DIFFERENTIAL MEMBRANOLYTIC EFFECTS OF MICROCRYSTALLINE SODIUM URATE AND CALCIUM PYROPHOSPHATE DIHYDRATE*

By WALTER R. WALLINGFORD,‡ M.D., and DANIEL J. McCARTY, M.D.

(*From the Section of Arthritis and Metabolism, Department of Medicine, Division of the Biological Sciences, University of Chicago, Chicago, Illinois 60637*)

(Received for publication 17 August 1970)

Intraleukocytic monosodium urate (MSU)[1] and calcium pyrophosphate dihydrate (CPPD) crystals are a constant feature of acute gout and pseudogout, respectively (1–3); phagocytosis of both types of crystal occurs rapidly in vitro when crystals are incubated with human buffy coat leukocytes, and is often seen in wet smears prepared from exudate aspirated from affected joints (2). Exudative leukocytes from joints of volunteers injected with synthetic MSU or CPPD crystals contain phagocytosed crystals, which appear identical by light microscopy with those found during the natural attack (3–5). As observed with phase-contrast and polarized light microscopy, a substantial number of CPPD crystals lie within phagosomes (3), but many CPPD and *all* MSU crystals appear free in the cytoplasm (6).

Recently, this concept of crystal-cell interaction has been challenged by Riddle et al., who, using electron microscopy, found no limiting membrane surrounding most intraleukocytic MSU crystals in natural gout, although a few MSU crystals lying within phagosomes were seen (7). Phagosomes were found uniformly when synthetic MSU was added to canine buffy coat leukocytes, or in exudate obtained from dog joints after crystal injection, and also when natural crystals were phagocytosed by human polymorphonuclear leukocytes in Rebuck skin windows (8). Bluhm et al. have also described the formation of intraleukocytic crystals *de novo* when hyperuricemic joint fluid or serum containing polymorphonuclear leukocytes was stored in the cold for many hours (9). These findings have led to the hypothesis that the primary event in gouty inflammation is the intracellular formation of MSU, phagocytosis representing only a secondary phenomenon.

The studies reported here were performed to explore an alternative explanation for these observations, i.e., that urate crystals might be phagocytosed

* Supported in part by United States Public Health Service Research Grant AM-13069 and United States Public Health Service Training Grant AM-05621-01.

‡ Formerly Instructor in Medicine and Trainee in Rheumatology, University of Chicago; presently Assistant Professor of Medicine, University of Wisconsin, Madison, Wis.

[1] *Abbreviations used in this paper:* CPPD, calcium pyrophosphate dihydrate; D, dextran; EA, ellagic acid dihydrate; H, heparin sodium; MSU, monosodium urate; PVP, polyvinylpyrrolidone; PVPNO, polyvinylpyridine-*N*-oxide; RBC, human erythrocytes; S, silicon dioxide.

and then interact with the phagosomal membrane, resulting in their liberation into the cytoplasm. Rupture of phagolysosomes induced in monocytes by silica crystals has been described recently by Allison et al. (10), and both colloidal and crystalline silica have been shown to lyse erythrocyte membranes (11–15). Because erythrocytes provide a convenient model for the detection and study of membrane lysis, they were used in the studies presented here.

Materials and Methods

General.—Doubly siliconized glassware was used throughout. A modified Hanks' buffer, pH 6.8, was prepared as described by Phelps, except that doubly distilled, deionized water was used and the calcium concentration was reduced to 0.23 mM (16). Penicillin and streptomycin, in final concentrations of 50,000 units and 50 mg per liter, respectively, were added to the buffer in all experiments performed at temperatures above 20°C.

Crystals.—Monosodium urate monohydrate (MSU) was prepared according to previously published methods (17). Samples of these crystals (30–100 μ in diameter) were ground in an agate mortar to produce particles 2–6 μ in length. These were heated for 2 hr to 200°C to eliminate any trace of pyrogen or other organic material that may have been trapped during crystal growth.

Calcium pyrophosphate dihydrate (CPPD) (triclinic dimorph) was supplied as a gift from James R. Lehr, National Fertilizer Development Center, Muscle Shoals, Alabama. Samples of these were reduced in length from approximately 100–200 μ to 1–20 μ by grinding in an agate mortar.

Silicon dioxide crystals (S) were obtained as a gift from the Pennsylvania Glass Sand Corp., Pittsburgh, Pa., as Min-U-Sil (Pennsylvania Pulverizing Co., Pittsburgh, Pa.), 1–10 μ in diameter, 99.9% pure SiO_2.

Chemicals.—

Polyvinylpyridine-*N*-oxide (PVPNO) was a generous gift from Dr. E. Grundmann of the Institute for Experimental Pathology, Farbenfabriken Bayer AG, 56 Wuppertal Elberfeld, West Germany.

Heparin sodium (H)—Liquaemin Sodium; 10,000 units/ml; Organon Inc., West Orange, N. J.

Dextran (D)—Type 200 C; average mol wt 204,000; Sigma Chemical Co., St. Louis, Mo.

Polyvinylpyrrolidone (PVP)—Pharmaceutical grade; average mol. wt 40,000; Sigma Chemical Co., St. Louis, Mo.

Disodium ethylenediaminetetraacetic acid dihydrate (EDTA)—Baker Analyzed Reagent, Phillipsburg, N. J.

Ellagic acid dihydrate (EA)—Aldrich Chemical Co., Inc., Milwaukee, Wis.

Human erythrocytes (RBC).—35 ml of blood from a normal human volunteer was drained into a 50 ml conical centrifuge tube, containing 1 ml of heparin sodium, through an 18 gauge disposable needle, and 7 ml of 6% dextran in isotonic sodium chloride was added. The cells were gentiy mixed and then allowed to settle at room temperature for 30 min. The supernate, which contained most of the leukocytes and platelets, was discarded. The RBC were washed three times with Hanks' buffer, pH 6.8 (centrifugation at 4°C, 400 *g*). The concentration of packed cells was then adjusted with buffer to provide 3 or 6% suspensions.

Incubations.—

Tumbling system: Most experiments were performed in 10 × 75 mm glass test tubes, sealed with Parafilm-covered corks, and rotated end over end, using a multipurpose rotator (Arthur Lapine Co.), at 3.5 rpm in an incubator (A. H. Thomas Co., Philadelphia, Pa.) at 37°C.

Stationary system: One experiment was performed without mechanical agitation by simply adding cells to crystals in the wells of a plastic disposable serology tray.

Shaking system: One experiment was performed by the addition of 1 ml of cells to 1 ml of crystals, with agitation in a Dubnoff metabolic shaker at 12 strokes/min for 14 hr at 37°C.

Experiments.—

Hemolytic effect of crystals: In order to determine whether urate and CPPD crystals behaved like silica and produced hemolysis of human erythrocytes (RBCs), 0.5 ml of the following were added to 0.5 ml of a 3% suspension of RBC in the tumbling system described above, yielding a final concentration per milliliter of: S, 2.5 mg; MSU, 10 mg; CPPD, 10 mg. Tubes were removed at ½, 1¼, 2¼, 3½, 4½, 6½, and 20½ hr, centrifuged at 400 *g* for 10 min at 4°C, and the supernate was read at 541 nm in a Beckman Spinco model 151 spectrocolorimeter. The effect of pH and the concentration of hemoglobin on the observed optical density was determined, and a standard curve was constructed. The results are expressed as a per cent of total hemolysis; a 6% suspension of RBC was diluted with two volumes of distilled water.

Hemolysis in a stationary system: To determine whether the hemolysis was due to mechanical effects, an experiment was performed in the stationary system described above—final concentrations were: RBC, 1.5%, and silica, MSU, and CPPD, each 10 mg/ml. Incubation at 37°C for 14 hr was performed, and supernates were processed as above. The effect of PVPNO (final concentration, 0.05%) was determined in each instance.

Hemolytic effect of crystal surfaces compared with solutions: In this experiment 1 ml of RBCs (final concentration, 1.5%) was placed in dialysis sacs, which in turn were placed in 13 × 100 mm test tubes, each containing 1 ml of buffer. In one-half of the tubes the crystals, MSU, or silica, were placed within the sacs (final concentration, 10 mg per ml); in the remainder, the crystals were placed in the buffer surrounding the sacs. Several tubes containing only RBC within sacs served as controls. All tubes were tumbled for 14 hr at 37°C.

Inhibition of crystal effect on RBC membranes: The effect of the strong hydrogen acceptor, PVPNO, was determined, as it has been described, as an effective inhibitor of the membranolytic effect of silica (10–12). PVPNO and the other substances studied were added to the RBC and to the crystal suspensions and incubated for 30 min at 20°C (the final concentration of each was 0.25 mg/100 ml, except ellagic acid which was 0.00025 mg/100 ml); the RBC and crystal suspensions were then mixed and tumbled for 14 hr at 37°C. The final concentrations of RBC and crystals were as listed in the first paragraph of this section.

Solutions of PVPNO (0.005%) in Hanks' buffer, pH 6.8, gave an absorption maximum at 260 nm in a Beckman DU (Gilford modification) spectrophotometer; 4 ml of this PVPNO solution were added to tubes containing 100 mg of silica, MSU, or CPPD (dry weight), and to 0.4 ml of packed red blood cells. The crystals were suspended by whirling in a vortex shaker, and then tumbled for 60 min, centrifuged at 1300 *g* at 4°C for 15 min, and the OD of the supernatant solution was determined.

The effect of plasma on the crystal–cell interaction was determined. MSU crystals (final concentration, 10 mg/ml) were opsonized in 50% plasma in Hanks' buffer, pH 7.3, at 37°C for 30 min, with frequent mixing using a vortex shaker, centrifuged twice at 1300 *g* for 10 min at 4°C, and resuspended in 2.5% plasma in Hanks' buffer, pH 7.3, each time. The opsonized crystals were added to an equal volume of RBC, producing final concentrations of: RBCs, 2%, silica, 10 mg/ml, and MSU, 5, 3.75, and 2.5 mg/ml. Controls were processed identically with the experimentals but in the absence of plasma. Incubation was performed in 25 ml Erlenmeyer flasks in a Dubnoff metabolic shaker at 37°C for 16 hr. Hemoglobin release into the supernate was determined as before.

RESULTS

Hemolytic Effects of Crystals.—Curves depicting the time course of hemolysis are shown in Fig. 1. All curves show a rapid initial phase followed by a sharp

inflection and a second, more gradual, slope. Silica crystals produced a marked hemolytic effect (curve *a*), confirming previous reports (11–15); MSU crystals exerted similar but less marked effects (curve *b*), whereas CPPD showed a lesser and slower effect (curve *c*). A difference from the hemolysis occurring in the samples of RBC incubated without particles was statistically significant immediately for silica and MSU, and at 2.25 hr for CPPD. Hemolytic effects were linear until 1.25 hr for silica and until 3.5 hr for MSU. Thereafter the rates of hemolysis in samples containing silica and MSU showed a sharp decline, although additional hemolysis occurred throughout the period of incubation. Curve *c*, produced by CPPD, and curve *d*, produced by RBC incubated with-

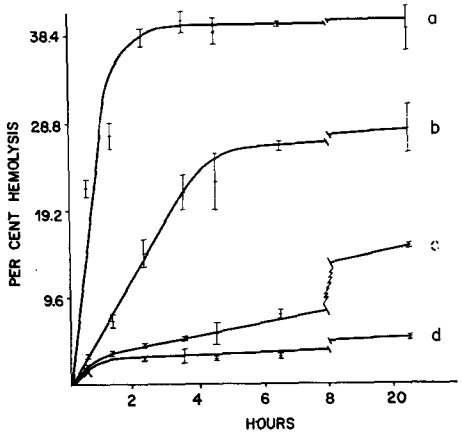

FIG. 1. Curves depicting the time course of hemolysis of a 1.5% suspension of human RBC in Hanks' buffer, pH 6.8, incubated with (*a*) silica, (*b*) MSU, (*c*) CPPD, (*d*) control. See text for discussion. The cross-bars in this and other figures indicate the mean ±SD; $n = 3$.

out particles, also showed a very small, but definite, steep initial slope. The rate of hemolysis produced by CPPD crystals then proceeded throughout the period of incubation, producing a curve which was steeper than the second phase of the silica and MSU curves.

Hemolysis in a Stationary System.—Silica and MSU produced definite hemolysis in a system where any possible mechanical effect was minimized. Silica was far more effective than MSU (Fig. 2); the CPPD effect was not statistically different from control lysis. Data pertaining to PVPNO will be presented below.

Effects of Crystal Surfaces vs. Urate and Silica Solutions.—Human RBC, separated physically from crystals in a tumbling system, showed no hemolysis compared to controls. Hemolysis occurred as expected when crystals and RBC were mixed as before (Fig. 3).

Inhibition of Crystal Effect on RBC Membranes.—Hemolysis of human

RBC was produced by silica, monosodium urate, and calcium pyrophosphate in the ratio 12:6:1.5, respectively, as compared to the control (Fig. 1). PVP and PVPNO markedly suppressed hemolysis in all groups, including the control (Fig. 4, *a* and *b*). This inhibition was identical for both the control and

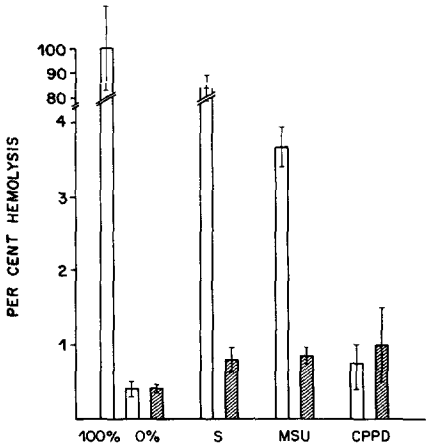

FIG. 2. Hemolytic effect on RBC produced after 14 hr of incubation in a stationary system designed to reduce mechanical effects to a minimum is shown with microcrystalline silica (*S*), *MSU*, *CPPD*, and 100% (total hemolysis in distilled water), and 0% (control hemolysis in the absence of particles). The hatched bars represent identical preparations with polyvinylpyridine-*N*-oxide added in a final concentration of 0.05%. See text for discussion. $n = 3$.

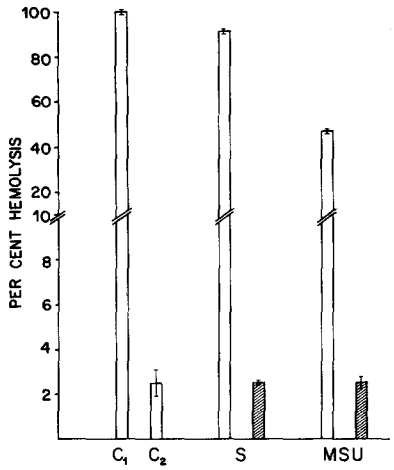

FIG. 3. Physical separation of human RBC and crystals was accomplished by placing the former in dialysis bags. After 14 hr of incubation, no significant hemolysis could be detected as compared to control (hatched bars). Cells not isolated were lysed (open bars). *C:* = 100% hemolysis control, C_2 = RBC in dialysis sac, *S* = silica crystals, *MSU* = monosodium urate.

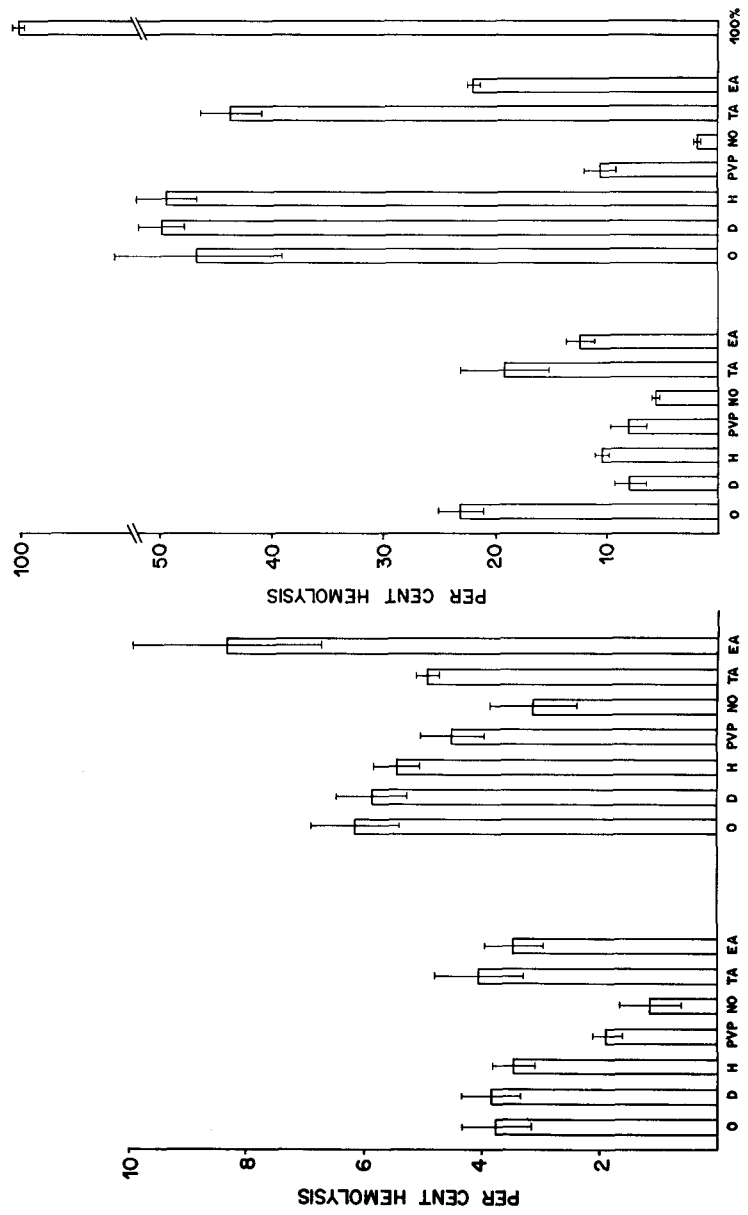

FIG. 4 *a*. Effect of various substances on hemolysis of human RBC produced by no particle control (*C*), and calcium pyrophosphate dihydrate (*CPPD*). See text for discussion. $n = 3$.

FIG. 4 *b*. Effect of various substances on hemolysis of human RBC produced by monosodium urate (*MSU*) and silica (*S*) crystals. See text for discussion.

CPPD groups, indicating that PVPNO had no inhibitory effect on CPPD hemolysis per se. Dextran and heparin inhibited (approximately 60%) the RBC lysis due to urate, but had no effect on silica, CPPD, or control lysis (Fig. 4 *a* and *b*). Ellagic acid inhibited silica and MSU, but not control or

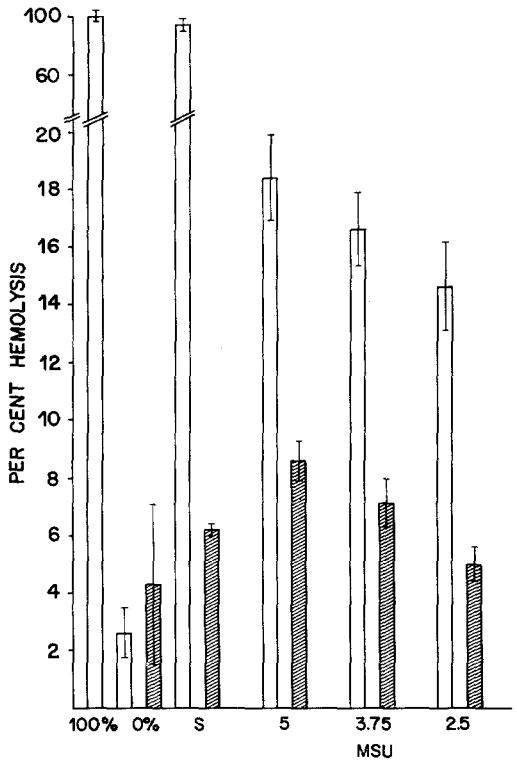

FIG. 5. Inhibitory effect of plasma on MSU (final concentrations of 5, 3.75, and 2.5 mg/ml) and silica-induced hemolysis. Hatched bars represent incubation of erythrocytes with particulates which had been opsonized with intermittent mixing at 37°C for 30 min in 2.5% plasma and resuspended in the same. The open bars represent incubation of erythrocytes with crystals suspended in buffer. See text for discussion.

CPPD, hemolysis. EDTA had no effect on any group, even when divalent cations were omitted from the buffer.

The inhibitory effect of PVPNO was confirmed using the stationary system. MSU and silica hemolysis were reduced markedly, but no inhibition of control or CPPD hemolysis was noted (Fig. 2).

Human plasma inhibited MSU- and silica-induced hemolysis; its effect on CPPD was not studied (Fig. 5). Hemolysis due to MSU was proportional to

the dose of crystals, and the degree of inhibition by plasma was proportional to the degree of hemolysis, i.e., the degree of hemolysis produced by each dose of crystals minus the degree of inhibition by plasma at that dose was nearly identical.

Differential adsorption studies demonstrated that silica and MSU crystals adsorbed PVPNO, and that CPPD crystals and RBC failed to bind this substance (Fig. 6).

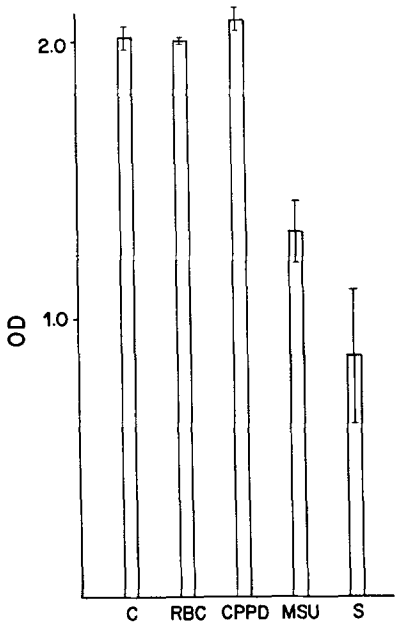

FIG. 6. Absorption of PVPNO by silica, MSU, CPPD, and erythrocytes. See text for discussion.

DISCUSSION

It was first shown in 1904 by Landsteiner and Jagic(13), that colloidal silica agglutinated washed red cells, and that the addition of fresh serum produced lysis. The importance of particle size as a determinant of biological effect was first clearly shown by Harley and Margolis (14); colloidal silica less than 3 nm in diameter blocked the effects of larger colloids but itself had no effect on washed red cells; colloidal silica 3–7 nm in diameter produced agglutination characterized by a prozone, and larger colloids produced hemolysis. The direct hemolytic effect of silica as contrasted to complement-mediated silica hemolysis was later shown to be a function of particle size by Charache and colleagues; large silica colloids produced direct hemolysis on washed red cells, which was

inhibited by protein, whereas small polymers produced hemolysis only after excess silica was removed and fresh serum or whole guinea pig complement was added (15).

An understanding of these phenomena at a molecular level is based on the work of Nash et al., who suggested that polymers which are extremely weak acids (phenolic character), and therefore effective hydrogen donors, are incompatible with cellular membrane integrity; on the other hand, hydrogen acceptors are compatible (11). Biomembranes themselves act as hydrogen acceptors by virtue of their protein and phospholipid components. The materials studied in this paper may be divided on the basis of structural characteristics into two groups: (*a*) the phenolic hydrogen donors—orthosilicic acid ($pK_a \cong 10^{-10}$) (11), MSU ($pK_{a2} = 1.78 \times 10^{-10}$)[2], and (possibly) ellagic acid; and (*b*) the hydrogen acceptors—PVPNO, phospholipid, protein, and CPPD. Hydrogen donors are polarized because the highly electrophilic phenyl radical and oxygen and silicon atoms result in a shift of the electron cloud relative to the hydrogen nucleus, producing a small positive charge. The hydrogen acceptors are characterized by unshared electrons held tightly by electrophilic atoms, giving them a small negative charge. The dative bond in PVPNO makes this molecule a hydrogen acceptor with marked affinity. The small electrostatic charge developed in this manner allows the electropositive hydrogen donors to bind to the electronegative hydrogen acceptors. A model system has shown that the affinity of silica for PVPNO, phospholipid, and protein is in the ratio 100:80:2 (11). The formation of hydrogen bonds between PVPNO and orthosilicic acid has been shown by ultraviolet spectroscopy (18).

Hydrogen bonds are weak compared to covalent bonds, and therefore require donors or acceptors with a number of closely associated subunits (i.e. crystals, colloids, polymers, or biomembranes) in order to produce stable complexes, as exemplified by the DNA double helix. The affinity of silica colloids for biopolymers and biomembranes appears to be a function of particle size, and this affinity appears to determine biological effects; monomers are not bound and have no effect, larger colloids may bind proteins (e.g. complement) and exert blocking effects; still larger colloids will bind red cell-to-red cell; and, finally, at a critical size the silica particle will produce sufficient distortion of the membrane to allow leaks. That spherocytosis precedes hemolysis by larger colloids was first noted by Charache and colleagues (15), and suggests that hemoglobin release is preceded by a phase in which the membrane becomes freely permeable to cations, which results in swelling and, ultimately, in lysis. This mechanism of hemolysis has been documented by vitamin A, although some controversy still exists as to whether or not osmotic stabilizers such as sucrose inhibit lysis (19, 20).

Our observations are consistent with the above considerations. PVPNO binding clearly suggests that silica and urate are hydrogen donors and that red cells and CPPD are not. As expected, urate-produced hemolysis and silica hemolysis were again confirmed, but unexpectedly CPPD produced a small

[2] Sorensen, L. B. Unpublished data.

degree of hemolysis. The lack of PVPNO inhibition of CPPD hemolysis suggests that this effect is not mediated by hydrogen bonds; furthermore, the difficulty encountered in the production of uniformly small CPPD microcrystals suggests that the hemolysis produced by this compound may be related to the impact of hard particles on red cell membranes.

The absence of hemolysis when red cells were separated from crystals by dialysis membranes is consistent with the reported inability of solutions of monomers to produce lysis. That hemolysis was not purely related to crystal impact on red cells is demonstrated by the occurrence of both lysis and inhibition of lysis in a stationary system. The inhibitor studies (Fig. 4 *a* and *b*) clearly showed that PVP and PVPNO inhibited all groups. Viscosity effects may have been responsible for this in the control and CPPD groups (Fig. 4, *a* and *b*, contrasted with Fig. 2), but clearly the inhibition of CPPD hemolysis is identical with that of the controls. EDTA had no inhibitory effect. The polysaccharides, dextran and heparin, inhibited MSU hemolysis but not that of the other groups; whether this represents binding of polysaccharide to urate or to the red cell is not yet known. Inhibition by ellagic acid is probably effected by its binding at the receptor sites on the red cell membrane to which silica and urate bind; this is not an unexpected finding, since ellagic acid contains phenolic hydrogen, is a dimer, and is closely related chemically to tannic acid which is commonly used to bind proteins to red cells. Finally, plasma is capable of inhibiting both silica and urate hemolysis.

A technical problem should be mentioned; it is known that hemolytic rate is a function of specific surface (12), whereas our observations were made on the basis of equal masses. It seems likely that this shortcoming probably minimizes the hemolytic effect of MSU as compared to silica, as the latter was monodispersed, whereas urate crystals tended to aggregate.

The physicochemical reactivity of the red cell membrane resembles that of the lysosomal membrane, based on the similarity of reaction to a number of substances. Agents which produce lysis of both red cells and primary lysosomes are vitamin A (19–22), polyene antibiotics (20, 21), lysolecithin (19, 22), streptolysins S and O (22), and weak acids (23). But the outer membrane of the polymorphonuclear leukocyte appears to be different; it is relatively resistant to lysis by weak acids and streptolysins S and O (24). One might speculate that the incorporation of lysosomal membrane into the inverted outer membrane (phagosome) results in a phagolysosome with physicochemical properties which resemble those of the primary lysosome and red cell. This analogy seems quite clear for silica; in vitro hemolysis and phagolysosome rupture, and fibrogenesis in vivo are blocked by PVPNO (10, 12, 25). Moreover, a recent electron micrographic study by Schumacher and Phelps directly demonstrated that opsonized urate crystals lyse polymorphonuclear leukocyte phagolysosomes, subsequently causing severe damage to the phagocyte (26). Further

support for this concept derives from the observation that phagocytosis of zymosan, heat-killed *Bacillus megaterium*, and diamond dust do not result in phagolysosome rupture (10, 27).

We suggest that in gout, the following sequence of events occurs. Protein-coated crystals are phagocytosed, and subsequent fusion of lysosomes with the phagosome produces a phagolysosome that contains enzymes. These enzymes digest the protein coat on the crystal, allowing hydrogen bond–mediated membranolysis to occur. Phagolysosome lysis is accompanied by the release of hydrolytic enzymes into the cytoplasm, resulting in cellular autolysis, increased permeability of the outer membrane, and release of enzymes into the extracellular medium. This concept does not rule out the possibility of intracellular crystallization of urate as suggested by Bluhm et al. (9).

A previously undescribed effect of ellagic acid, inhibition of urate and silica hemolysis, has been shown. According to a recent report, the formation of foreign-body granulomas is blocked by this agent (28). One wonders if this effect might have been due, at least in part, to stabilization of phagolysosomes.

The striking difference in the effects of MSU and CPPD on red cell membranes is consistent with the observed absence of phagolysosomes surrounding MSU crystals in gouty effusions, and the frequent finding of CPPD crystals inside phagosomes in pseudogout. This difference may be of importance in understanding other dissimilar features which characterize the pathophysiology of inflammation in these two crystal deposition diseases.

SUMMARY

Microcrystals of sodium urate produced direct lysis of erythrocyte membranes, as had been described previously for silica. Calcium pyrophosphate crystals induced modest erythrocyte hemolysis, also, and time-course experiments showed a markedly different reaction curve from those produced by silica and urate. Polyvinylpyridine-*N*-oxide, a strong hydrogen acceptor, was bound from solution to urate and silica, but not to calcium pyrophosphate crystals; this compound effectively blocked urate and silica, but not calcium pyrophosphate or control hemolysis. Dextran and heparin inhibited urate- but not silica-induced hemolysis.

If erythrocyte and lysosome membranes react similarly to these particles, then the absence of phagosomes in gouty synovial fluid leukocytes, and the presence of these structures in pseudogout, may be explained.

The authors are indebted to Pierre Jacques, M.D., of Louvain, Belgium who drew their attention to the previous mark on the membranolytic effect of silica.

BIBLIOGRAPHY

1. McCarty, D. J., and J. L. Hollander. 1961. Identification of urate crystals in gouty synovial fluid. *Ann. Intern. Med.* **54**:452.

2. McCarty, D. J. 1962. Phagocytosis of urate crystals in gouty synovial fluid. *Amer. J. Med. Sci.* **243**:288.
3. McCarty, D. J., N. N. Kohn, and J. S. Faires. 1962. The significance of calcium phosphate crystals in the synovial fluid of arthritic patients: the "pseudogout syndrome." I. Clinical aspects. *Ann. Intern. Med.* **56**:711.
4. FAIRES, J. S., D. J. McCarty. 1962. Acute arthritis in man and dog after intrasynovial injection of sodium urate crystals. *Lancet.* **2**:682.
5. Seegmiller, J. E., R. R. Howell, and S. E. Malawista. 1962. The inflammatory reaction to sodium urate. *J. Amer. Med. Ass.* **180**:469.
6. McCarty, D. J., R. A. Gatter, J. M. Brill, and J. M. Hogan. 1965. Crystal deposition diseases. Sodium urate (gout) and calcium pyrophosphate (chondrocalcinosis, pseudogout). *J. Amer. Med. Ass.* **193**:129.
7. Riddle, J. M., G. B. Bluhm, and M. I. Barnhart. 1967. Ultrastructural studies of leukocytes and urates in gouty arthritis. *Ann. Rheum. Dis.* **26**:389.
8. Bluhm, G. B., J. M. Riddle, and M. J. Barnhart. 1969. Ultrathin study of exudative leukocytes provoked by acicular urate crystals. *Arthritis Rheum.* **12**:657. (Abstr.)
9. Bluhm, G. B., J. M. Riddle, and M. J. Barnhart. 1969. Intracellular formation of urate crystals. *Arthritis Rheum.* **12**:283. (Abstr.)
10. Allison, A. C., J. S. Harington, and M. Birbeck. 1966. An examination of the cytotoxic effects of silica on macrophages. *J. Exp. Med.* **124**:141.
11. Nash, T., A. C. Allison, and J. S. Harington. 1966. Physicochemical properties of silica in relation to its toxicity. *Nature (London).* **210**:259.
12. Stalder, K., and W. Stöber. 1965. Haemolytic activity of different silica modifications and inert dusts. *Nature (London).* **207**:874.
13. Landsteiner, K., and N. Jagic. 1904. Über Analogien der Wirkungen Kolloidaler Kieselsäure mit den Reaktionen der Immunkörper und Verwandter Stoffe. *Wien. Klin. Wochenschr.* **17**:63.
14. Harley, J. D., and J. Margolis. 1961. Hemolytic activity of colloidal silica. *Nature (London).* **189**:1010.
15. Charache, P., C. M. MacLeod, and P. White. 1962. Effects of silicate polymers on erythrocytes in the presence and absence of complement: *J. Gen. Physiol.* **45**:1117.
16. Phelps, P., and D. Stanislaw. 1969. Polymorphonuclear leukocyte motility in vitro. I. Effect of pH, temperature, ethyl alcohol, and caffeine, using a modified Boyden chamber technic. *Arthritis Rheum.* **12**:181.
17. McCarty, D. J., and J. S. Faires. 1963. A comparison of the duration of local anti-inflammatory effect of several adrenocorticosteroid esters—a bioassay technique. *Curr. Ther. Res. Clin. Exp.* **5**:284.
18. Holt, P. F., and E. T. Nasrallah. 1966. Interaction of poly-2-vinylpyridine-1-oxide with silicic acid. *Nature (London).* **211**:878.
19. Dingle, J. T., and J. A. Lucy. 1962. Studies of the mode of action of excess vitamin A. 5. The effect of vitamin A on the stability of the erythrocyte membrane. *Biochem. J.* **84**:611.
20. Kinsky, S. C. 1963. Comparative responses of mammalian erythrocytes and microbial protoplasts to polyene antibiotics and vitamin A. *Arch. Biochem. Biophys.* **102**:180.

21. Weissmann, G., R. Hirschhorn, M. Pras, G. Sessa, and V. A. H. Bevans. 1967. Studies of lysosomes VIII—the effect of polyene antibiotics on lysosomes. *Biochem. Pharmacol.* **16:**1057.
22. Weissmann, G., B. Becher, and L. Thomas. 1964. Studies on lysosomes V—the effects of streptolysins and other hemolytic agents on isolated leukocyte granules. *J. Cell Biol.* **22:**115.
23. Cohn, A. A., and J. G. Hirsch. 1960. The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. *J. Exp. Med.* **112:** 983.
24. Hirsch, J. G., A. W. Bernheimer, and G. Weissmann. 1963. Motion picture study of the toxic action of streptolysins on leukocytes. *J. Exp. Med.* **118:**223.
25. Schlipkoter, H. W., and A. Brockhaus. 1961. Die Hemmung der experimentellen Silikose durch subcutane Verabreichung von Polyvinylpyridin-N-Oxyd. *Klin. Wochenschr.* **39:**1182.
26. Schumacher, H. R., and P. Phelps. 1970. Sequential changes in polymorphonuclear leukocytes after urate crystal phagocytosis: an electron microscopic study. *Arthritis Rheum.* **13:**347. (Abstr.)
27. Hirsch, J. G. 1962. Cinemicrophotographic observations on granule lysis in polymorphonuclear leukocytes during phagocytosis. *J. Exp. Med.* **116:**827.
28. Kellermeyer, R. W., and K. S. Warren. 1970. The role of chemical mediators in the inflammatory response induced by foreign bodies: comparison with the schistosome egg granuloma. *J. Exp. Med.* **131:**21.