level on this disease. We recently described the ultrastructural appearance of initial calcification in a case of calcinosis universalis accompanying dermatomyositis.[7] In our report, the ultrastructure was described as follows: the calcified materials were distributed on collagen fibers and seemed to have a positive relationship with foci of fibrinoid degeneration. Globular and/or membranous structures, considered to have derived from the degenerated cells of the stroma, were observed in these calcified areas, and some of them contained electron-dense materials, Therefore, we proposed that the globular and/or membranous structures might be involved in the initial calcification in this case.

In the present paper, we examined the mineral deposits in the same case of calcinosis universalis by analytical electron microscopy. Through EDS analysis, mineral deposits of the specimen were found to consist of sodium, phosphorus, calcium, rhenium, and some also contained sulphur and magnesium. The deposits were mainly composed of hydroxyapatite according to the results of the field-limited area electron diffraction and X-ray diffraction examinations. It was proposed by Loewi and Dorling [10] that the mineral deposits in calcinosis universalis are built up from hydroxyapatite; and Nielsen et al.[9] reported the mineral part to be calcium apatite (calcium-hydroxyapatite or calcium fluoroapatite). The above-mentioned reports are in accord with our present findings. Pinter

et al.[11] investigated the calcified masses in a case of calcinosis universalis using X-ray diffraction, and found them to be composed of a rare kind of carbonate apatite. Therefore, it seems that the composition of mineral deposits is different depending on the case investigated. However, we believe the mineral deposits in most cases are composed of hydroxyapatite.

Regarding our results of the mineral composition of the stromal tissue, we have a strong suspicion, especially in view of the quantitative relationship between stromal elements and those in calcified deposits, that the mineral elements in the stromal tissue are involved in the calcification mechanism. So, in our continuing work, the relationship between the mineral components and the ultrastructure in both deposits and surrounding stroma should be investigated, which should give us a better understanding of mechanism of calcification in calcinosis universalis.

Acknowledgement

This study was suported in part by a grant (No. 61570901) from the Ministry of Education, Culture and Science, Japan. The authors wish to express their thanks to Dr. T. Yamazaki, Shinshu University School of Medicine, for suplying of the surgical material from and clinical data on the patient.

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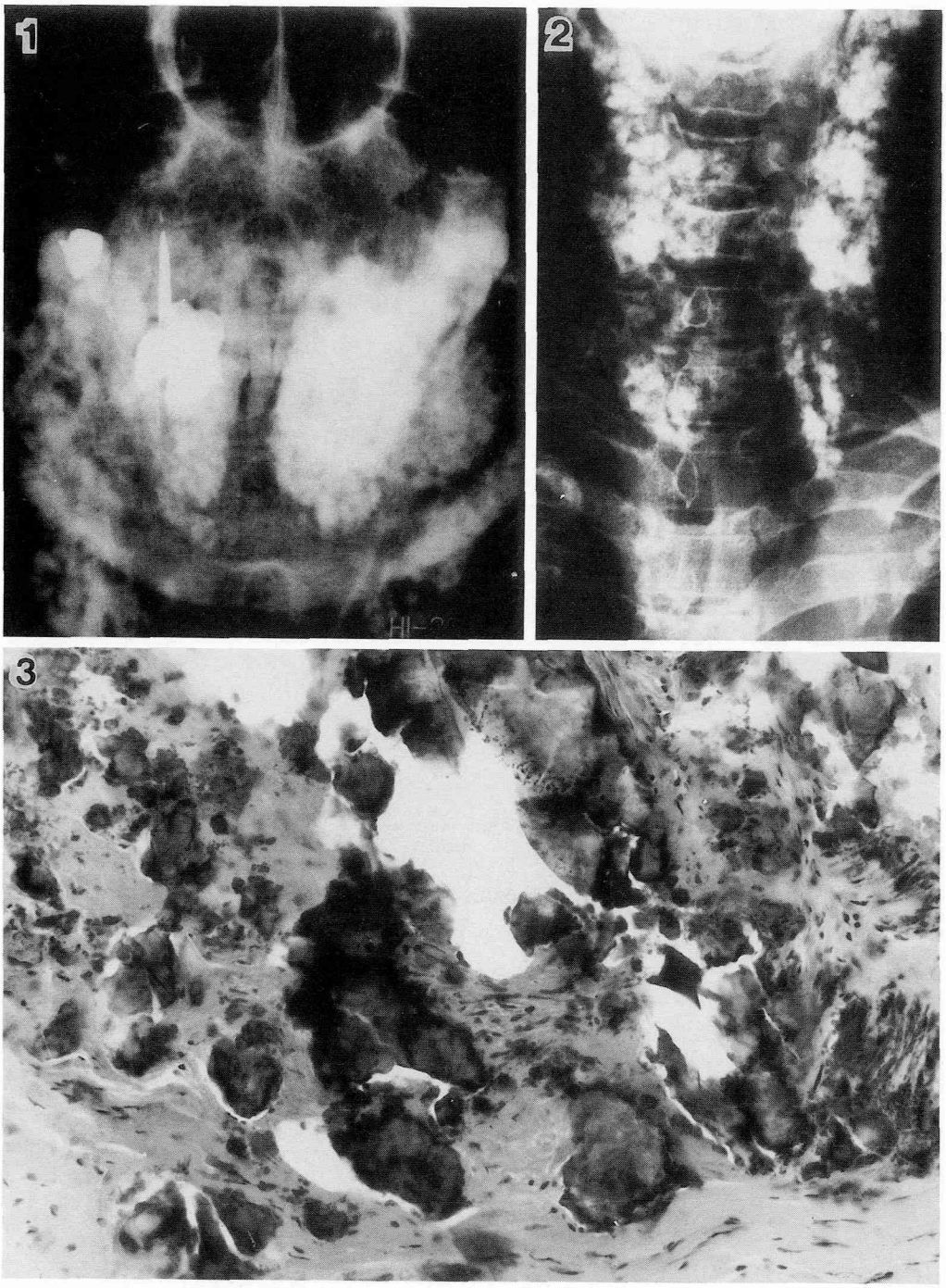


Fig. 1. X-ray photograph showing small radiopaque granules located in the sublingual region
Fig. 2. X-ray photograph revealing radiopaque granules located in the neck region
Fig. 3. Calcified globular masses strongly stained with hematoxylin (H-E. undemineralized section, ×220)

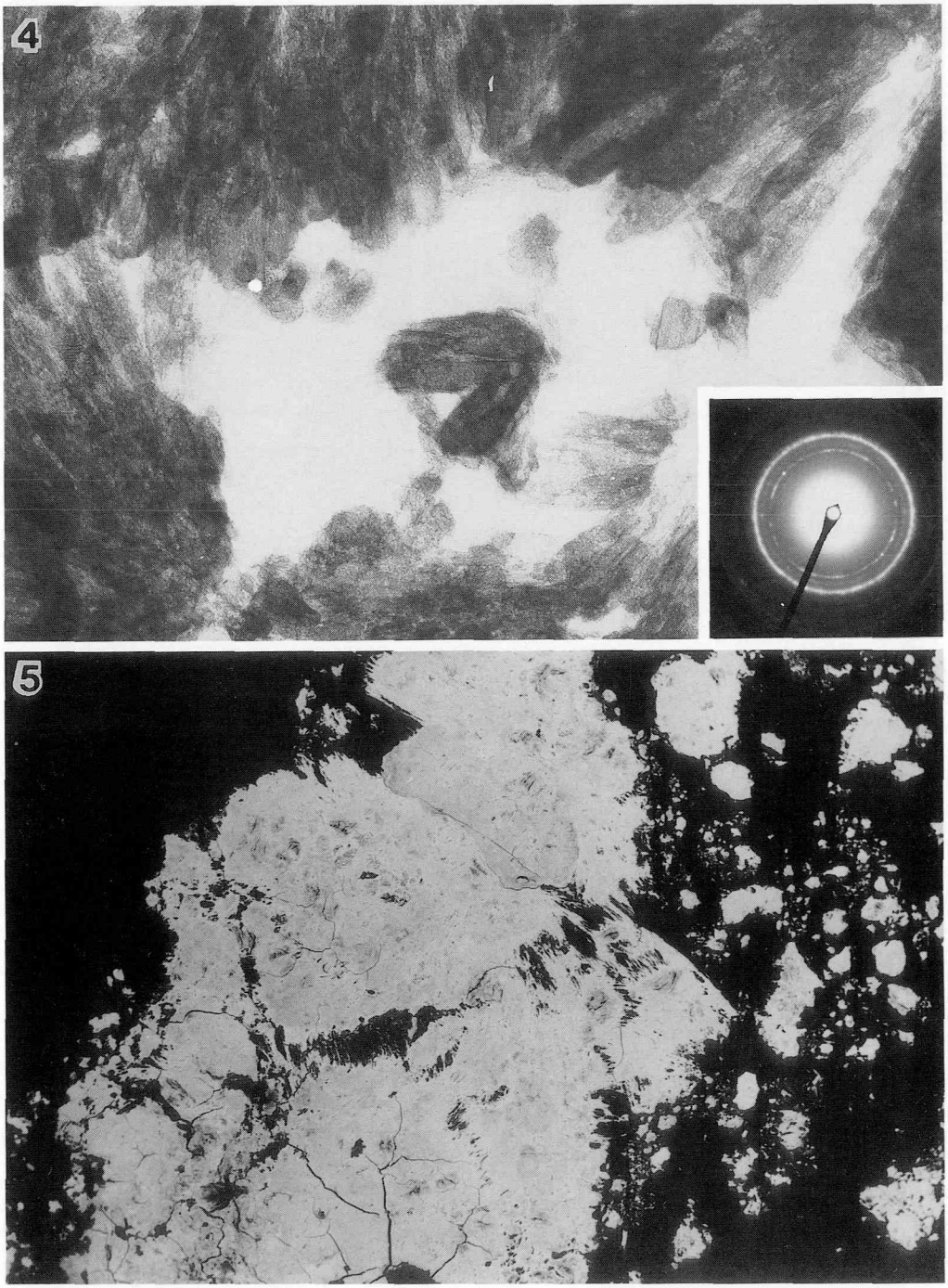
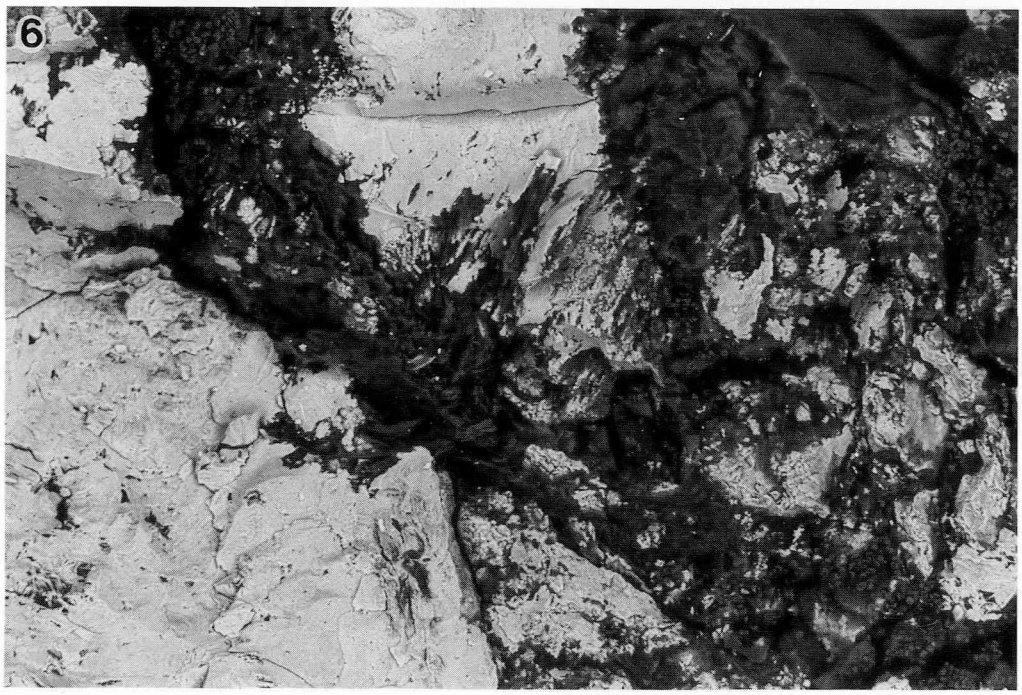


Fig. 4. Transmission electron microscopic photograph showing columnar crystals and their field-limited area electron-diffraction pattern (insert) indicating them as hydroxyapatite (unstained & undemineralized section, ×150,000)

Fig. 5. Scanning electron microscopic composition image of polished surface of embedded calcified materials (×50)



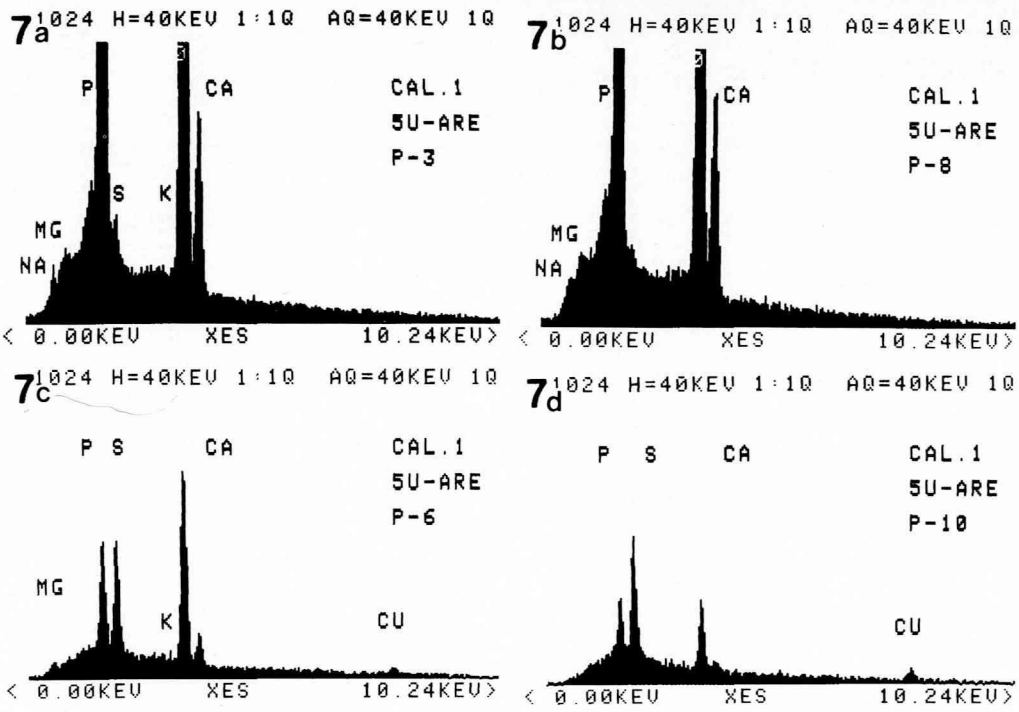


Fig. 6. Scanning electron microscopic composition image of the fractured surface of a calcified mass showing the bright mass surrounded by a dark matrix (×200)

Fig. 7. EPMA by EDS of calcified material (a and b) and of stromal tissues (c and d)

8a

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PROBE CURRENT : 2.230E-08 (A)
STAGE POS. :  X  16973  Y  25077  Z  11670
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CH(1) TAP				CH(2) PET				CH(3) LIF			
EL	WL	COUNT	INTENSITY(LOG)	EL	WL	COUNT	INTENSITY(LOG)	EL	WL	COUNT	INTENSITY(LOG)
Y	6.45	39	*********	TI	2.75	15	******	BI	1.14	14	******
RE	6.73	370	************+++	BA	2.78	12	******	PB	1.17	11	******+
SR	6.86	31	********	CS	2.89	12	******	TL	1.21	11	******
W	6.98	30	********	SC	3.03	19	*******+	HG	1.24	12	******+
SI	7.13	28	********	I	3.15	13	******	AU	1.28	13	******
TA	7.25	26	********+	TE	3.29	15	******	PT	1.31	10	*****
RB	7.32	26	********+	CA	3.36	3480	************++++++++++	IR	1.35	11	******
HF	7.54	22	********	SB	3.44	7	*****	OS	1.39	11	******
LU	7.84	15	******	SN	3.60	9	*****	ZN	1.44	13	******
YB	8.15	13	******	K	3.74	10	*****	CU	1.54	12	******+
AL	8.34	16	********	IN	3.77	7	*****+	NI	1.66	7	*****
BR	8.37	13	******	U	3.91	7	*****+	TM	1.73	8	*****
ER	8.82	15	******+	CD	3.96	4	****	CO	1.79	6	****
SE	8.99	12	******+	TH	4.14	5	****	FE	1.94	5	***+
HO	9.20	12	******	AG	4.15	5	****	GD	2.05	4	****+
DY	9.59	6	****	PD	4.37	5	***+	MN	2.10	7	******+
AS	9.67	9	*****	RH	4.60	3	***	EU	2.12	4	*+++
MG	9.89	53	**********+	CL	4.73	3	**+	SM	2.20	4	****
TB	10.00	11	******	RU	4.85	1	*	CR	2.29	2	++
GE	10.44	6	****	S	5.37	13	*******+	ND	2.37	2	*+
GA	11.29	5	****	MO	5.41	2	*+	PR	2.46	1	+
NA	11.91	23	********+	NB	5.72	2	*+	V	2.50	1	*
**	15.80	2	*+	ZR	6.07	1	+	CE	2.56	0	
F	18.32	4	*+++	P	6.16	273	**************	LA	2.67	2	++

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RESULTS:

THE FOLLOWING ELEMENTS ARE PRESENT
  NA  MG  P   S   CA  RE

THE FOLLOWING ELEMENTS ARE PROBABLLY PRESENT
  F   CR  MN  LA  EU
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8b

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PROBE CURRENT : 2.220E-08 (A)
STAGE POS. :  X  17153  Y  25230  Z  11728
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CH(1) TAP				CH(2) PET				CH(3) LIF			
EL	WL	COUNT	INTENSITY(LOG)	EL	WL	COUNT	INTENSITY(LOG)	EL	WL	COUNT	INTENSITY(LOG)
Y	6.45	55	**********+	TI	2.75	4	****	BI	1.14	4	****
RE	6.73	57	**********	BA	2.78	4	****	PB	1.17	5	****
SR	6.86	43	**********	CS	2.89	6	****	TL	1.21	4	****
W	6.98	29	*********	SC	3.03	4	***+	HG	1.24	5	****
SI	7.13	37	*********+	I	3.15	4	***+	AU	1.28	5	****
TA	7.25	35	*********	TE	3.29	3	***	PT	1.31	4	****
RB	7.32	34	*********	CA	3.36	142	************	IR	1.35	4	****
HF	7.54	34	*********	SB	3.44	3	***+	OS	1.39	3	***
LU	7.84	17	********	SN	3.60	3	***+	ZN	1.44	2	**
YB	8.15	23	********	K	3.74	6	****+	CU	1.54	5	****+
AL	8.34	15	******	IN	3.77	2	**	NI	1.66	4	****+
BR	8.37	17	*******+	U	3.91	2	**	TM	1.73	4	*+++
ER	8.82	13	******	CD	3.96	3	***	CO	1.79	3	***+
SE	8.99	13	******	TH	4.14	3	***	FE	1.94	5	*+++
HO	9.20	12	******	AG	4.15	1	*	GD	2.05	4	*+++
DY	9.59	9	*****	PD	4.37	1	*	MN	2.10	1	+
AS	9.67	13	******	RH	4.60	1	*	EU	2.12	3	***+
MG	9.89	18	*******+	CL	4.73	8	**+++	SM	2.20	2	++
TB	10.00	9	*****	RU	4.85	1	*	CR	2.29	2	**
GE	10.44	7	*****	S	5.37	7	+++++	ND	2.37	2	**
GA	11.29	4	****	MO	5.41	2	**	PR	2.46	1	*
NA	11.91	58	**********+	NB	5.72	1	+	V	2.50	2	*+
**	15.80	1	*	ZR	6.07	0		CE	2.56	1	*
F	18.32	1	*	P	6.16	13	++++++	LA	2.67	0	

```
RESULTS:

THE FOLLOWING ELEMENTS ARE PRESENT
  NA  P   S   CL  CA

THE FOLLOWING ELEMENTS ARE PROBABLLY PRESENT
  MG  K   FE  SM  GD
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Fig. 8. EPMA by WDS of a mineral deposit (a) and stromal tissue (b) demonstrating element composition