# Crystals and vessel walls

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Hitherto the concept of crystal-induced diseases has had its main impact in rheumatology. It is becoming obvious, however, that crystals have a part to play in the production of disease in other systems. We thought that the crystalline form of some of the constituents of atherosclerotic plaques may play a part in their formation—for example, cholesterol crystals are capable of activating polymorphs[1] and promoting collagen production by fibroblasts.[2] Hence as part of an investigation into the role of crystals in vessel walls we undertook a preliminary study using polarising light microscopy and present the results.

Early non-calcified plaques of uncomplicated atheroma of the aorta were obtained at necropsy from both sexes with an age range of 28–84 years.

These plaques were subjected to rapid freezing and 5 $\mu$m thick cryostat sections both unstained and stained (haematoxylin and eosin, oil red 'O') were examined using a polarising light microscope. The oil red 'O' demonstrated lipid, and crystals of cholesterol and monosodium urate. Urate crystals were identified by their characteristic appearance under polarised light microscopy and solubility in uricase.

In addition to the expected cholesterol crystals we describe what appeared to be crystals of monosodium urate occurring in samples of early atheromatous plaques from human aortas at necropsy. We believe that this may be of some importance in view of the long speculated association between hyperuricaemia and atherosclerosis.[3] The situation may be analogous to that occurring in tophi, where cholesterol and hydroxyapatite[4] are often found in conjunction with urate crystals suggesting common nucleation factors. The capabilities of these crystals to trigger inflammatory and sclerotic reactions suggests a possible role in the production of atherosclerotic lesions.

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# An immunoelectron microscopical study of the orientation of IgG molecules on the surface of monosodium urate crystals

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The binding of IgG to monosodium urate (MSU) crystals has been demonstrated by several techniques.[1–3] It is thought to play an important part in the pathophysiology of the gouty attack, as IgG coating of MSU crystals could modulate crystal interactions with cells, in particular by reacting with IgG Fc receptors. The availability of Fc fragments on the crystal-bound IgGs thus appears to be an important factor in the part IgG might play in gouty inflammation. We recently presented data in favour of such an availability.[3] We set out to investigate further the orientation of IgG molecules from serum, bound to MSU crystals, by using immunoperoxidase techniques directed against the Fc and $F(ab')_2$ fragments of IgG.

Unheated synthetic MSU crystals were incubated with normal human serum for one hour at 4°C, washed, and then processed with an indirect immunoperoxidase technique using rabbit antihuman IgG, at a concentration of 120 $\mu$g/ml, directed either against the $F(ab')_2$ or the Fc fragments, followed by horseradish peroxidase conjugated goat antirabbit IgG. Next, crystals were incubated with DAB + $H_2O_2$ and were post fixed

with osmium tetroxide. To prevent non-specific binding of the antibodies to crystals, samples were incubated with normal goat globulins before and during the antibody incubation steps. Samples were washed between each step with buffers saturated with urate to avoid dissolution of the crystals. Appropriate controls were used, including blocking with unconjugated goat antirabbit antibodies and replacement of the primary antibodies by normal rabbit globulins. All samples were dehydrated, embedded in Spurr medium and thin sectioned for observation on transmission electron microscopy. About 100 crystals were analysed from samples of each type of processing. Each crystal was classified as negative, weakly positive, or strongly positive, according to the intensity of peroxidase reaction results without knowledge of the processing done. Results were compared by the $\chi^2$ test (see Table 1).

Crystals dissolved during the dehydration and embedding procedures, but most of their silhouettes could still be identified in thin sections. In samples processed to react with $F(ab')_2$ fragments, most of the crystal sites were strongly outlined by dark reaction products which showed the IgG coating and proved the functional availability of the Fc fragments of the crystal-bound IgG. In contrast, positive crystals were very significantly less frequent ($p<0{\cdot}00001$) in samples treated to react with $F(ab')_2$ fragments than in those processed to demonstrate Fc fragments (see Table 1). Controls were negative or had only weak reaction products.

These data are consistent with previous studies using different techniques[4] and might be explained by electrostatic forces that are known to be important in protein adsorption to MSU crystals.[1 2] Fab fragments have a more positive charge than Fc fragments[5] so that the Fab extremity may bind preferentially to the negatively charged crystal, thus leaving the Fc end free.

Table 1 *Intensity of IgG Fc and $F(ab')_2$ reaction with immunoperoxidase after incubation of MSU crystals with serum*

| *Types of processing* | *Total No of crystals analysed* | *No of negative crystals* | *No of weakly positive crystals* | *No of strongly positive crystals* |
|---|---|---|---|---|
| Anti-IgG Fc | 101 | 17 | 24 | 60 |
| Anti-Ig $F(ab')_2$ | 129 | 97 | 22 | 10 |
| Normal rabbit globulin control | 92 | 85 | 7 | — |
| Blocking control | 126 | 84 | 36 | 6 |

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# Crystal-induced oxygen uptake by animal neutrophils

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Crystals and other types of particulate material are often seen in synovial fluids. At least three types of crystal are known to be pathogenic: monosodium urate monohydrate, calcium pyrophosphate dihydrate, and hydroxyapatite. Phagocytosis of crystals by leucocytes or other interactions between crystals and leucocytes in joint tissue are likely to be involved in the inflammatory response.[1 2]

We have prepared neutrophils from pig blood[3] and examined the effect on their oxygen metabolism of crystal additions. The crystals, a gift from Dr Paul Dieppe, had the following dimensions: monosodium urate monohydrate, 5–10 $\mu$m length; calcium pyrophosphate dihydrate (CPPD), average 15 $\mu$m length; brushite, 5–15 $\mu$m length; diamond, 2–7 $\mu$m length; and cholesterol, average 20 $\mu$m length. Crystals were suspended in the modified Krebs–Ringer buffer used in neutrophil preparation and sonicated briefly before use. Oxygen uptake was measured using a Clarke type electrode, superoxide production determined as superoxide-sensitive cytochrome with reduction and peroxide fluorimetrically by coupling it to the peroxidation of 4-OH, 3-methoxyphenylacetic acid.

The addition of crystals of urate, brushite, or CPPD to neutrophils caused a great increase in oxygen uptake which was insensitive to 2