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 K_m 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 sedimentation² 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 1.5×10^6 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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