

# Functional Role of Hydroxyapatite Crystals in Mönckeberg's Arteriosclerosis

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## Abstract

The etiology of Mönckeberg's arteriosclerosis and its relationship to atherosclerosis are controversial. In our view a great deal of Mönckeberg's arteriosclerosis may be the crystal-induced angiopathy caused by a genetically determinate complex metabolic disorder. The aim of this study was to determine the prevalence of crystal deposits in the wall of arteries of patients with histologically diagnosed Mönckeberg's sclerosis.

Medium size arteries with the histological diagnosis of Mönckeberg's sclerosis in 35 amputated lower legs of 28 patients were studied. Under polarized light, large amounts of hydroxyapatite (HA) crystals were identified in 45.71% of tissue samples. In a few cases HA crystals were accompanied sporadically by some calcium pyrophosphate dihydrate (CPPD) and /or cholesterol crystals.

According to our observations Mönckeberg's sclerosis should be divided in two groups based on the presence of HA. In one group large amounts of HA crystals may be detected in unstained sections viewed under polarized light. These cases should be regarded as a crystal induced angiopathy and a manifestation of a metabolic disorder. In the second group of patients HA crystals are not detectable; they have a simple manifestation of general atherosclerosis.

**Keywords** — Mönckeberg's arteriosclerosis, (hydroxyapatite) crystal-induced angiopathy, method of "not-staining", polarizing microscope

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## Introduction

The German pathologist Johann Georg Mönckeberg (1877-1925) described a special form of arteriosclerosis – characterized by calcification and ossification of the media of medium size arteries mainly of the lower extremities – in

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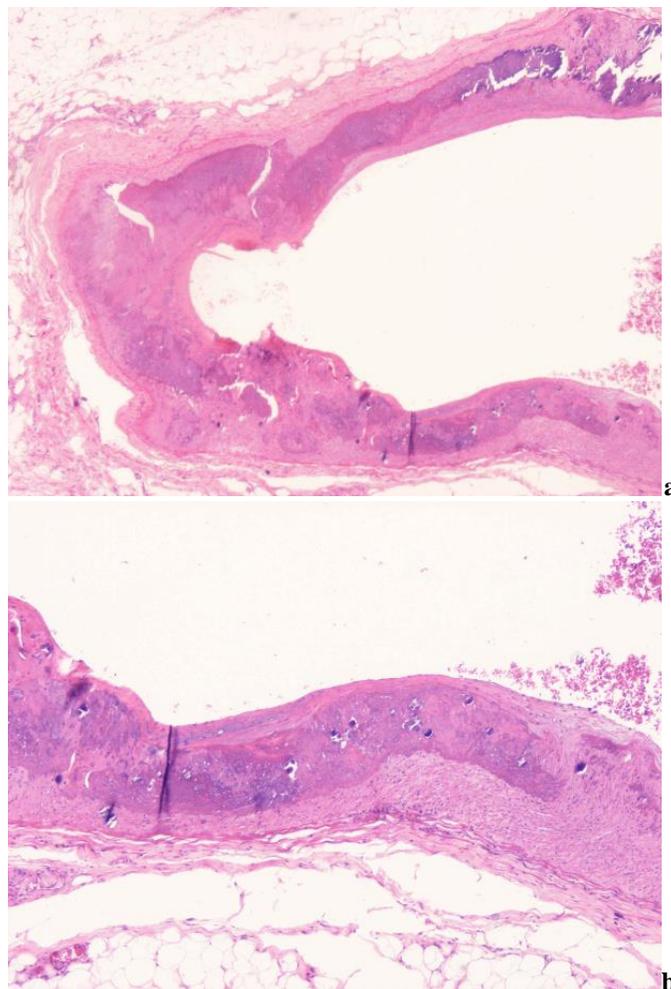
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1903.<sup>1</sup> The etiology of Mönckeberg's arteriosclerosis and its relationship to atherosclerosis are not exactly known and are controversial. This type of atherosclerosis is probable consequence of an underlying metabolic disorder.<sup>2</sup> According to McCullough and co-workers Mönckeberg's sclerosis is "a manifestation of accelerated atherosclerosis in patients with chronic kidney disease".<sup>3</sup>

According to our observations some cases of Mönckeberg's sclerosis may be a crystal-induced angiopathy caused by a genetically determinate complex metabolic disorder<sup>4</sup>; and a part of them is a simple manifestation of systemic atherosclerosis (with or without chronic kidney disease).



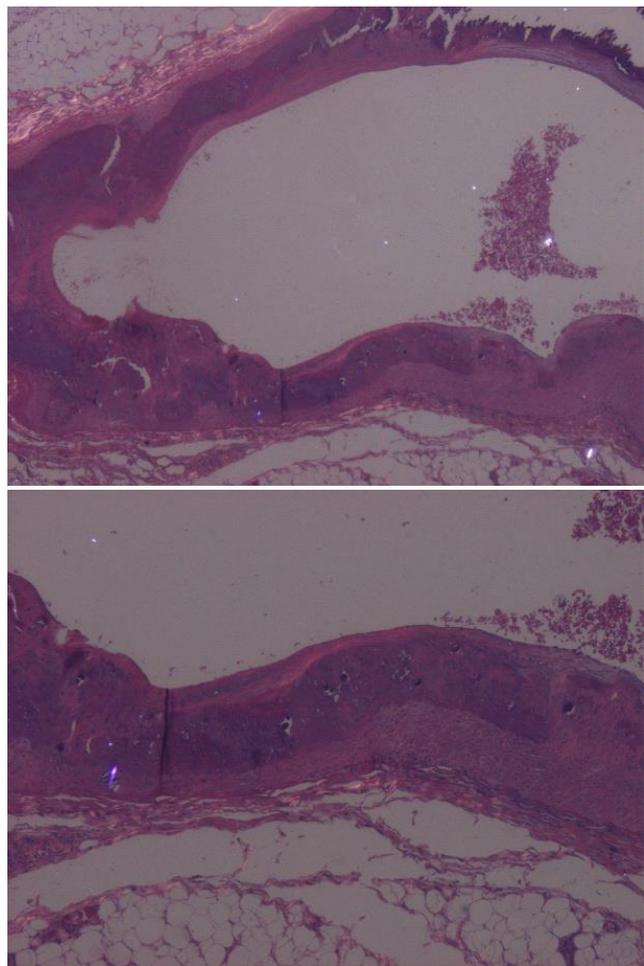
**Fig. 1.** Mönckeberg's sclerosis (hydroxyapatite crystal induced angiopathy), femoral artery, H-E, viewed under light microscope

(a) x20, (b) same as Figure 1a x40.

The aim of this study was to determine the prevalence of crystal deposits in the wall of arteries of patients with histologically diagnosed Mönckeberg's sclerosis.

Demonstration of crystal deposits in haematoxylin-eosin stained sections is unsuccessful in most cases because the vast majority of the crystals are dissolving in conventional fixatives (aqueous formaldehyde solution), in acetone, or in solutions of dyes.<sup>5-6</sup>

The probability of identifying crystals is much higher in unstained sections viewed under polarized light. This simple and sensitive method led to recognition of crystal deposits in numerous metabolic disorders or crystal-induced arthropathies.<sup>4</sup>



**Fig. 2.** Mönckeberg's sclerosis (hydroxyapatite crystal induced angiopathy), femoral artery, H-E, viewed under polarized light (a) Same as Fig. 1a, x20, (b) same as Fig. 1b, x40

## Material and methods

Medium size arteries (*A. femoralis*, *A. poplitea* and/or *A. tibialis anterior or posterior*) with Mönckeberg's sclerosis in 35 amputated lower legs of 28 patients (females 15, average age: 79.13 years, range 86 – 62; males 13, average age: 71.17 years, range 85 – 54) were studied.

The formaldehyde fixed and paraffin embedded tissue samples were studied in serial sections stained with haematoxylin-eosin (H-E) according to Mayer<sup>7</sup> and in unstained sections<sup>4</sup> viewed under polarized light with an Olympus BX51 polarization microscope.

The amorphous calcium phosphate or carbonate deposits in the wall of arteries were demonstrated by Alizarin Red staining (specific for calcium)<sup>8</sup> or the von Kossa reaction (specific for phosphate and carbonate).<sup>9</sup>

The association between Alizarin Red and von Kossa reaction positivity, furthermore the link between the amorphous calcium phosphate or carbonate deposits and the presence of crystals was calculated by  $\chi^2$  test.

## Results

Diagnosis of Mönckeberg's sclerosis with characteristic changes of medium size arteries was confirmed histologically in all 35 tissue samples of 28 patients (**Fig. 1a-b**). All of these were considered "crystal negative" with haematoxylin-eosin stain viewed under polarized light (**Fig. 2a-b**).

In formaldehyde fixed and paraffin embedded unstained sections viewed under polarized light large amounts of hydroxyapatite [ $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ] (HA) crystals were identified in 16 (45.71%) (**Figs. 3a-d and 4a-b**), but not detected in 19 (54.29% of 35) tissue samples.

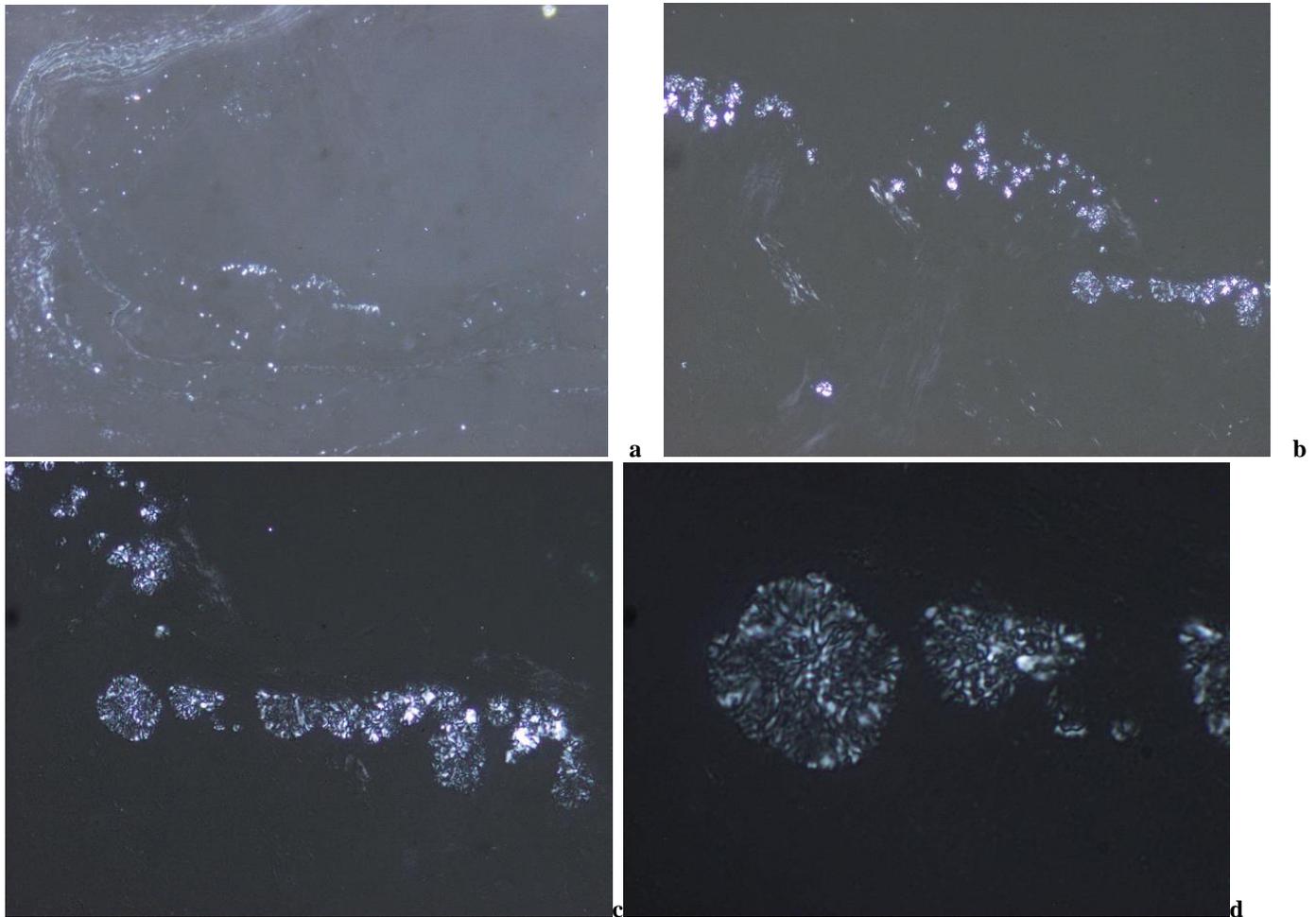
In a few cases (in 5 of 35 tissue samples) HA deposits were accompanied scantily (sporadically) with some calcium pyrophosphate dihydrate [ $\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$ ] (CPPD) crystals.

In unstained sections viewed under polarized light cholesterol crystals – with or without HA (or CPPD) – were detected in 10 tissue samples of 28 patients.

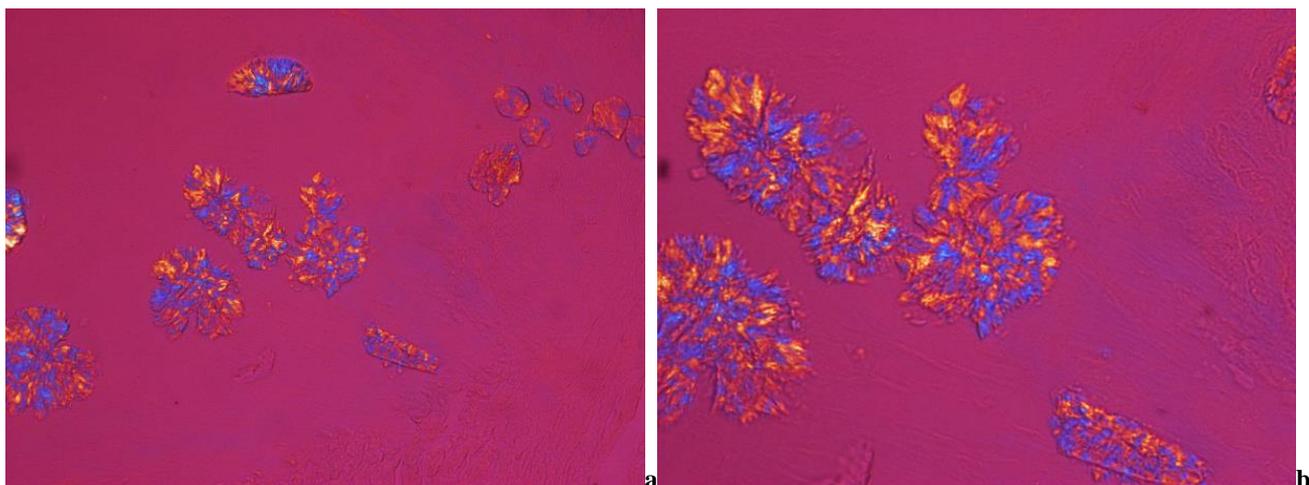
In haematoxylin-eosin stained sections – viewed under polarized light – HA, CPPD or cholesterol crystals were never detected in tissue samples of 28 patients.

Amorphous calcium phosphate or carbonate deposits were demonstrable in the wall of arteries in 23 (65.71%) of 35 formalin fixed tissue specimens stained with Alizarin Red (**Fig. 5a-b and 6a-b**) and were present in 17 (48.57%) of 35 tissue specimens stained by the von Kossa reaction.

There was a strong significant correlation (association's coefficient: 0.923,  $\chi^2=9.5118$ ,  $p<0.002$ ) between calcium and phosphate or carbonate contents of amorphous deposits.



**Fig. 3.** Mönckeberg's sclerosis (hydroxyapatite crystal induced angiopathy), femoral artery, unstained section, viewed under polarized light (a) Same as Fig. 1a, x20, (b) same field as 3a, x100, (c) same field as 3b, x200 (d) same field as 3c, x600. The small 50-500 nm, rod-shaped HA crystals are arranged typically in 1-5  $\mu$ m spheroid microaggregates.

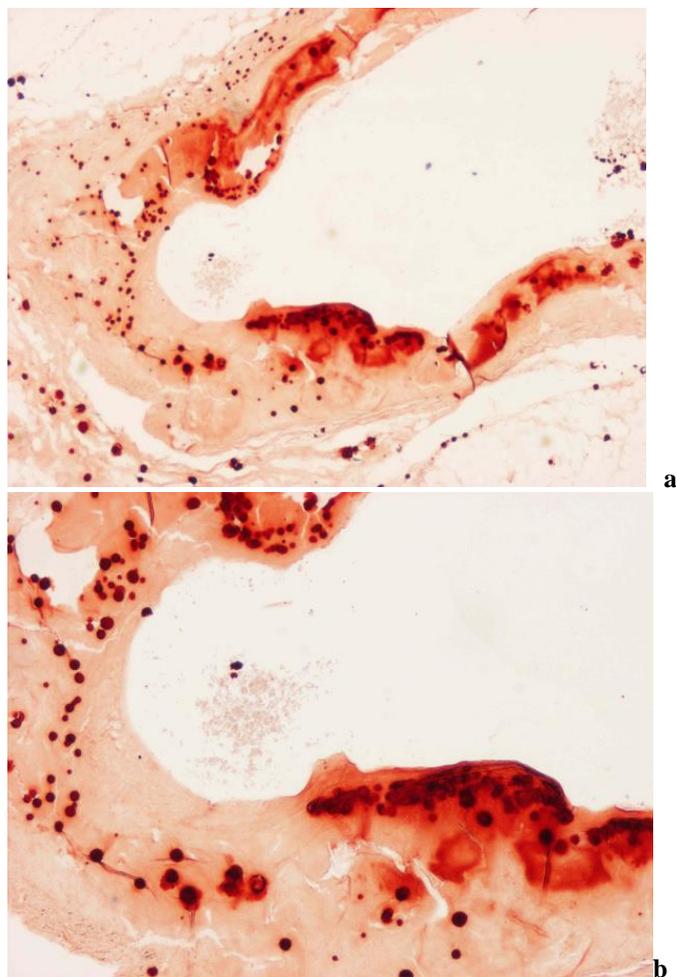


**Fig. 4.** Mönckeberg's sclerosis (hydroxyapatite crystal induced angiopathy), femoral artery, unstained section, Rot I compensator, viewed under polarized light (a) x100, (b) same field as 4a, x600. Under polarized light HA crystals show positive birefringence (the intensity of birefringence is much weaker in comparison with CPPD).

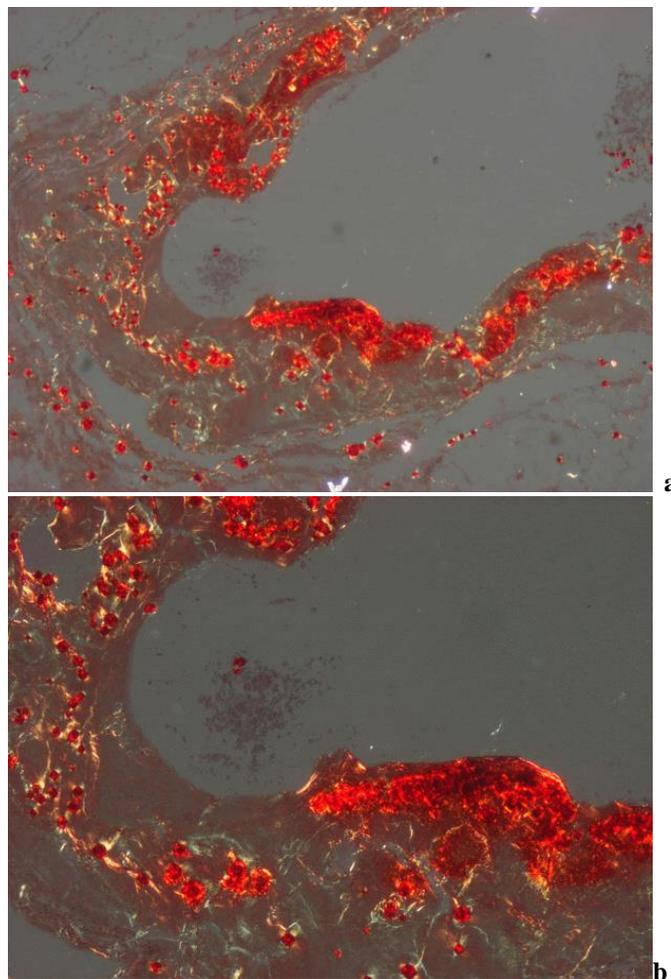
In unstained sections viewed under polarized light HA or CPPD crystals were staining with Alizarin Red in 13, and did not stain in 9 of 35 tissue samples. The correlation between HA or CPPD crystals and calcium content of amorphous deposits was not significant ( $\chi^2=3.1574$ ,  $p<0.07$ ).

The HA or CPPD crystal deposits associated with von Kossa reaction positivity in 11, and not in 13 of 35 tissue samples. The correlation between HA or CPPD crystals and phosphate or carbonate content of amorphous deposits was significant ( $\chi^2=4.8043$ ,  $p<0.02$ ).

The HA or CPPD crystals hidden in sections stained with Alizarin Red or by von Kossa reaction; the amorphous masses of calcium phosphate and carbonate masked the crystals with the von Kossa reaction, there was no detectable birefringence. The HA or CPPD crystals may be incorporated by phagocytes (**Fig. 7a-b**).



**Fig. 5.** Mönckeberg's sclerosis (hydroxyapatite crystal induced angiopathy), femoral artery, Alizarin Red staining (specific for calcium), viewed by light microscope  
(a) Same as Fig. 1a, x20, (b) same field as 5a, x40  
The HA or CPPD crystals are hidden in sections stained with Alizarin Red or by von Kossa reaction.



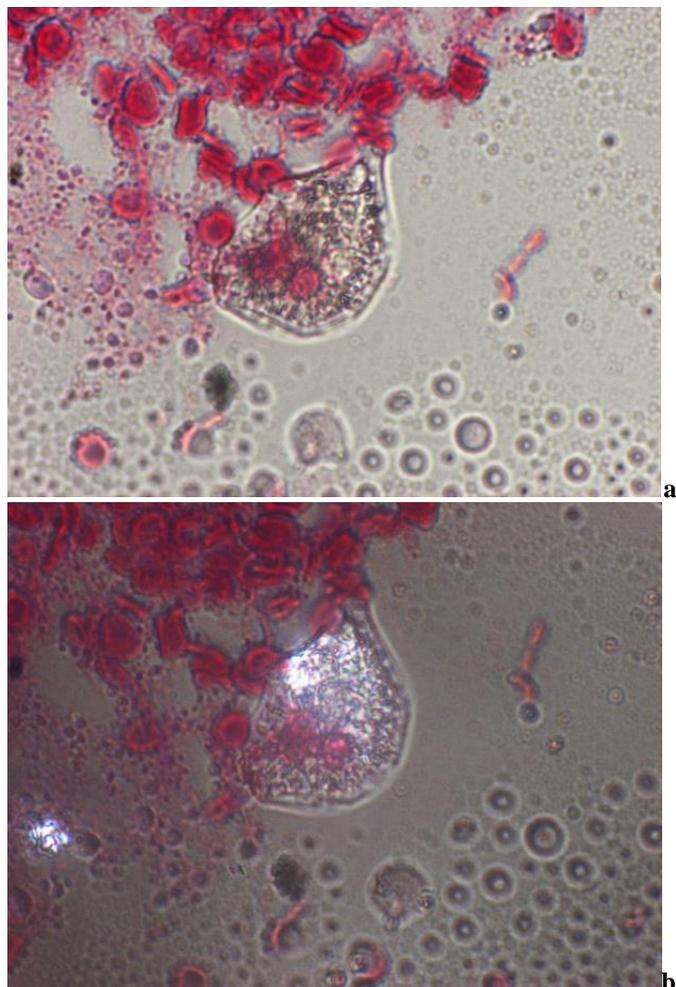
**Fig. 6.** Mönckeberg's sclerosis (hydroxyapatite crystal induced angiopathy), femoral artery, Alizarin Red staining (specific for calcium), viewed under polarized light  
(a) Same as Fig. 5a, x20, (b) same Fig. 5b, x40  
The crystals are masked by amorphous masses of calcium phosphate and carbonate.

## Discussion

Mönckeberg's arteriosclerosis is characterized histologically by calcification and ossification of the media and/or intima of medium size arteries, with partial occlusion of the vessels. Stenosis may cause diminished blood flow to the periphery, with or without complications.

The presented observations indicate that the so-called "Mönckeberg sclerosis" consists of two different entities. In one group of patients a large amount of HA crystals may be detected in unstained sections viewed under polarized light. These cases should be regarded as crystal induced angiopathy, a manifestation of a metabolic disorder. In the second group of patients HA crystals are not detectable. These cases present a manifestation of a general atherosclerosis (with or without chronic kidney disease). The two entities may overlap and occur in combination in the same patient.

CPPD sporadically may be associated with HA, and cholesterol crystals may be present in both groups.



**Fig. 7.** Mönckeberg's sclerosis (hydroxyapatite crystal induced angiopathy), femoral artery, von Kossa reaction (specific for phosphate and carbonate) Incorporated HA crystals in phagocytes.  
(a) Light microscope, x600, (b) same field as 7a, viewed under polarized light x600..

Presumably HA, CPPD and other crystals – similarly to crystal deposition induced arthropathy<sup>10-16</sup> – cause fibrosis with or without intimal proliferation and contribute to progressive occlusion of blood vessels. Based on these observations, in the presence of HA crystals, Mönckeberg's sclerosis may be defined as a crystal-induced angiopathy.

Calcification in the wall of arteries may be identified with Alizarin Red staining or by the von Kossa reaction as amorphous calcium phosphate or carbonate deposits. The strongly significant correlation between calcium and phosphate or carbonate contents of amorphous deposits indicates that they belong together. The lack of significant correlation between HA crystals and Alizarin Red staining positivity suggests that the crystal and the amorphous calcium containing deposition are an independent phenomenon and suggest a different pathogenesis supporting the view that Mönckeberg's arteriosclerosis is not a single entity.<sup>17-19</sup>

It seems that calcium in a crystalline structure does not bind Alizarin Red;  $[\text{Ca}_5(\text{PO}_4)_3(\text{OH})]$  does not stain with calcium specific Alizarin Red. The weak but significant correlation between HA crystals and the von Kossa reaction suggests that the phosphate content of crystals may slightly be modified.

In case of clinically or histologically suspected metabolic or crystal induced disease the tissue specimens should be evaluated (examined) in sections stained by haematoxylin-eosin and in unstained sections as well. The probability of crystal positive cases is much higher in unstained sections viewed under polarized light in comparison with stained ones. This approach may also be useful in other crystal deposition induced diseases. Textbooks of histologic methods and histochemistry do not mention this simple technique.<sup>7-9, 20-22</sup>

### Conclusions

The so-called Mönckeberg's sclerosis – according to our observations – can be divided in two groups based on the presence of HA. In one group large amounts of HA crystals may be detected in unstained sections viewed under polarized light. These cases can be regarded as a crystal induced angiopathy, a manifestation of a metabolic disorder. In the second group of patients HA crystals are not detectable; they simply manifest general atherosclerosis.

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