Analysis of Calcific Deposits in Calcifying Tendinitis

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The precise composition of calcific deposits in calcifying tendinitis is still unknown. However, analysis of such deposits can help to elucidate the disease's pathogenesis. Twenty-five calcific deposits from various phases of the disease were analyzed by several methods. The macroscopic appearance of the specimens during the acute phase of calcifying tendinitis resembled a milky emulsion; in contrast, it resembled a granular conglomerate during the chronic phase. X-ray diffraction showed a poorly crystallized hydroxyapatite lattice (resembling that in bone) in both phases. Infrared spectroscopy revealed variable H_2O, CO_3, and PO_4 contents in all samples, but no significant differences in these proportions were seen in the two phases of the disease. Organic molecules were seen in addition in all samples. Scanning electron microscopy showed similar morphologies of the crystalline conglomerates of both phases, with somewhat round, nongeometric structures. The macroscopic difference was not reflected in the mineralogic structure. Neither a chemical compositional change nor a change in the crystal lattice was observed. The disintegration of the conglomerates probably depends on a change in the bonding capacity of the organic molecules, which in turn initiates phagocytosis in the resorptive phase.

The different phases of calcifying tendinitis of the shoulder have been histologically documented by various authors.[1,3,5–10] In the chronic phases of the disease, metaplasia of tenocytes to chondrocytes has been observed. Within the intercellular substance, calcific deposits are formed, which in turn are resorbed during the acute phase. So far, histologic investigations have failed to reveal a factor inducing the resorptive activity. However, the elucidation of such a factor would be of great therapeutic importance. The aims of the present study were to investigate the crystal chemistry of such deposits and to find the answer to two questions of major importance: (1) What is the composition of this calcific material? (2) Are there any structural or chemical differences among the calcific deposits found in the various phases of the disease?

MATERIALS AND METHODS

Specimens were taken from 25 calcific deposits of patients suffering from calcifying tendinitis of the rotator cuff tendon. Nine patients were in the chronic stage (formative phase) of the disease and had had pain for many months. The roentgenographic appearance of the deposits was well circumscribed, homogenous, and dense. The other 16 patients were in the acute stage (resorptive phase). They had experienced increased pain for several days, and the deposits had a fluffy, cloudy appearance. The calcific materials were obtained by either aspiration under local anesthesia (using image-intensifier control) or during surgery. All samples were washed several times with distilled water and were dried in an oven at 50°. The specimens were then inspected in the visible ranges and

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various ranges of the invisible magnetoelectrical wave spectrum. Samples of synthetic hydroxyapatite (HA) were subjected to the same analyses.

X-Ray Powder Diffraction

In addition to routine inspection of the deposits by light microscopy, x-ray powder diffraction was performed. This technique reveals information about the structure and, indirectly, about the chemical composition of the crystals. The incident x-ray beam is diffracted by the crystal lattice, in a process similar to the diffraction of light by a prism.

The diffraction angles obtained are plotted against the relative intensity of the x-rays. Each structurally and chemically different crystal displays its own specific pattern that can be used for identification (a technique similar to fingerprinting).[4]

Infrared Spectroscopy

Further information on the molecular structure of the crystals was obtained by infrared spectroscopy.

In this method, the sample is irradiated and absorption (the reciprocal of transmittance) is caused by the energetically induced vibrations of molecules. Molecular groups within the crystal or at its surface are monitored as a function of wavelength of the infrared light. Particular chemical groups of the crystal can thus be allocated to certain wavelengths of the infrared spectrum.

Scanning Electron Microscopy

Investigations of the morphology of the specimens were also performed by scanning electron microscopy (SEM). The specimens were affixed to a sample holder with a double-sided table and were sputtered with gold. Magnifications ranged up to 5000 times.

RESULTS

Macroscopic and Microscopic Aspects

The macroscopic appearance of the calcific deposits is well known. In the acute phase of calcifying tendinitis, their appearance resembles a milky emulsion, which becomes a fine powder after careful dehydration (Fig. 1).

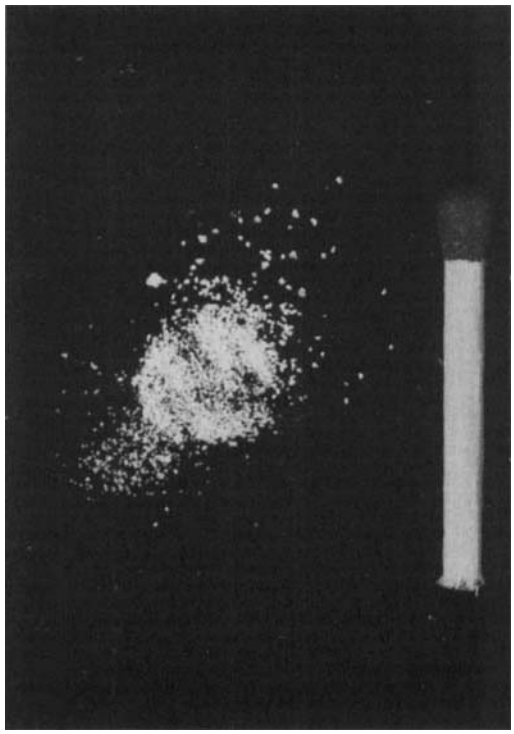


FIG. 1. Dehydrated calcific material, obtained through aspiration during the acute phase of calcifying tendinitis, compared with a matchstick of 3.5-cm length.

In the chronic phase of the disease, either small particles like granules or a more defined area (slightly spherical in shape) is found in the tendon (Fig. 2). The morphology of these granules does not change after careful dehydration.

The light-microscopic image yields no further information regarding analysis of the calcific deposits.

X-Ray Powder Diffraction

In all samples, HA ($Ca_{10}[PO_4]_6(OH)_2$) is found as the only inorganic constituent of the calcific deposits.

The diffraction pattern further demonstrates poorly crystallized HA (small crystalline particles), as indicated by the relative

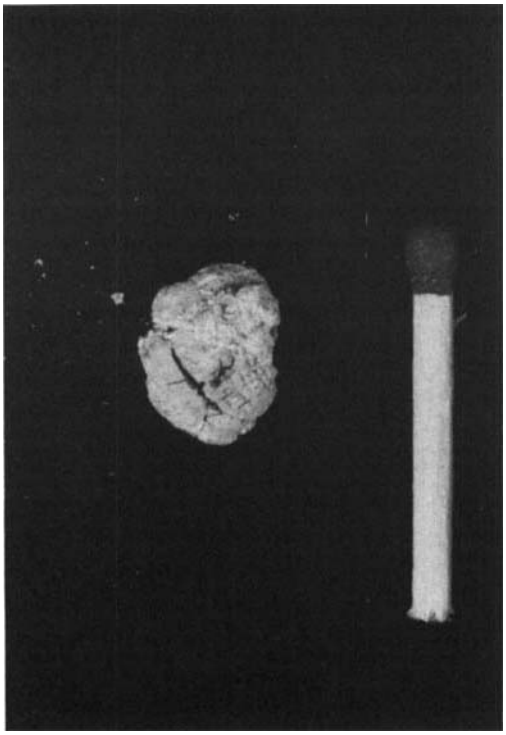


FIG. 2. An operative specimen (a dehydrated conglomerate) obtained during the chronic phase of calcifying tendinitis, compared with a matchstick of 3.5-cm length.

broadness of peaks in comparison to those of synthetic HA (Figs. 3A and 3B). The patterns of the calcific deposits are similar to those of bone. The HA crystalline structure, which is imperfect because of lattice defects, is also similar to that in bone.

The x-ray diffraction measurements do not reveal any difference between the acute and chronic stages of calcifying tendinitis.

INFRARED SPECTROSCOPY

Using the infrared technique, all samples from the acute and chronic phases revealed HA that differed somewhat from a pure synthetic HA (Fig. 4).

The content of adsorbed H_2O is significantly higher in natural HA than in synthetic HA. In addition, CH vibrations resulting from organic molecules observed in natural HA are absent in synthetic HA. The natural HA occurring in both phases of calcifying tendinitis shows considerable $(CO_3)^2$, which replaces up to 10% of the $(PO_4)^3$ content. On the other hand, $(CO_3)^2$ does not occur in the crystal lattice of pure synthetic HA. The proportions of H_2O, CO_3, and PO_4 show considerable variations in all samples. No correlation between the chronic and acute stages of the disease, however, could be found (Figs. 5A and 5B).

SCANNING ELECTRON MICROSCOPY

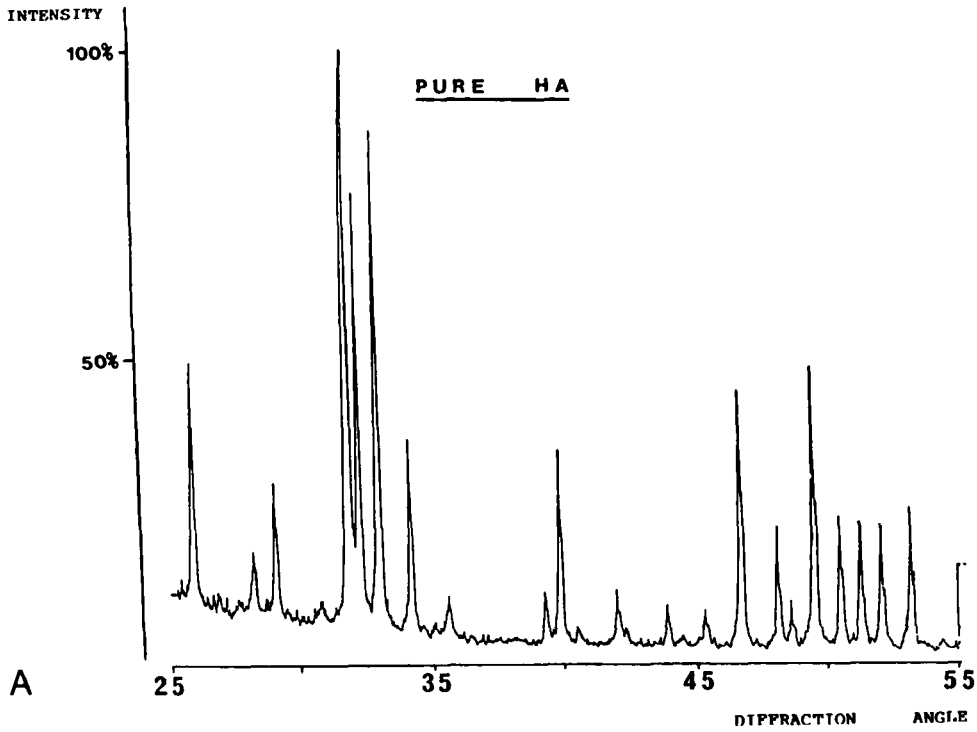
To elucidate the surface structure and morphology of the calcific deposits, the samples were investigated by SEM. No significant differences in the surface structure of samples from the acute and chronic phases of calcifying tendinitis were observed. A relatively greater abundance of somewhat round structures, partly with smoother surfaces, was seen in the acute phase (Figs. 6A, 6B, and 7). In some cases, additional structures with a spherical appearance (Fig. 8) were found in both phases.

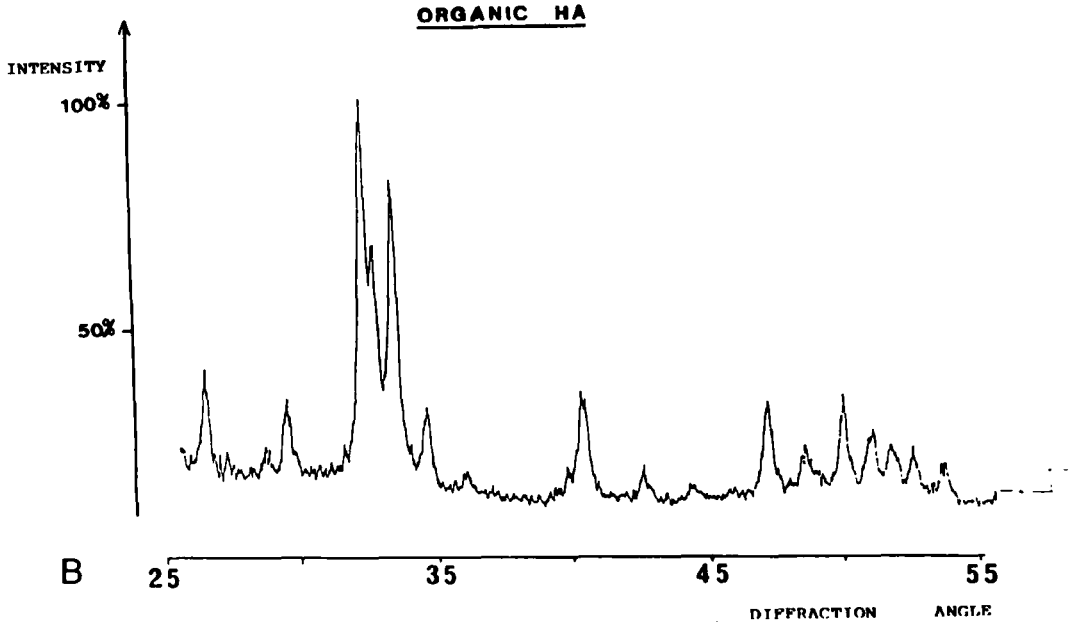
Calcific deposits analyzed from the two stages of calcifying tendinitis were composed of crystallites more or less equal in size and comparable to those of HA in bone.

One unusual observation was attributed to a preparational artifact. In scanning images of samples from the acute phase, rodlike and needlelike crystals were observed (Fig. 9). However, these were crystals of the local anesthetic used, mepivacain hydrochloride, which crystallizes during drying of the milky emulsion. Consequently, the samples were washed carefully with distilled water to remove the anesthetic.

DISCUSSION

The macroscopic and microscopic appearances of calcific deposits of calcifying ten-





FIGS. 3A AND 3B. X-ray diffractograms of (A) synthetic (pure) HA and (B) natural (organic) HA from a calcific deposit. There is no difference between x-ray diffraction results from the acute and chronic stages of calcifying tendinitis.

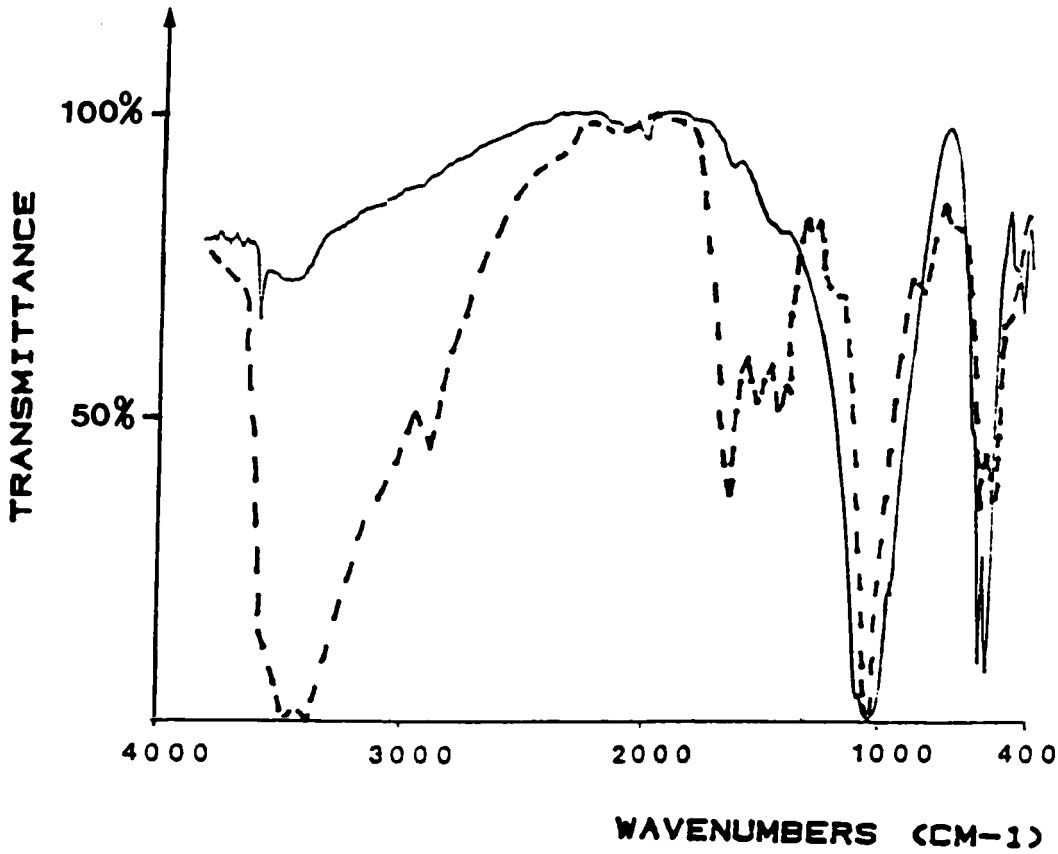
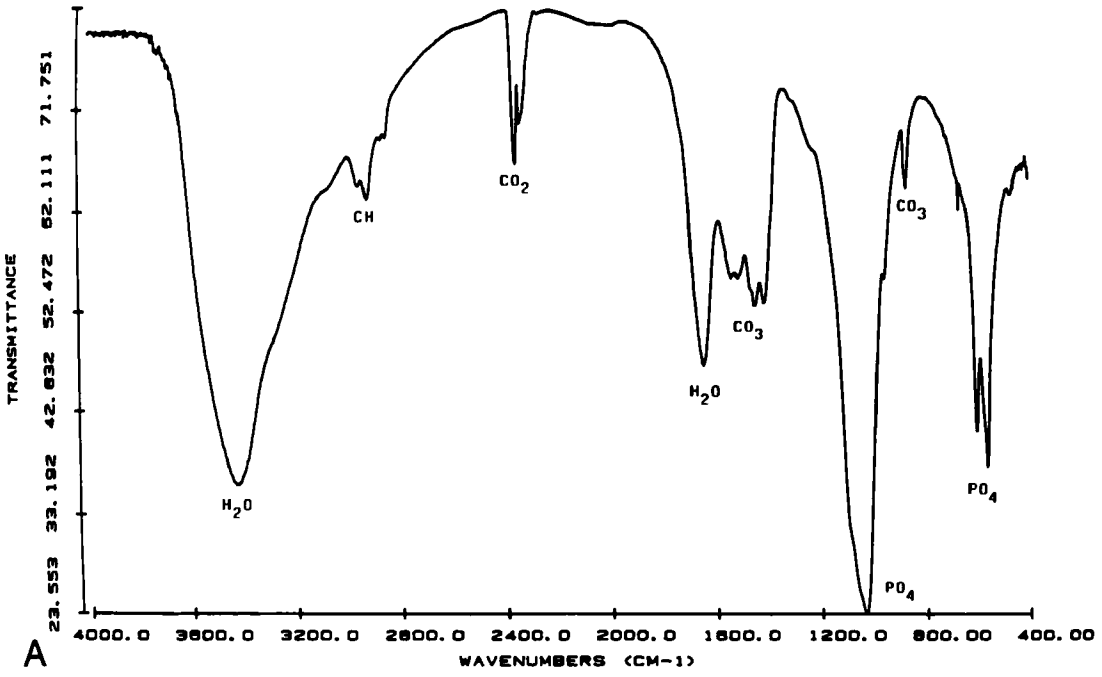


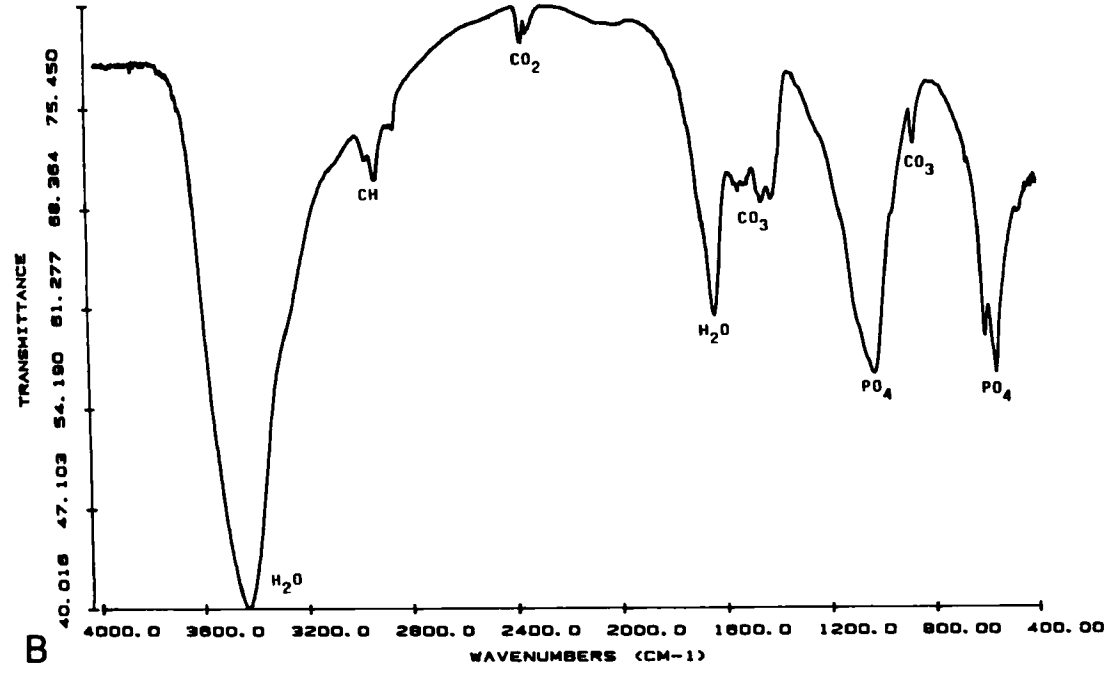
FIG. 4. A graph of infrared spectroscopy results, with a solid line representing synthetic HA and an interrupted line representing natural HA. The x axis shows the wave number (the reciprocal of wavelength). The percentage of the infrared transmittance (the reciprocal of absorption) is shown on the y axis. Differences in spectra of natural and synthetic HA in the wave range are due to the higher $(CO_3)^2$ and H_2O contents of the organic material. Wave ranges for natural and synthetic HA, respectively, were: H_2O, around 1640 and 3400 cm^{-1}; $(CO_3)^2$, around 875 and 1450 cm^{-1}; and $(PO_4)^{3-}$, around 1045 cm^{-1}.

dinitis are well known.[1-3,5-10] Whereas granular conglomerates are found in the chronic phase, a milky emulsion is observed in the acute phase.

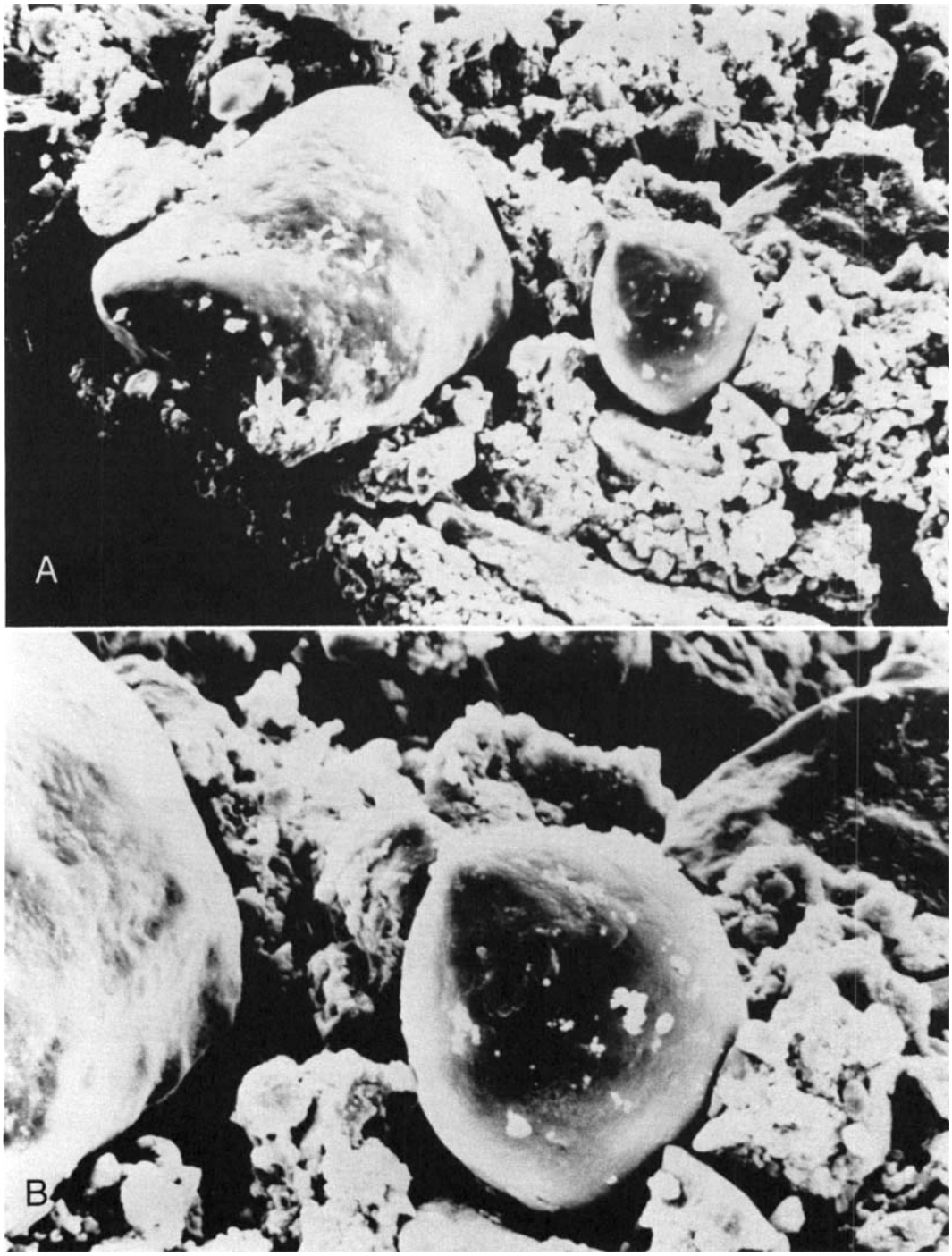
Histologic investigations demonstrated a special cycle of the disease process. Phagocytosis of the crystals appears in the acute phase. However, the cause of stimulation of this resorptive activity is not known. Accordingly, the aim of the present mineralogic study was to determine whether or not differences in crystal structure and composition exist among the various stages of the disease and whether they could be responsible for the resorption occurring in the acute phase.

Twenty-five calcific deposits of the rotator cuff tendon were submitted to light microscopy, x-ray powder diffraction, infrared spectroscopy, and SEM. In the literature, only one x-ray diffraction of the calcific deposit has been reported.[7] In the present study, HA of low crystallinity was observed by x-ray diffraction and infrared examinations in all phases of calcifying tendinitis. In addition, infrared spectroscopy revealed a high CO_3 content in all samples, comparable to HA in





FIGS. 5A AND 5B. Infrared spectroscopy results from studies of calcific deposits from (A) the acute phase and (B) the chronic phase. The H_2O, CO_3, and PO_4 contents are not constant; there is no correlation among the different phases of the disease. Similar CH vibrations result from attached organic molecules in both phases. These peaks are due to contaminations from the air during preparation.



FIGS. 6A AND 6B. SEM images of calcific deposit in the acute phase reveal somewhat round structures with a smooth surface. (A) Magnification, ×640; (B) magnification, ×1250.

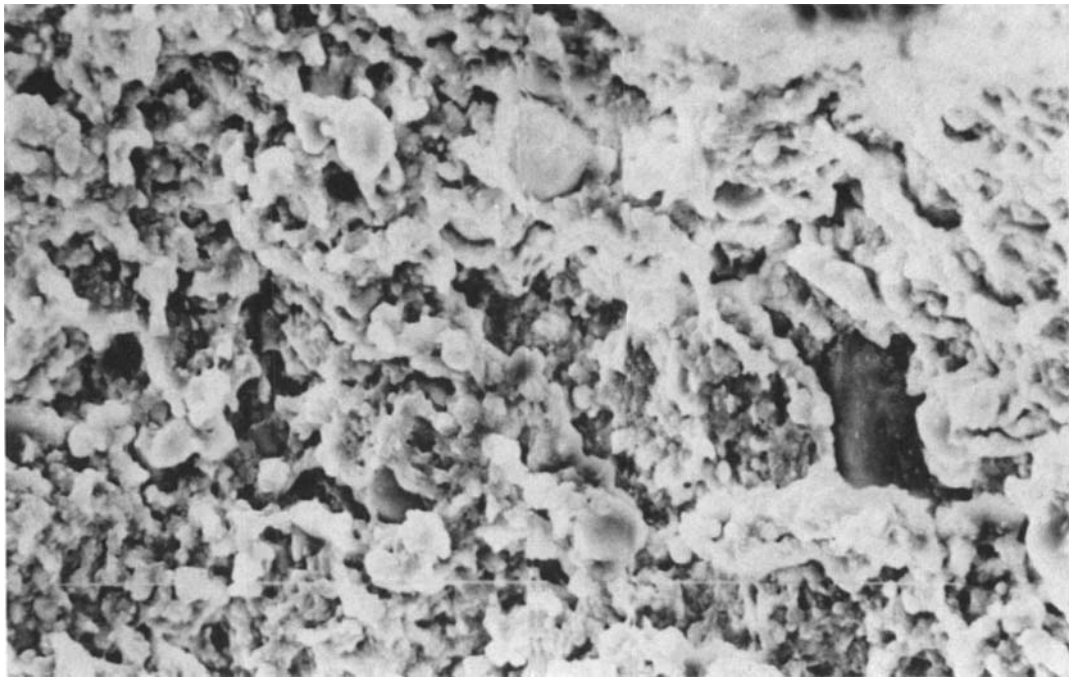


FIG. 7. An SEM image of a calcific deposit in the chronic phase reveals somewhat roundish structures and small conglomerates. (Magnification, ×1250.)

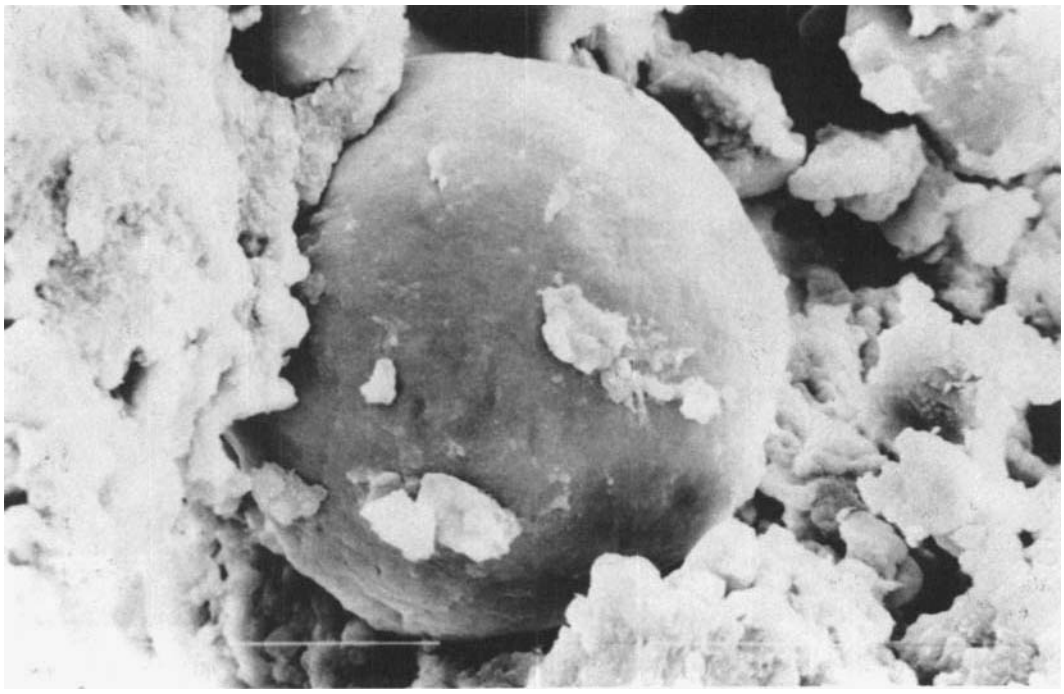


FIG. 8. An SEM image of a calcific deposit with a spherical structure, which is found in both phases of calcifying tendinitis. (Magnification, ×2500.)

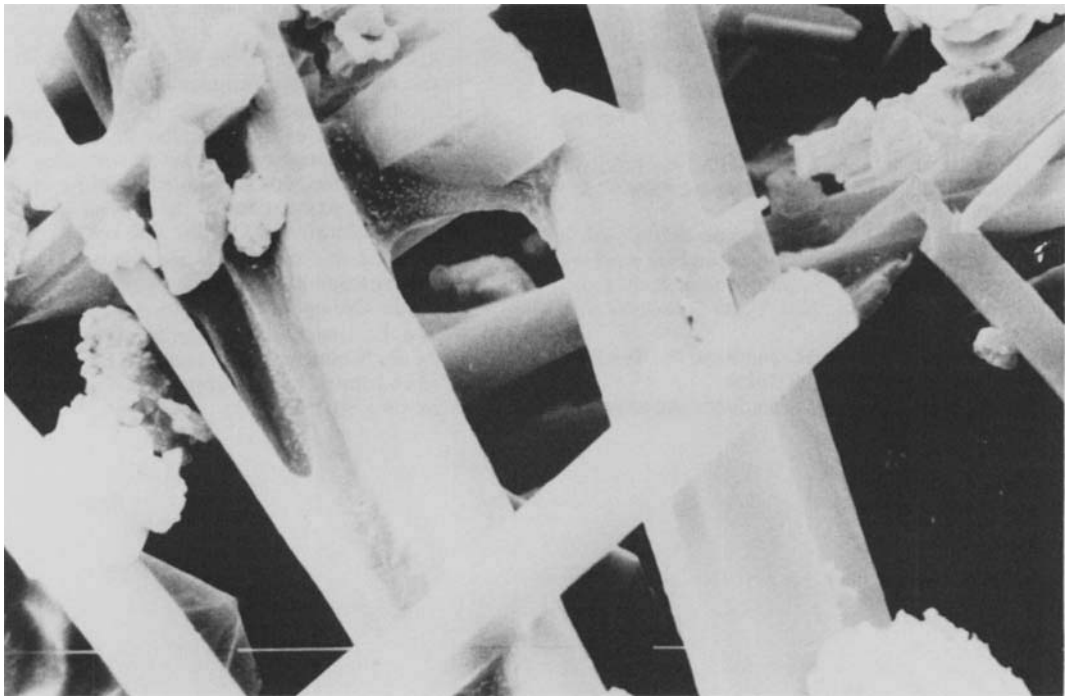


FIG. 9. An SEM image obtained with local anesthetic reveals typical rod-shaped crystals. (Magnification, ×2500.)

bone. H_2O, CO_3, and PO_4 concentrations were highly variable in all samples, but no significant differences in these proportions were seen among the various stages of calcifying tendinitis. A high content of adsorbed H_2O and organic CH was found, in contrast to the low content in synthetic HA.

In the acute phase, HA does not dissolve but has the consistency of an emulsion. No intermediate-phaselike tricalcium phosphate or other phosphates could be detected.

In the SEM study, similar morphologies of the crystalline conglomerates were observed in all phases of the disease. The crystallites as well as crystalline conglomerates were irregularly distributed, in contrast to the regular arrangement of HA in bone.

All observations of this study demonstrated that the macroscopic difference in calcific deposits is not reflected in their mineralogic structure. Neither a chemical compositional change nor a change in the crystal lattice occurs in calcifying tendinitis. Only minor differences are noted in SEM studies.

It is therefore concluded that no chemical dissolution process of the inorganic material is responsible for the resorption activity in the acute phase. The bonding of all HA deposits seems to be governed by organic constituents. This is indicated by CH vibrations seen by infrared spectroscopy of all samples.

The disintegration of the conglomerates probably depends on a change in the bonding capacity of the organic molecules, which initiates phagocytosis in the resorptive phase.

ACKNOWLEDGMENT

The authors would like to dedicate this paper to Professor W. Blauth, M.D., on the occasion of his 65th birthday.

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