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Different types of microcalcifications observed in breast pathology*

Correlations with histopathological diagnosis and radiological examination of operative specimens

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Summary. Microcalcifications taken from 50 systematized mammary excisions were submitted to light microscopic and scanning electron microscope analysis. Microprobe and x-ray diffraction analyses were also performed. Two main types were observed:

– Type I microcalcifications composed of weddellite crystals. They were observed in benign breast leasions only (11 cases out of 21) or, in lobular carcinomas in situ (L.C.I.S.) of the breast (5 cases out of 6). They were not seen in 3 cases of intraductal carcinoma (I.D.C.) nor in infiltrating (I.C.) carcinomas (20 cases).

– Type II microcalcifications, non-cristalline in nature, composed of calcium, phosphate, hydroxyapatite or of phosphorus and calcium associated with other elements, were observed in benign lesions (10 cases out of 21) and in all cases of infiltrating carcinomas.

The microcalcifications observed on mammography were also found on the radiographs of systematised mammary excisions from the lesion or from its immediate vicinity, but only when using the appropriate technique. Microcalcifications are therefore an excellent marker of breast lesions but they cannot be simply divided into "benign" or "malignant" types. Nevertheless, the presence of a visible crystalline structure on the radiograph of the specimen argues in favour of a benign breast lesion or of a lobular carcinoma in situ.

Key words: Breast – Breast cancer – Microcalcifications

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Introduction

Since the beginning of the century, several authors have observed radiographic breast microcalcifications (Salomon 1913; Warren 1930). In 1949, Leborgne demonstrated that microcalcifications enable breast cancer to be detected.

In order to differentiate between benign and malignant lesions only the following criteria were used: size, number, shape, localisation and mode of grouping. These criteria, which are not very specific, were taken into account in Le Gal's classification (1976, 1984).

In this study we analyse the structure and composition of breast microcalcifications using light microscopy, scanning electron microscopy, microprobe analysis and x-ray diffraction; the aim of this work was to look for a possible correlation between the type of microcalcification and the histopathological diagnosis. An accurate radiological technique allows the different morphological types of microcalcifications to be distinguished on observation of the specimens.

Material and methods

Material. Fifty systematized mammary excision specimens, taken between 24th October 1982, and 1st July 1985 at the Gynecology Clinic (Prof. Y. Rochet, Prof. A. Bremond) and at the Clinique Mutualiste (Dr. C. Palayer), were studied.

In all the cases studied, microcalcifications were observed on the mammograms of patients consulting for various symptoms: mastodynia, palpable tumour, bleeding from the nipple. Occasionally screening had taken place at the time of a systematic examination in a "high risk" context.

In every case, the presence of microcalcifications on mammography resulted in a zonectomy, a quadrantectomy, a subcutaneous mastectomy or a radical mastectomy.

Lesions were classified according to the histopathological

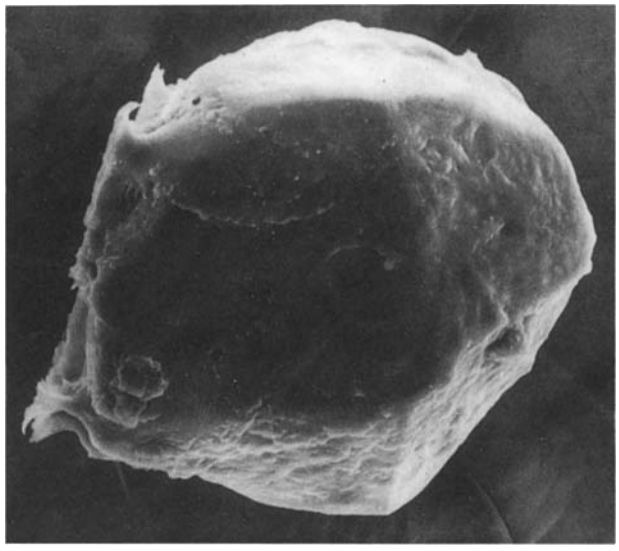


Fig. 1. Scanning electron microscope – M × 300. Type I microcalcification – L.C.I.S

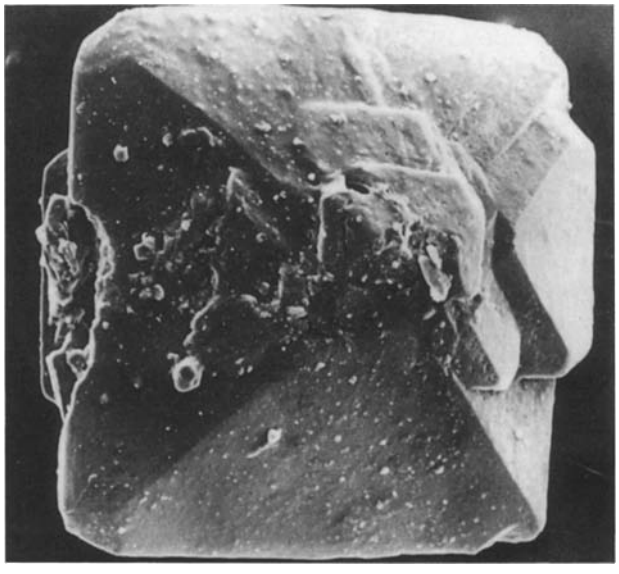


Fig. 2. Scanning electron microscope – M × 330. Type I microcalcification – sclerocystic mastopathy

classification of breast tumours, WHO (1983): 21 benign lesions, including 5 lobular carcinomas in situ (L.C.I.S.), 1 in situ lobular carcinoma associated with an intraductual carcinoma (I.D.C.), 3 intra-ductal and 20 infiltrating carcinomas were included in this study.

One to six microcalcifications were extracted from each tumour or from its immediate vicinity. Only those microcalcifications visible on mammography, i.e., with a diameter equal to or greater than 0.1 mm are included in this study.

Methods. Preopérative mammography revealed a site of microcalcification in all cases. Following surgical excision, the operative specimen was at once radiographed with a Faxitron 43804 N (Hewlett Packard), allowing low voltages to be used (10–30 KV), in order to check the excision of the microcalcification site. Slices of 0.5 cm, thickness were made. The macroscopic appearance was noted and the slices were radiographed.

Fine grain films were used (Kodak X – Omat – Ready Pack). After development, the radiographs were examined by Galkin's technique (1983) using a Wild M 410 macroscope fitted with a camera and a Macrozoom. Microcalcifications were examined and photographed at high magnification (M × 40), the shape and pattern of grouping were also noted.

Other thin slices were required to localise microcalcifications so as to proceed to their extraction.

Microcalcifications were removed quickly by means of microsurgical instruments, under strong light, using a Wild – M 410 macroscope. They were then placed in a dry tube. In this way their structure and chemical composition was preserved intact as no chemical products were used which might modify the microcalcification in any way.

The region where the microcalcification was found was sampled and semi-serial sections were studied in order to characterisation the type of lesion.

The microcalcification was carefully placed on glass slides and examined for shape, colour and transparency in the light microscope. Photographs were made with an Ektachrome 50 ASA Tungsten film. The sample was then examined under polarised light, and for each microcalcification the presence or absence of birefringence, was noted.

For scanning electronmicroscopy microcalcifications were fixed on aluminium supports (Balzers Union) with double-sided adhesive tape. They were then metallized in a Hummer II (Technics) cathodic pulverisation apparatus using gold palladium. Contants were as follows: vacuum, less than 100 millitorrs; intensity, 10 mA; duration, 210 seconds. The preparations were then examined at a voltage of 25 KV using a JEOL 35 CF. Photographs were taken.

The principle of microprobe analysis consists of irradiating the sample by a mono-kinetic beam and studying the X rays given off by the sample (Roomans and Shelburne 1983). The apparatus used in this study was a Cameca MBX (Camebax) probe equipped with WDS (Wawelength – Dispersive Spectometry) and E.D.S. (Energy Dispersive Spectrometry) systems (Tracor Northern TN 2000). The analysis was performed using E.D.S. only, with a solid detector Si (Li). Experimental conditions were: estimated probe diameter, 1 μ: electron acceleration voltage, 15 KV; current, 27 nA. The methods of sample preparation were such as to preserve intact the chemical composition as found in *in vivo* conditions. Each sample was fixed on an aluminium support with carbon glue (Balzers Union), then covered with a thin layer of carbon in an evaporator (Edwards) so as to make the sample conductive and not disturb the X signal sent off by the sample.

Each microcalcification was subjected to three series of measures at different points. The standard used was a tooth composed of hydroxyapatite: the P/Ca ratio was equal to 0.7. Formula: 3 $Ca_3(PO_4)_2Ca(OH)_2$.

Counts were made for those regions of the spectrum presenting some interest (area situated below the peak corresponding to the element being investigated). The values retained were in the following ranges: for calcium: between 3.44 and 3.90 Kev; for phosphorus: between 1.85 and 2.18 Kev; for sulphur: between 2.16 and 2.50 Kev; for silicum: between 1.62 and 1.88 Kev.

X-ray powder and for single crystal patterns were recorded with a cylindric camera (∅ 180 mm). For x-ray diffraction Cu K radiation (0.1540 nm) was produced by an Enraf Nonius generator at 30 KV and 24 mA. The microcalcifications were enclosed in sealed Lindeman tubes to prevent alteration by the atmosphere.

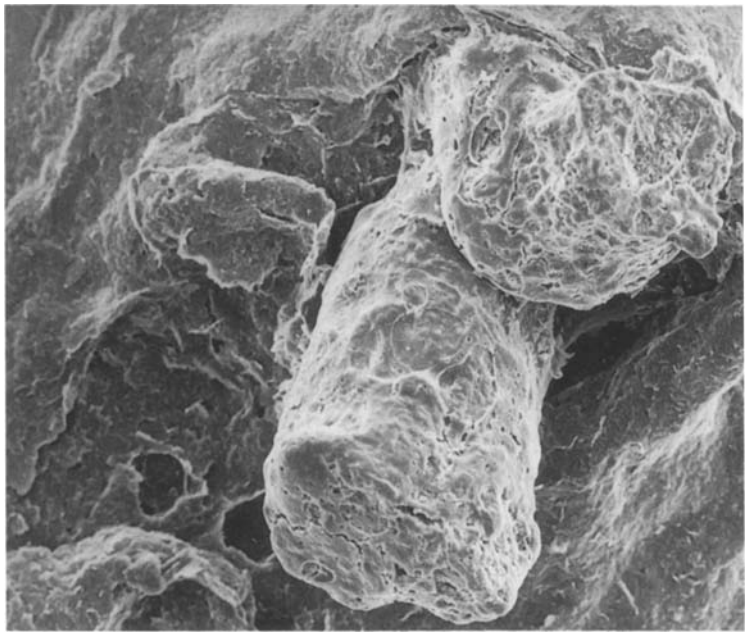


Fig. 3. Scanning electron microscope – M × 195. Type II microcalcification – I.D.C

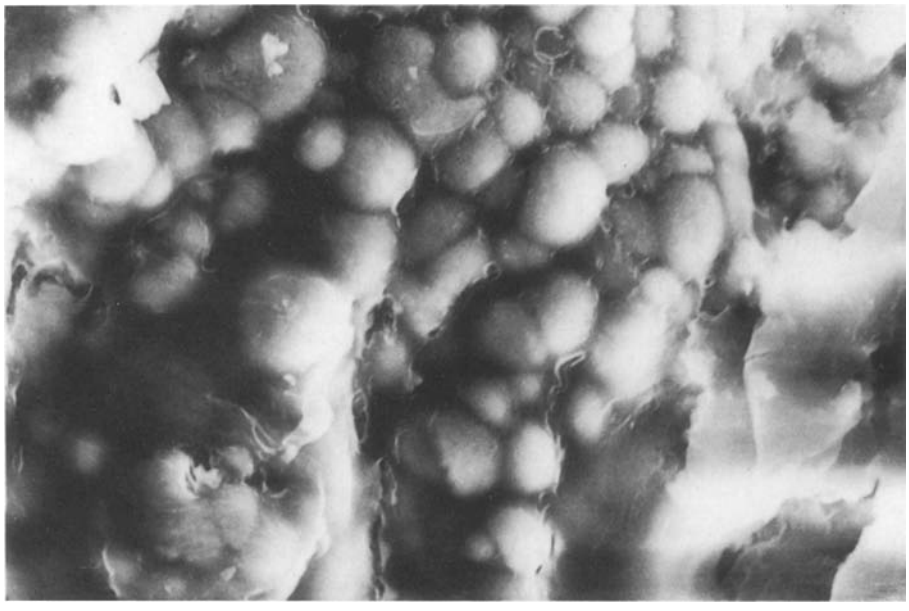


Fig. 4. Scanning electron microscope – M × 1,500. Type II microcalcification composed of oolites – Infiltrating carcinoma

Results

Two main types of microcalcification were observed: *Type I* includes microcalcifications whose crystalline structure is immediately apparent on radiographic examination of the operative specimen. On light microscopy, they are amber, light yellow or translucid, partially transparent and birefringent under polarised light. On scanning electron microscopy, their crystalline structure is also evident, in the form of pyramids which can combine together to form dipyramids or sometimes geometrical figures with a symmetry of order four; the surfaces of these pyramids is generally smooth but may show resorption images. On microprobe analysis, only the calcium peak was observed. X-ray diffraction revealed that these crystals are made up of weddellite, in this case a particular form of calcium oxalate: $CaC_2O_4\,2\,H_2O$. This was done in comparison with the standards given in the A.S.T.M. which classifies the 4 most intense rays.

Type II microcalcifications are generally ovoid without an evident crystalline structure on radiography. On light microscopy they are greyish,

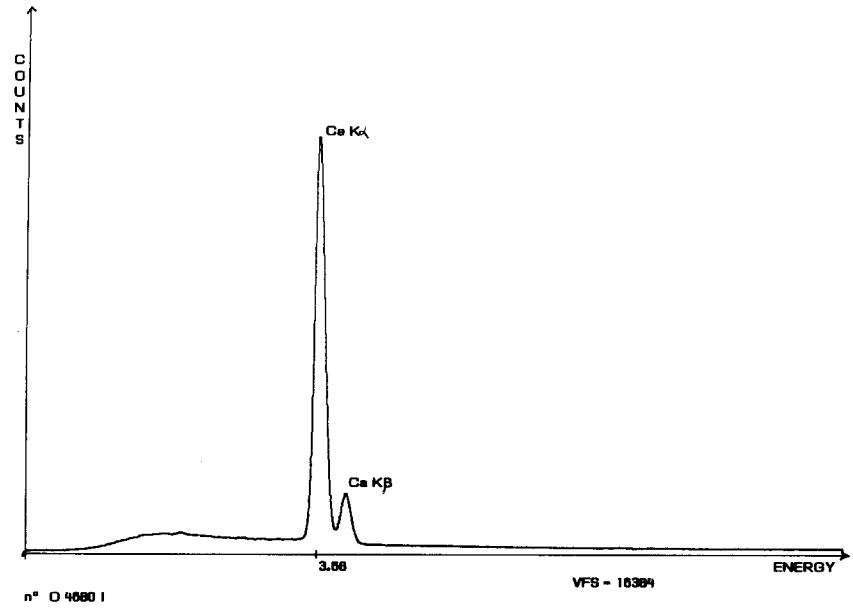


Fig. 5. Microprobe analysis: sclerocystic mastopathy. Type I microcalcification. Only one calcium peak is observed

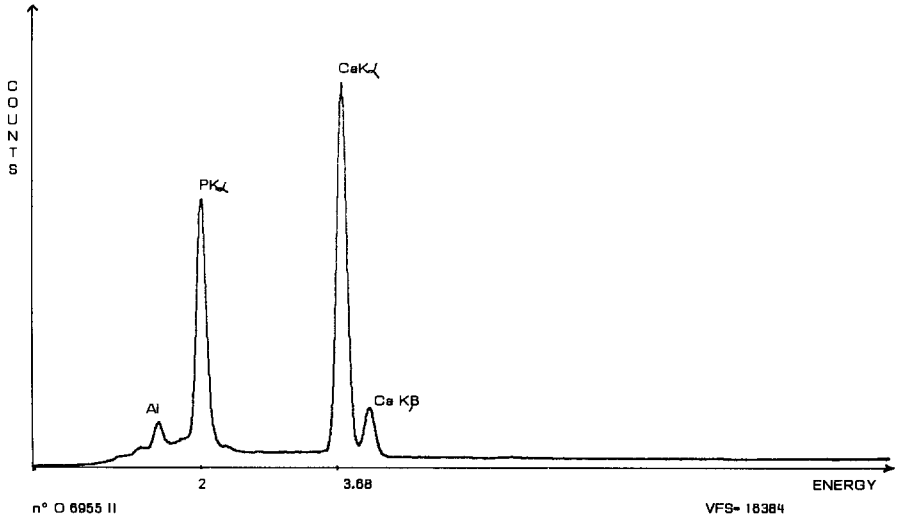


Fig. 6. Microprobe analysis: infiltrating carcinoma. Type II microcalcification. Two peaks are observed: Ca, P

opaque to luminous rays and non-birefringent under polarised light. Scanning electron microscopy disclosed the absence of any crystalline structure; their surface are irregular with small mounds; in a few instances, the surface is composed of finy spheres or oolites; exceptionnaly, fine needles may be observed. On microprobe analysis, findings were variable: either two isolated peaks were found, calcium and phosphorus, forming different types of calcium phosphate whose P/Ca ratio is 0.5, 0.7, 1.0 or 1.5; or the calcium and phosphorus are associated with other elements: sulphur, silicum, sodium, magnesium, chlorine or potassium in small amounts. As aluminium could have come from the support used this value was not retained.

In correlating the type of microcalcification and the histopathological diagnosis microcalcifications from 21 benign lesions were studied and it was found that there were 11 cases of type I, with a crystalline structure (7 cases of sclerocystic mastopathy, 2 cases of fibroadenoma, 1 intraductal papilloma and 1 periductal mastitis. 3 cases of L.C.I.S. were associated with type I microcalcifications, one case with type II microcalcifications; in one case, we observed both types of microcalcification (in association with two microcalcifications presenting a crystalline structure there was noted a round non crystalline microcalcification, with small mounds on the surface). The L.C.I.S. associated with an in situ intraductal carcinoma and the three cases of isolated I.D.C. revealed type II microcalcifications.

Table 1. The higher total number of cases is explained by the fact that within a given lesion may be found microcalcifications of different composition

Diagnosis	Type I weddellite	Type II		
		Hydroxyapatite $P/ca = 0.7$	Phosphorus calcium	Phosphorus calcium + other elements
Benign lesions 21 cases	11	2	3	6
Lobular carcinoma in situ (L.C.I.S.) 5 cases	4	0	2	0
Intraductual carcinoma (I.D.C.) 3 cases	0	0	1	2
L.C.I.S. + I.D.C. 1 case	0	1	0	0
Infiltrating carcinoma 20 cases	0	5	6	13

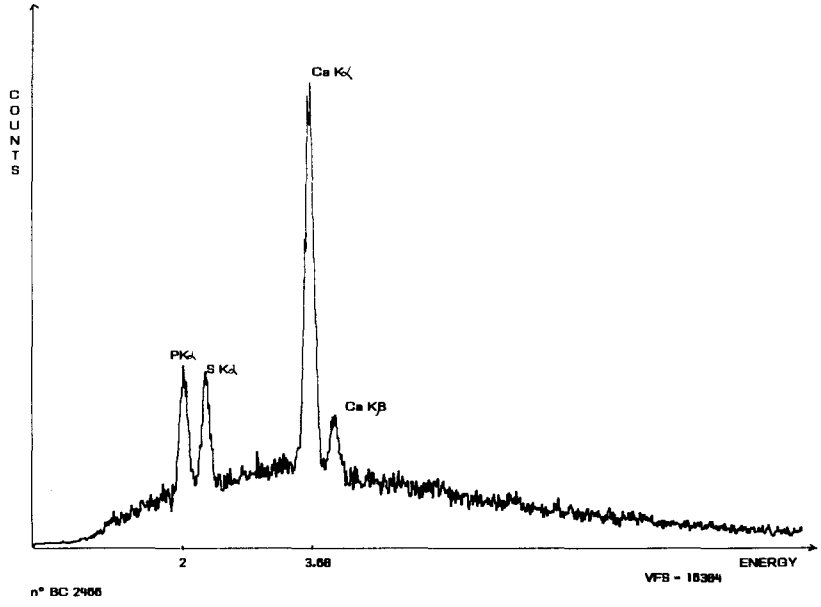


Fig. 7. Microprobe analysis – Infiltrating carcinoma. Type II microcalcification, three peaks are observed: Ca, P, S

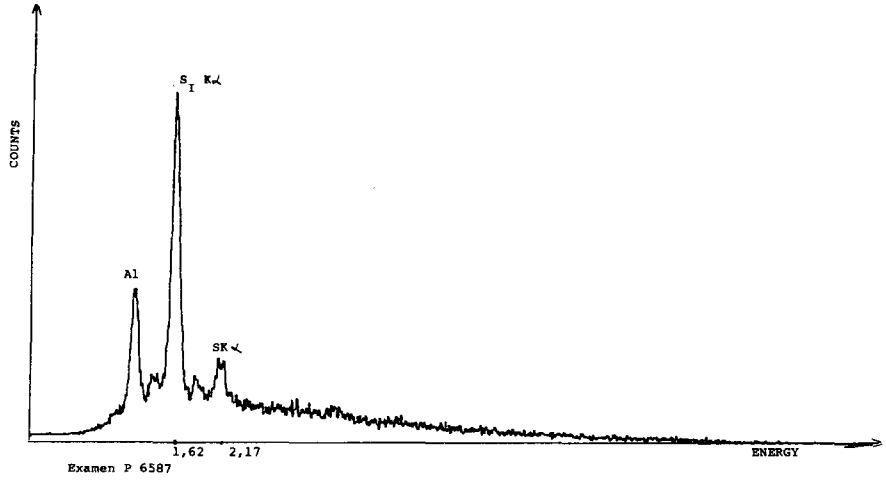
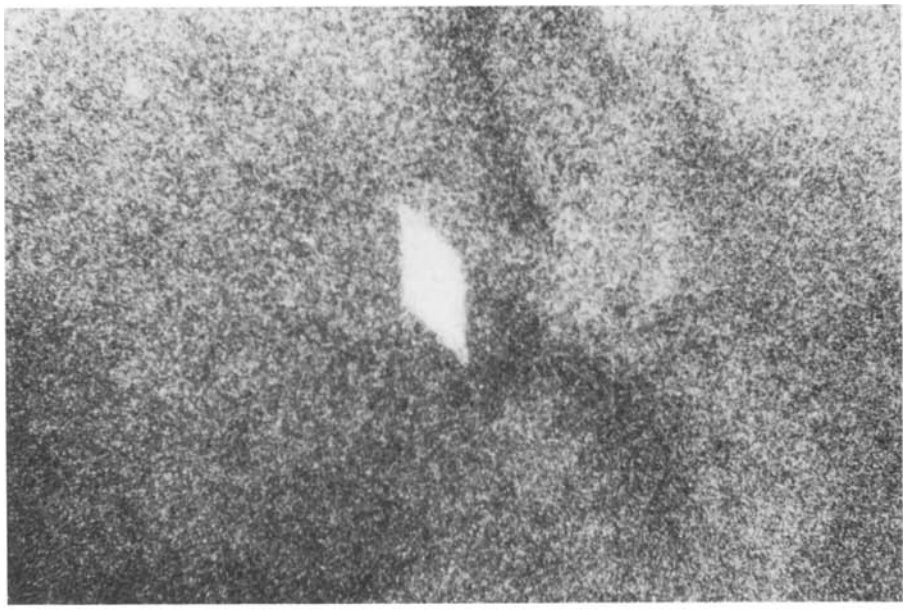


Fig. 8. Microprobe analysis – Infiltrating carcinoma. Type II microcalcification – three peaks are observed: Al, S_i, S



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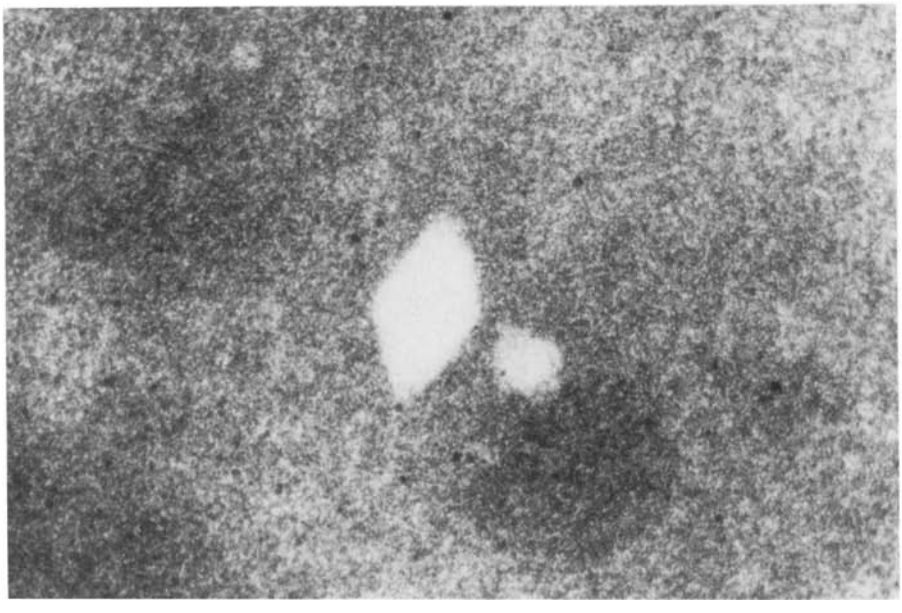

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Figs 9, 10. Sclerocystic mastopathy – magnification of the radiographic image (specimen) with an optical microscope. Type I microcalcifications: crystalline shape (**Fig. 9**: M × 25, **Fig. 10**: M × 40)

In the 20 cases of infiltrating carcinomas all the microcalcifications were of type II, non-crystalline.

On radiography of the operative specimen all type I microcalcifications presented a uniform crystalline aspect with sharp, regular contours, separated from each other or grouped together in an organised fashion either in a line or forming a bee's nest. They were sometimes observed in the lumen of microcysts. Type II microcalcifications revealed an irregular non-uniform aspect, their contours were angular and ill-defined. They were separated from each other or grouped together in a random fashion. Their average diameter was greater than that of type I microcalcifications. A rod-like form was observed in the lumen of dilated galactophores. We also noted tube-like forms (periductal mastitis).

Discussion

Although microcalcifications have been studied for many years in radiology, there are few works which deal with their structure and chemical composition and the possible correlation between these two factors and the histopathological diagnosis. Further,

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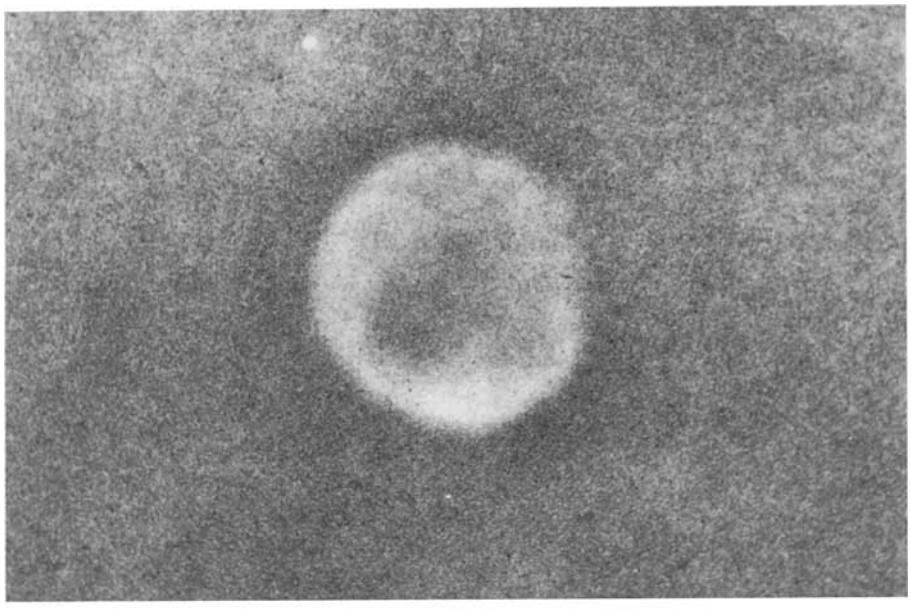

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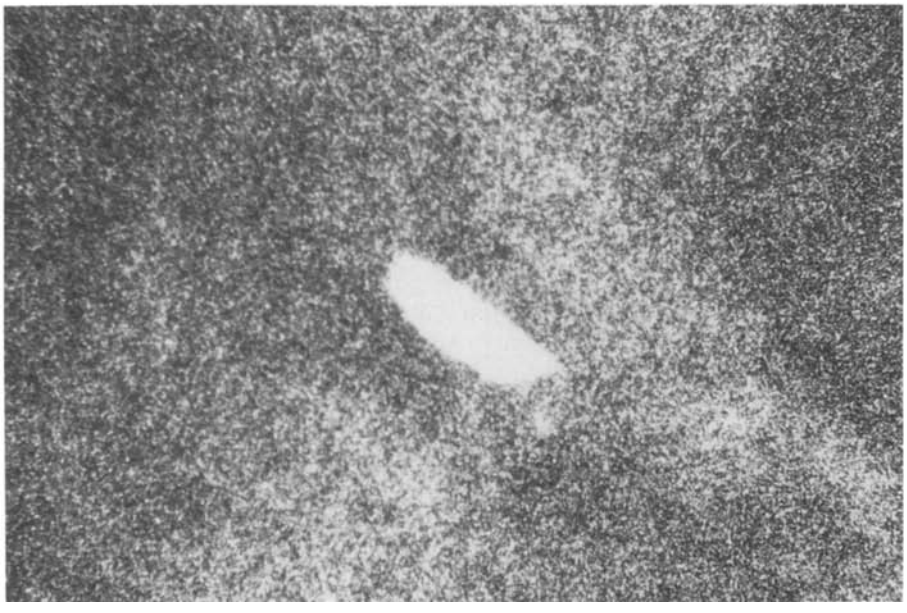

Figs. 11, 12. Infiltrating carcinoma – magnification of the radiographic image (specimen) with an optical microscope. Type II microcalcifications. (**Fig. 11**: round shape: M × 20, **Fig. 12**: linear shape: M × 40)

it is now possible to analyse their radiological structure with greater precision provided a suitable technique is used. Examination of an average quality radiographic film by means of a magnifying glass with a low power of magnification is not an up-to-date method.

Published work to date is open to criticism mainly for the small number of cases studied after going through the classic cycle of histological techniques. Only a single section of the microcalcification and not the entire-specimen was usually studied.

Hassler (1969) reported three cases of infiltrating carcinomas whose microcalcifications on X-ray diffraction were composed of 30, 50 and 70% hydroxyapatite, the remainder being made up of tricalcic phosphate. Ahmed (1975) noted, on transmission electron microscopy, that microcalcifications of carcinomas were composed of crystalline deposits, sometimes needle-shaped, associated with an amorphous material; on microprobe analysis, they were composed of calcium and phosphate in the form of hydroxyapatite. Barth (1977), in a case of benign mastopathy, observed on light microscopy, a crystalline-like microcalcification, transparent, well-delimited, dipyramidal and, on micro-

Table 2. Microcalcifications of the breast. Literature. I.C.: infiltrating carcinoma – L.C.I.S.: lobular carcinoma in situ – I.D.C.: intraductal carcinoma

	Type I Microcalcification	Type II Mcirocalcification
Hassler (1969)		3: I.C.
Ahmed (1975)		1: I.C.
Barth et al. (1977)	1: benign lesion	
Busing et al. (1981)	3: benign lesions 1: L.C.I.S.	6: I.C.
Galkin et al. (1982)		4: I.C. 2: Normal breast tissue
Frappart et al. (1984) (1985) present study	11: benign lesions 3: L.C.I.S. one case of L.C.I.S. with type I and type II	10: Benign lesions 1: L.C.I.S. 1: L.C.I.S. + I.D.C. 3: I.D.C. 20: I.C.

probe analysis, composed of calcium oxalate in the form of weddellite. In the work of Büsing et al. (1981), microcalcifications in three cases of sclerocystic mastopathy and one case of in situ lobular carcinoma turned out to be, homogeneous bodies on light microscopy birefingent under polarised light. On microprobe analysis they were composed of calcium oxalate. Microcalcifications from the six infiltrating carcinomas studied corresponded, on light microscopy, to irregular clusters, non-birefringent under polarised light. In five of six cases, they were composed of hydroxyapatite, on microprobe analysis, and in one case of calcium phosphate. Galkin (1980) studied 40 samples of 4 infiltrating carcinomas of the breast and two supposedly normal cadaver breasts. He distinguished several categories of microcalcifications found both in infiltrating carcinomas and in tissue considered to be normal taken from the two cadavers. These included microcalcifications composed of calcium and phosphorus in the form of hydroxyapatite or of tricalcic phosphate, microcalcifications composed of calcium but with very little phosphorus, more complex microcalcifications composed of calcium and numerous other elements varying in amount from one microcalcification to another, including aluminium, potassium, sulphur, silicium and titanium. Finally, particles were found containing no calcium but other elements associated together in variable quantities: aluminium, magnesium, silicium, copper, chrome, titanium, nickel, lead, gold, chlorine, lodine and molybdenum.

Four microcalcifications of the benign lesion type have been analysed in the literature: one was composed of weddellite, the others of calcium oxalate. In our work, in 11 out of 21 cases of benign lesions we observed type I microcalcifications composed of weddellite, and the 10 others were type II composed of calcium phosphate associated with sulphur, silicium and chlorine in 1 case, and with sulphur, silicium and magnesium in 1 case. Only one case of L.C.I.S. has been reported in the literature, the microcalcifications were composed of weddellite as in two of our observations. In a third observation we found type II microcalcifications (calcium phosphate); finally, in our fourth observation, the two types were associated.

In three I.D.C. of which one was associated with an L.C.I.S., all the microcalcifications were composed of calcium phosphate.

Microcalcifications extracted from 35 I.C. (15 in the literature and 20 personal cases) were composed of hydroxyapatite, calcium phosphate, calcium associated with various elements (Al, Ti, K, S, Si). Some particles devoid of calcium have been observed by Galkin et al. (1982) and by us in one case. No crystalline microcalcification was noted in infiltrating carcinomas.

Thus, type I microcalcifications can be observed in benign lesions of the breast or in L.C.I.S. which, as Haagensen et al. (1978) has shown, has a very long evolution. Type I is never observed in infiltrating carcinomas. Type II microcalcifications may be seen in the different types of lesion. As we have shown here, radiological study of the specimen reveals the morphological type of the microcalcification, provided that a high performance technique is used. Galkin et al. (1983), working on benign lesions, isolated a particular type of crystalline microcalcification on radiography; a partial

extension of these results to mammography seems a distinct possibility. Further, Galkin et al. (1983) appears to have detected a crystal contained inside the first structure after a fracture of a non-crystalline microcalcification.

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