Activation of the alternative pathway of complement by monosodium urate monohydrate crystals and other inflammatory particles

M. DOHERTY,¹ J. T. WHICHER,² AND P. A. DIEPPE¹

From the University ¹Department of Medicine and the ²Department of Chemical Pathology, Bristol Royal Infirmary, Bristol

SUMMARY Activation of serum C3 by monosodium urate monohydrate (MSU) crystals and other particles was determined by immunofixation following electrophoretic separation of C3 and its activation products. Densitometry allowed quantitation of results. MSU, hydroxyapatite, brushite, and calcium pyrophosphate dihydrate crystals split C3 under conditions which demonstrate activation via the alternative pathway (AP). Quantitatively similar results were obtained in immunoglobulin deficient serum. Activation was crystal specific and was reduced by heating, grinding, sonication, and aging of crystals. Other inflammatory particles (e.g., blackthorn) activated C3 via the AP: noninflammatory particles (e.g., diamond) caused insignificant activation. It is suggested that particle-induced activation of the alternative pathway of complement may be important in the initiation of crystal-induced synovitis.

The precise way in which monosodium urate monohydrate (MSU) and other crystals cause an inflammatory synovitis has not been determined. The polymorph is usually afforded a central role in the inflammatory response,^{1,2} but reports of crystal synovitis in the absence of synovial fluid polymorphs,^{3,4} the occurrence of significant crystal-induced inflammation despite neutropenia,⁵ and the sequence of events following injection of MSU crystals into the canine joint⁶ suggest that other mediators of inflammation may also be important, particularly in the initial stages of the response.⁶

That complement may be one of these mediators is supported indirectly by the finding of decreased complement together with complement split products in gouty synovial fluid⁷⁻⁹ and by the demonstration of diminished urate-induced inflammation in complement-depleted animals.^{10,11} More direct evidence comes from the demonstration that complement may be activated by MSU and other crystals *in vitro*.¹²⁻¹⁴ The mechanism of activation, however, remains uncertain, and experimental evidence on this point is conflicting.

Naff and Byers demonstrated disproportionate decreases in C1, C4, and C2 haemolytic activities

when MSU crystals were added to serum, raising the possibility of a unique method of activation.¹² Hasselbacher, using crossed immunoelectrophoresis, has shown calcium-dependent activation of serum C3 by MSU, hydroxyapatite (HA), and calcium pyrophosphate dihydrate (CPPD) crystals, concluding that C3 activation occurs via the classical pathway¹³ and is dependent on adsorbed immunoglobulin.¹⁵ Giglas *et al.* proposed that activation by MSU crystals arises by direct interaction between the crystal surface and the C1q component of intact C1 independently of IgG.¹⁴ Thus despite uncertainty over the part played by immunoglobulin the consensus of opinion appears to favour activation via some part of the classical pathway. This method of activation is perhaps surprising in that other inflammatory particles such as cholesterol crystals,¹⁶ particulate polysaccharide,¹⁷ and hay dusts¹⁸ are known to activate the alternative pathway (AP) of complement. There have been no reports, however, of AP activation by the crystalline particles of MSU, HA, and CPPD.

This study investigates further the complement-splitting activity of MSU crystals and a variety of other particles, employing a recently developed immunofixation technique that allows quantitative estimation by densitometry of the conversion product of C3.¹⁹ Adjustment of calcium and magnesium

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Correspondence to Dr M Doherty, Department of Medicine, Bristol Royal Infirmary, Bristol BS2 8HW.

(Mg) ion concentration allowed demonstration of AP activation. The effect of prior physical manipulation on the complement-splitting potential of MSU crystals was also investigated.

Materials and methods

PARTICLES

MSU crystals (2–20 μm long), CPPD crystals (triclinic and monoclinic), brushite, and HA crystals were prepared by standard techniques^{20–22} and examined by polarised light microscopy and infrared spectrophotometry. Calcium orthophosphate, 'spheroidal' HA, cholesterol crystals, and zymosan A from *Saccharomyces cerevisiae* were obtained from Sigma. Diamond dust (1–6 μm diameter) was a generous gift from de Beers of London. Pyrex glass filter wool was obtained from BDH, Poole. Inorganic dusts, fibres, and carbonyl iron were kindly provided by the MRC Pneumoconiosis Unit, Penarth. Black-thorns were harvested from wild shrubs (*Prunus spinosus*) and the tips were ground in a pestle and mortar to fragments 1–12 μm long. The tips of date palm fronds (*Phoenix dactylifera*) were similarly ground to fragments 1–20 μm long.

Particles were prepared and handled in clean conditions in sterilised polypropylene and glass ware; they were not heated to destroy pyrogens because of the known effect of heat on crystal structure and surface.^{23, 24} The effect of heating was studied on MSU crystals heated to 200°C for 2 hours. Grinding of crystals was manual, with a pestle and mortar. Crystals were sonicated at 1.5 MA (1/8 inch (3 mm) exponential probe, 10 μm peak to peak amplitude) for 10 minutes in sterile saline with cooling. 'Old' crystals were those stored in airtight containers at room temperature for 9 months.

ACTIVATION OF COMPLEMENT

50 μl samples of a 1% suspension of the test particles in sterile normal saline were incubated in polypropylene tubes at 37°C for 30 min with 0.45 ml fresh normal human serum (NHS). Incubation was performed concurrently under 3 different conditions: (1) Alone, with free interaction allowed between particle and serum. (2) In the presence of 100 μl of 0.05 M ethylene diaminetetraacetic acid (EDTA), which chelates calcium and Mg. This prevented activation of C3 via both the calcium-dependent classical pathway and the Mg-dependent AP. (3) In the presence of 100 μl of 0.05 M ethyleneglycoltetraacetic acid (EGTA), which preferentially chelates calcium, together with 10 μl of 0.0175 M MgCl_2 . Under these conditions classical pathway activation is blocked but AP activation can still proceed. Three control tubes containing 0.45 ml NHS + 50 μl saline were treated similarly. A positive control (0.45 ml NHS incubated with 20 μl of antialbumin) and a negative control (0.45 ml fresh normal plasma in 0.01 M EDTA) were included in each experiment.

Serum was obtained from a single healthy donor on the morning of the experiment and contained normal levels of complement and immunoglobulin. One set of experiments compared the activity of MSU crystals in 2 different sera—one being NHS, the other being fresh hypogammaglobulinaemic serum (HHS) from a patient with hypogammaglobulinaemia (IgA undetectable, IgG 0.2 g/l, IgM 0.1 g/l). Both sera contained similar quantities of C3 (0.85 and 0.86 g/l respectively) and C4 (0.40 and 0.38 g/l respectively).

The ability of this system to differentiate classical from AP activation was checked by using 20 μl of antialbumin (Dakopatts, Denmark) or zymosan (1%) (Sigma) as the test suspension (the former activates the classical pathway by generating soluble

Fig. 1 Typical plate obtained for MSU crystals 1% (M—untreated, H—heated, G—ground), diamond crystals 1% (D), and saline controls (S). The slower bands ($\beta 1$ mobility) correspond to native C3; the bands with greater anodal mobility correspond to the split products of C3. Incubation with EDTA prevents C3 splitting, whereas incubation with EGTA + Mg allows expression of AP activation. Incubation with saline (alone or with EGTA + Mg) causes some C3 activation. A negative and positive control are included.

antigen-antibody complexes in antigen excess, the latter activates the AP).

ELECTROPHORESIS AND IMMUNOFIXATION

Electrophoresis and immunofixation were performed as previously described.¹⁹ A portion of each incubate was applied by means of a slotted mask for 3 minutes to a 1 mm layer of 1% agarose. The buffer

Fig. 2 Densitometric scans obtained for diamond crystals 1% (above) and MSU crystals 3% (below). For each the right hand peak corresponds to native C3, the left to the split products of C3. Automated computation of these traces showed diamond to have split 14% of the total C3 present (the same as saline control) and MSU to have split 60.5% (i.e., 46.5% more than saline control).

was 0.06 M barbitone, pH 8.6, containing 0.1 M disodium EDTA. The gel was subjected to electrophoresis (10 v/cm with cooling) to allow separation of native C3 from its activation products (EDTA in the buffer is essential to prevent electrophoresis-induced C3 activation.¹⁹) The gel was then overlaid for one hour with cellulose acetate membrane soaked in rabbit antiserum to human C3 (Dakopatts, Denmark) diluted 1:2 with 0.12 M barbitone buffer (pH 8.6, containing 80 g/l polyethylene glycol). After removal of the membrane the gel was washed in hypertonic saline (50 g/l) for 2 hours to remove unfixed protein and then stained with Coomassie brilliant blue R (5 g/l in methanol:acetic acid:water, 450:100:450). Staining showed native C3 as a single band with β 1 mobility; activation of C3 was shown by the appearance of a second band with greater anodal mobility corresponding to the C3 activation products.¹⁹

Up to 4 different particles could be fully tested on a single plate (Fig. 1). Densitometric scanning with an automated computing densitometer (ACD-18, Gelman Instrument Co., USA) allowed estimation of the percentage of C3 present as the anodal activation product for each sample (Fig. 2).

Statistical analysis was done by Student's *t* test for paired observations (test particle compared with saline control). Results are given as mean $\pm$ SD.

Results

The ability of this system to differentiate classical from AP activation was confirmed (Table 1). Incubation with EDTA blocked C3 activation by either pathway, whereas EGTA + Mg selectively blocked classical pathway activation but allowed AP activation to proceed. The amount of available Mg in this system is crucial: insufficient will prevent AP activation, while excess will itself lead to C3 activation. The presence of 8 mM EGTA + 0.3 mM MgCl₂ was determined to be optimal.

Reproducibility on a single plate was excellent. Measurement of C3 activation in 15 replicate samples on a single plate showed a coefficient of variation of 3.62%. Greater variability occurred between

Table 1 Ability of system to differentiate classical and alternative pathway activation of C3

Activator	% of C3 split in excess of saline control (mean $\pm$ SD)				Number of tests
	Incubated alone	<i>p</i>	Incubated with EGTA + Mg	<i>p</i>	
Zymosan (1% suspension)	43.7 $\pm$ 3.67	<0.01	36.4 $\pm$ 2.91	<0.01	4
Antialbumin (20 μ l)	47.4 $\pm$ 4.61	<0.01	0.4 $\pm$ 1.56	*NS	25

*NS=not significant.

experiments. Measurements of C3 activation produced by incubation of NHS with saline alone in 33 experiments showed a coefficient of variation of 17.82%.

Incubation of NHS with saline alone (Fig. 1) produced some C3 activation (mean of 33 experiments = $15.4\% \pm \text{SD } 2.75\%$). Results for test particles are therefore expressed as the percentage of C3 split in excess of the saline control incubated concurrently under the same ionic conditions.

MSU crystals caused significant activation of C3 (compared with saline control) when incubated with NHS alone (Table 2). Incubation with EDTA prevented C3 activation, but incubation with EGTA + Mg allowed splitting of C3 by MSU crystals, thus demonstrating activation via the AP. Activation was dose dependent. The sensitivity of this system

allowed detection of significant C3 activation by as little as $10 \mu\text{g/ml}$ of MSU crystals. No significant C3 splitting was produced by the supernatant from a 2% MSU crystal suspension or by 1% uric acid, indicating that complement activation by MSU is crystal specific.

HA, CPPD, and brushite crystals similarly split C3 and activated the AP (Table 3), though to a lesser extent than MSU on an equal weight comparison. 'Spheroidal' HA and amorphous powders of calcium phosphate salts caused insignificant activation, suggesting that activation by HA, CPPD, and brushite is also specific to their structurally ordered crystal state.

Various other particles were tested (Table 4). Plant thorns, α quartz, asbestos, and cholesterol crystals were shown to split C3 and activate the AP. Of all particles tested zymosan was the most potent activator of C3 on an equal weight comparison

Table 2 C3 splitting activity of MSU crystals

Test particle/Solution	% of C3 split in excess of saline control (mean $\pm$ SD)				Number of tests
	Incubated alone	p	Incubated with EGTA + Mg	p	
MSU 0.01%	3.2 $\pm$ 0.67	<0.01	1.3 $\pm$ 0.53	<0.01	4
0.10%	5.3 $\pm$ 0.84	<0.01	3.7 $\pm$ 1.14	<0.01	5
0.50%	23.6 $\pm$ 1.93	<0.01	10.7 $\pm$ 1.57	<0.01	5
1%	37.2 $\pm$ 4.39	<0.01	21.4 $\pm$ 2.77	<0.01	12
3%	45.1 $\pm$ 2.95	<0.01	26.7 $\pm$ 1.97	<0.01	4
MSU 1% sonicated	34.5 $\pm$ 1.36	<0.01	15.8 $\pm$ 1.36	<0.01	3
ground	13.3 $\pm$ 1.05	<0.01	10.4 $\pm$ 2.06	<0.01	3
heated	6.2 $\pm$ 1.25	<0.01	5.8 $\pm$ 1.64	0.01	3
9 month-old MSU 1%	29.2 $\pm$ 2.98	<0.05	15.7 $\pm$ 1.75	<0.05	2
Uric acid 1%	-0.4 $\pm$ 1.19	*NS	-0.2 $\pm$ 1.69	NS	2
MSU 2%—supernatant	0.2 $\pm$ 1.12	NS	-0.3 $\pm$ 1.00	NS	2

*NS=not significant.

Table 3 C3 splitting activity of calcium phosphate salts

Test particle (1% suspension)	% of C3 split in excess of saline control (mean $\pm$ SD)				Number of tests
	Incubated alone	p	Incubated with EGTA + Mg	p	
HA	22.1 $\pm$ 2.89	<0.01	14.7 $\pm$ 5.60	<0.01	5
CPPD	16.2 $\pm$ 2.97	<0.01	11.1 $\pm$ 1.78	<0.01	5
Brushite	20.2 $\pm$ 8.36	<0.01	10.1 $\pm$ 2.89	<0.01	5
'Spheroidal' HA	-0.6 $\pm$ 0.31	*NS	1.1 $\pm$ 3.05	NS	2
Calcium orthophosphate	0.4 $\pm$ 0.21	NS	0.8 $\pm$ 2.76	NS	2
Calcium pyrophosphate	-0.6 $\pm$ 1.62	NS	-0.3 $\pm$ 0.83	NS	2
9-month old HA	17.2 $\pm$ 1.47	<0.05	13.2 $\pm$ 4.18	<0.05	2
9-month old brushite	11.9 $\pm$ 0.25	<0.01	6.1 $\pm$ 0.98	<0.05	2

*NS=not significant.

Table 4 C3 splitting activity of a variety of inflammatory and noninflammatory particles

Test particle (1% suspension)	% of C3 split in excess of saline control (mean±SD)				Number of tests
	Incubated alone	p	Incubated with EGTA + Mg	p	
1. Inflammatory particles					
Blackthorn (ground)	17.0±1.64	<0.01	10.8±1.43	<0.01	8
Date palm (ground)	22.0±1.82	<0.05	12.6±1.34	<0.05	2
Cholesterol crystals	15.2±1.37	<0.01	10.8±1.06	0.01	2
α Quartz	12.9±0.11	<0.01	12.9±0.11	<0.01	2
Anthophyllite (asbestos)	17.0±1.23	<0.05	15.8±0.79	<0.05	2
2. Noninflammatory particles					
Diamond crystals	0.6±1.38	*NS	0.9±1.06	NS	4
Carbonyl iron	0.3±1.93	NS	-0.2±1.14	NS	4
Glass wool (ground)	-0.3±1.12	NS	-0.1±0.77	NS	2
Silicon carbide	-0.3±1.32	NS	1.2±1.80	NS	2

*NS=Not significant.

Table 5 Comparison of crystal-induced C3 activation in normal and hypogammaglobulinaemic sera

Particle 1%	% of C3 split in excess of saline control (mean±SD)		Number of tests	Difference (NHS-HHS)
	NHS†	HHS‡		
MSU: 1. Incubated alone	39.5±3.48	38.3±4.38	5	*NS
2. Incubated with EGTA + Mg	20.1±3.35	18.7±2.43	5	NS
Brushite:				
1. Incubated alone	17.6±2.54	17.5±2.31	3	NS
2. Incubated with EGTA + Mg	13.6±2.19	14.6±2.58	3	NS

*NS=Not significant. †NHS=Normal human serum. ‡HHS=Hypogammaglobulinaemic human serum.

(Table 1). Several particles, including diamond crystals, did not produce significant C3 activation.

Both MSU and brushite crystals produced C3 activation in the HHS (Table 5). Quantitatively there was no significant difference from results obtained with NHS tested concurrently with the same crystal suspensions.

Prior physical treatment of particles was shown to affect their C3 splitting potential. Sonication, grinding, or heating (200°C for 2 hours) of MSU crystals reduced their capacity to activate C3 by 10%, 64%, and 83% respectively (Table 2). Aging also modified their activity. MSU crystals lost 21% of their C3 splitting potential over a 9-month period. Storage of HA and brushite crystals for the same period resulted in a reduction in C3 splitting potential of 22% and 41% respectively (Table 3).

Discussion

Activation of complement may be important in mediation of crystal-induced inflammation.¹² This

study confirms in-vitro activation of serum C3 by MSU, HA, and CPPD crystals in accord with previous reports¹²⁻¹⁴ and also demonstrates complement activation by brushite crystals, plant thorns, and other inflammatory particles. The finding that some crystals and particles (e.g., diamond, carbonyl iron) do not activate C3 suggests that complement activation by active agents depends on some special characteristic(s) of their presenting surface and not merely on their crystalline or particulate state.

Other investigators have concluded that complement activation by inflammatory crystals occurs via the classical pathway.^{15, 16} In the present study, however, the demonstration of significant C3 activation in the presence of EGTA + Mg suggests that complement activation by these agents is mediated at least in part via the AP. Although the AP is known to be activated by a variety of injurious particles,¹⁶⁻¹⁸ AP activation by MSU, HA, CPPD, or brushite crystals has not previously been described.

The discrepancy between our findings and those of others who report classical but not AP activation by

MSU,^{13 14} HA,¹³ and CPPD¹³ crystals may well reflect differences in experimental method. In the system employed by Hasselbacher¹³ the addition of EGTA caused only minimal reduction in zymosan-induced AP activation, and further addition of Mg had no effect. By contrast, in the present system addition of EGTA caused marked reduction in AP activation by zymosan, but the addition of Mg (0.3 mM) with the EGTA (8 mM) allowed nearer normal expression of AP activity while still inhibiting classical pathway activation. In such a system insufficient Mg will prevent AP expression, while excess will activate the AP and possibly displace calcium from EGTA. Variability in such factors may well be critical in permitting expression of AP activity, particularly by less potent activators with a higher restriction index than zymosan.

The quantitatively lower values obtained on incubation with EGTA + Mg may arise because chemical manipulation and volume alteration prevents optimal AP expression or because activation has proceeded via both pathways. The disparity in values obtained for zymosan would seem to support the former explanation, while for MSU crystals the body of evidence in favour of classical pathway activation¹⁴⁻¹⁶ might support the latter.

Several investigators stress the importance of adsorbed immunoglobulin in conferring phlogistic potential to crystals,^{25 26} while others suggest that activity arises as a direct effect of the crystal surface.¹⁴ Although crystal surfaces avidly attract protein, including immunoglobulin,²⁷ such protein binding is nonspecific³⁶ and unlikely to result in significant stereochemical alteration of IgG or IgM, a supposition that is supported by lack of correlation between C3 activation and levels of adsorbed IgG or C1q.²⁸ In this study we share the finding by Naff and Byers¹² of quantitatively similar C3 activation by MSU (and brushite) crystals in normal and hypogammaglobulinaemic sera; although hypogammaglobulinaemic rather than agammaglobulinaemic serum was used, this finding supports the concept that the crystal surface alone may be sufficient to confer activity.

Classical pathway activation is primarily an adaptive immune response mediated via IgG or IgM combined with specific antigen, whereas the phylogenetically more primitive AP provides a means of nonimmune response to injurious agents.²⁹ For activation of the AP an ordered surface configuration of repeating polysaccharide or other polymeric units appears to be important,^{29 30} and, although immunoglobulin may have a facilitatory role,^{31 32} activation normally proceeds in the absence of specific antibody. Crystals, with their rigid repetitive surface structure (required by definition) would thus appear well suited for AP activation, and we

have shown that loss of this ordered crystal morphology (i.e., the amorphous state) results in inability to activate complement. Demonstration of C3 activation by other particulate agents such as plant thorns and asbestos fibres further emphasises the importance of a structured surface; irregularity of this surface may be a further requirement for complement activation, perhaps accounting for lack of activity by diamond.³³ Mandel has suggested that hydroxyl groups (hydrogen bond acceptors) on the crystal may be important in interaction between crystal and cell membrane,³³ and these same hydroxyl groups would be good candidates for covalent linkage with the putative thioester bond on the metastable binding site of C3b.³⁴ Such a reaction would be independent of immunoglobulin. Activation of the AP by inflammatory crystals and particles would thus appear theoretically attractive.

Heating of MSU crystals is known to alter their chemical and physical state,^{13 23 35} to reduce their net negative surface charge,²⁴ and to reduce their phlogistic²⁴ and complement splitting activity.^{12 13} This study confirms the reduction in complement splitting potential of MSU crystals following heating and further demonstrates alteration in MSU crystal activity by 2 other laboratory techniques (grinding and sonication) commonly used in preparing crystal suspensions. These observations stress the importance of using fresh, untampered crystal preparations and must put in question the validity of much previous work where such manipulations have been rigorously employed. However, the problem of eliminating endotoxin without heating, and thus altering, crystals remains unresolved. Although endotoxin may activate the AP, the marked differences between crystals observed in the present study seems unlikely to be explained by action of endotoxin alone, since all crystals were treated identically (and should therefore have been equally contaminated), supernatant from a concentrated MSU suspension lacked C3 splitting activity, and stored crystals demonstrated reduction, rather than increase, in C3 activating potential.

In the present system the degree of C3 activation by crystals was paralleled by their known phlogistic potential.⁵ On an equal weight comparison MSU were more active than HA or CPPD crystals, and the noninflammatory diamond crystals produced no C3 splitting at all. It may therefore be that the initial event in crystal-induced synovitis is activation of the AP consequent upon direct interaction, independent of immunoglobulin, between C3 and special features present on the crystal surface. Subsequent production of chemotactic complement-split products could then lead to influx of phagocytic cells as a secondary event: phagocytosis and enzyme release would then follow. Such a hypothesis fits well with the known sequence of events following injection of MSU crystals into the dog knee⁶ and with the positive correlation observed between the level of

complement-split products and the number of phagocytic cells present in synovial fluid from patients with acute crystal-induced synovitis.⁸

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References

- Phelps P, McCarty D J. Crystal-induced inflammation in canine joints II. Importance of polymorphonuclear leukocytes. *J Exp Med* 1966; **124**: 115-25.
- Chang Y-H, Gralla E J. Suppression of urate crystal-induced canine joint inflammation by heterologous anti-polymorphonuclear leukocyte serum. *Arthritis Rheum* 1968; **11**: 145-50.
- Matthay M, Lindamood M, Steigerwald J C, *et al.* Acute pseudogout in the absence of synovial fluid leukocytes. *J Rheumatol* 1977; **4**: 303-6.
- Ortel R W, Newcombe D S. Acute gouty arthritis and response to colchicine in the virtual absence of synovial fluid leukocytes. *N Engl J Med* 1974; **290**: 1363-4.
- Giatt M, Dieppe P A, Willoughby D. Crystal-induced inflammation, enzyme release and the effects of drugs in the rat pleural space. *J Rheumatol* 1979; **6**: 251-8.
- Schumacher H R, Phelps P, Agudelo C A. Urate crystal induced inflammation in dog joints: sequence of synovial changes. *J Rheumatol* 1974; **1**: 102-13.
- Pekin T J, Zvaifler N J. Haemolytic complement in synovial fluid. *J Clin Invest* 1964; **43**: 1372-82.
- Hunder G G, McDuffie F C, Mullen B J. Activation of complement components C3 and factor B in synovial fluids. *J Lab Clin Med* 1977; **89**: 160-71.
- Hasselbacher P. Immuno-electrophoretic assay for synovial fluid C3 with correction for synovial fluid globulin. *Arthritis Rheum* 1979; **22**: 243-50.
- Webster M E, Maling H M, Zweig M H, *et al.* Urate crystal induced inflammation in the rat: evidence for the combined actions of kinins, histamine and the components of complement. *Immunol Commun* 1972; **1**: 185-98.
- Kellermeyer R W, Naff G B. Chemical mediators of inflammation in acute gouty arthritis. *Arthritis Rheum* 1975; **18**: 765-70.
- Naff G B, Byers P H. Complement as a mediator of inflammation in acute gouty arthritis. I. Studies on the reaction between human serum complement and sodium urate crystals. *J Lab Clin Med* 1973; **81**: 747-60.
- Hasselbacher P. C3 activation by monosodium urate monohydrate and other crystalline material. *Arthritis Rheum* 1979; **22**: 571-8.
- Giglas P C, Ginsberg M H, Cooper N R. Immunoglobulin independent activation of the classical pathway of monosodium urate crystals. *J Clin Invest* 1963; **32**: 759-64.
- Hasselbacher P. C3 activation by monosodium urate monohydrate is enhanced by surface IgG. *Arthritis Rheum* 1979; **22**: 620 (abstr).
- Hasselbacher P. Activation of the alternative pathway of complement by microcrystalline cholesterol. *Atherosclerosis* 1980; **37**: 239-45.
- Götte O, Müller-Eberhard H J. The C3-activator system: an alternative pathway of complement activation. *J Exp Med* 1971; **134**: 905-1085.
- Edwards J H. A quantitative study on the activation of the alternative pathway of complement by mouldy hay dust and thermophilic actinomycetes. *Clin Allergy* 1976; **6**: 19-25.
- Whicher J T, Higginson J, Riches P G *et al.* Clinical applications of immunofixation: detection and quantitation of complement activation. *J Clin Pathol* 1980; **33**: 781-5.
- Seegmiller J E, Howell R R, Malawista S E. The inflammatory reaction to sodium urate. *JAMA* 1962; **180**: 469-75.
- Cheng P T, Pritzker K P H, Adams M E, *et al.* Calcium pyrophosphate crystal formation in aqueous solutions. *J Rheumatol* 1980; **7**: 609-16.
- Blumenthal N C, Posner A S. Hydroxyapatite: Mechanism of formation and properties. *Calcif Tissue Res* 1973; **13**: 235-43.
- Mandel N S. Structural changes in sodium urate on heating. *Arthritis Rheum* 1980; **23**: 772-6.
- Dieppe P A, Doherty M, Swan A, *et al.* Changes in monosodium urate monohydrate crystals on heating or grinding. *Arthritis Rheum* 1981; **24**: 975-6.
- Wallingford W R, McCarty D J. Differential membranolytic effects of microcrystalline sodium urate and calcium pyrophosphate dihydrate. *J Exp Med* 1971; **133**: 100-12.
- Kozin F, Ginsberg M H, Skosey J L. Polymorphonuclear leukocyte responses to monosodium urate crystals: modification by adsorbed serum proteins. *J Rheumatol* 1979; **6**: 519-26.
- Hasselbacher P, Schumacher H R. Immunoglobulin in tophi and on the surface of monosodium urate crystals. *Arthritis Rheum* 1978; **21**: 353-61.
- Hasselbacher P. Crystal induced complement activation: lack of correlation with IgG or C1q adsorption. *Arthritis Rheum Abstracts* 1979; 620.
- Müller-Eberhard H J, Schreiber R D. Molecular biology and chemistry of the alternative pathway of complement. *Adv Immunol* 1980; **29**: 1-53.
- Kazatchkine M D, Fearon D T, Austen K F. Human alternative complement pathway: Membrane-associated sialic acid regulates the competition between B and β 1H for cell-bound C3b. *J Immunol* 1979; **122**: 75-81.
- Edwards M S, Nicholson-Weller A, Baker C J, *et al.* The role of specific antibody in alternative complement pathway-mediated opsonophagocytosis of type III, group B streptococcus. *J Exp Med* 1980; **151**: 1275-87.
- Nelson B, Ruddy S. Enhancing role of IgG in lysis of rabbit erythrocytes by the alternative pathway of human complement. *J Immunol* 1979; **122**: 1994-9.
- Mandel N S. The structural basis of crystal-induced membranolytic. *Arthritis Rheum* 1976; **19**: 439-45.
- Pangburn M E, Müller-Eberhard H J. Relation of a putative thioester bond in C3 to activation of the alternative pathway and the binding of C3b to biological targets of complement. *J Exp Med* 1980; **152**: 1102-14.
- Howell R R, Eanes E D, Seegmiller J E. X-ray diffraction studies of the tophaceous deposits in gout. *Arthritis Rheum* 1963; **66**: 97-103.