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 χ^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 μm length; calcium pyrophosphate dihydrate (CPPD), average 15 μm length; brushite, 5–15 μm length; diamond, 2–7 μm length; and cholesterol, average 20 μm 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

mmol KCN. The effects depended on the crystal concentration, but the maximum stimulation approached 50% of that given by the soluble stimulus phorbol myristate acetate. The results could be plotted using the form of the Michaelis–Menten equation to give a Km for each crystal. For urate this was 12·5 mg crystal/ml, for brushite 1·2 mg/ml, for calcium hydroxyapatite 1·2 mg/ml. Diamond and cholesterol crystals did not stimulate oxygen uptake.

Addition of brushite caused the production of superoxide and H_2O_2 by isolated neutrophils at concentrations similar to those found to stimulate oxygen uptake. Analysis of the temperature dependence of the stimulation of oxygen uptake showed that urate crystals were ineffective at temperatures below 23°C; phorbol was effective at, and above, −17·5°C. Urate and phorbol showed similar temperature dependence above this trigger temperature in Arrhenius plots.

Neither colchicine nor cytochalasin B inhibited the crystal-induced burst of oxygen uptake over the usual concentration range at which these inhibitors are used. Inhibitors of SH–dependent enzymes and flavin analogues were, however, potent inhibitors.

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Crystal interactions with polymorphonuclear leucocytes studied by luminol-dependent chemiluminescence

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In recent years the crystal arthropathies have emerged as a distinct group of diseases linked by common pathogenetic mechanisms. One topic thought to be important is the interaction between crystals and polymorphonuclear leucocytes in the production of inflammation.[1] We describe a method that allows the study of early events in this interaction by means of luminol-dependent chemiluminescence.

Polymorphonuclear leucocytes were obtained from fresh normal peripheral blood by dextran sedimentation[2] and suspended in phosphate buffered saline.

Luminol reacts with oxidising agents produced by the polymorph,[3] in the form of superoxides, hydrogen peroxide, and hydroxyl radicals, to produce an unstable intermediate which spontaneously releases to a ground state releasing photons in the process. The reaction was followed on an LKB 1250 luminometer linked to a flat bed recorder. The reaction cell of the luminometer was thermostatically controlled and the reactants held at 37°C. The reaction mixture consisted of 1 ml of phosphate buffered saline medium containing $1·5 \times 10^6$ cells, 0·1 ml of a standard luminol solution, and 0·1 ml of a 1% crystal suspension.

A series of dose-response curves with varying concentrations in a fixed volume showed a linear response until concentrations greater than 3% were used. We have used this technique to investigate the ability of different crystal preparations to induce release of superoxides and other oxygen radicals from polymorphs. Of those crystals implicated in joint disease monosodium urate crystals produced the largest response, the responses of CPPD, hydroxyapatite, brushite and cholesterol being less than 20% of the response to MSU.

The inflammatory potential of crystals as measured by animal models and their surface charges have been shown to be highly correlated.[4] We have demonstrated a high degree of correlation between the surface charge of MSU crystals as measured by electrophoretic mobility and the chemiluminescence response induced by the crystal preparations.

Luminol-dependent chemiluminescence appears to be a useful method for studying early events in the interaction of polymorphonuclear leucocyte membranes and crystal surfaces and may allow further clarification of factors initiating and modifying effector mechanisms of crystal induced disease.

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