

# INTERACTIONS OF C-REACTIVE PROTEIN WITH THE COMPLEMENT SYSTEM

## II. C-Reactive Protein-Mediated Consumption of Complement by Poly-L-Lysine Polymers and Other Polycations\*,‡

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The acute phase reactant, C-reactive protein (CRP),<sup>1</sup> is associated in man and other species with processes of inflammation and tissue destruction (3–5), and may increase as much as 1,000-fold in response to an inflammatory stimulus (6). Human CRP consists of 5 or 6 noncovalently bound subunits of approximately 21,500–23,500 daltons (7), and has an amino acid composition similar to that of immunoglobulin (8). It shows calcium-dependent binding to and/or precipitation with the pneumococcal C-polysaccharide (CPS) and phosphate monoesters, and at low ionic strength reacts with the polyanions heparin, dextran sulfate, and nucleic acids (9–11). CRP shares with antibodies the ability to initiate certain functions of potential significance to host defense and inflammation, including precipitation (12), agglutination (13), opsonization (14), capsular swelling (15), and complement (C) activation (16, 17). Substances which react with CRP to result in activation of the classical C pathway include CPS, lecithin, sphingomyelin, and protamine; this C consumption can be inhibited by phosphorylcholine (16, 17). CRP also combines with T lymphocytes and inhibits certain of their functions (18), and inhibits the aggregation of platelets induced by aggregated human gamma globulin and thrombin (19).

The ability of protamine to initiate C activation in the presence of CRP suggested that polycations generally might represent an additional reactivity, and that the interaction of CRP and polycations with C might represent a major function of this protein. In the present investigation, a variety of synthetic and

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<sup>1</sup> *Abbreviations used in this paper:* C, complement—the nomenclature for C components conforms to that agreed upon under the auspices of the World Health Organization (1968. *Bull. W. H. O.* 39:395); CPS, C-polysaccharide; CRP, C-reactive protein; D<sub>5</sub>W<sup>++</sup>, 5% dextrose; EGTA, ethylene glycol bis(β-aminoethyl ether) N-N'-tetraacetic acid; GGVB<sup>++</sup>, glucose-gelatin-veronal-buffered saline; GVB<sup>++</sup>, gelatin-veronal-buffered saline; 2/3 GVB<sup>++</sup>, 2 parts GVB<sup>++</sup> and 1 part D<sub>5</sub>W<sup>++</sup>; LCP, leukocyte cationic protein; MBP, myelin basic protein; PLL, poly-L-lysine; TLL, tetra-L-lysine; VBS<sup>++</sup>, veronal-buffered saline with optimum divalent cations.

naturally occurring polycations were surveyed for their ability to activate C in the presence of CRP.

### Materials and Methods

**L-Lysine and L-Lysine Polymers.** L-lysine HCl and lysyl-L-lysine HCl were purchased from the Sigma Chemical Co., St. Louis, Mo. Tetra-L-lysine HCl was purchased from Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill. Poly-L-lysine HBr (1,700 daltons) was purchased from ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio. Poly-L-lysine HBr polymers of 2,000, 4,000, 8,000, 23,000, 30,000, 70,000, 178,000, and 400,000 daltons were purchased from the Sigma Chemical Co.

**L-Arginine Polymers.** Poly-L-arginine HCl, 17,000 daltons, and poly-L-arginine sulfate, 65,000 daltons, were purchased from the Sigma Chemical Co. Poly-L-arginine sulfate, 15,000–50,000 daltons, was purchased from Miles Laboratories Inc., Miles Research Div. These polymers were not readily soluble in aqueous solutions, and were used as colloidal suspensions after pH adjustment and sonication.

**Poly-L-Ornithine.** Polymers of L-ornithine (5–20,000 and 165,000 daltons) were obtained as the hydrobromide salts from the Sigma Chemical Co.

**Histone.** Lysine-rich histones (type III) containing 22% lysine and 3.4% arginine, and arginine-rich histones (type VIII) containing 9% lysine and 16% arginine, were purchased from the Sigma Chemical Co.

**Lysozyme.** Hen egg white lysozyme was purchased from the Sigma Chemical Co. Human lysozyme was obtained from Laboratory & Educational Supplies, Inc., Los Angeles, Calif.

**Protamine.** Protamine sulfate was purchased from Calbiochem, Los Angeles, Calif.

**Phosphorylcholine.** Phosphorylcholine was purchased as the calcium salt from Sigma Chemical Co.

**Polybrene® (Hexadimethrine Bromide).** Polybrene was purchased from Aldrich Chemical Co., Milwaukee, Wis.

**Leukocyte Cationic Proteins (LCP).** LCP were obtained from lysosomal granules of rabbit leukocytes (20–22), and suspended in 0.15 M acetate buffer. The protein concentration was determined by a modification of the assay described by Fields (23) using bovine serum albumin as a standard. Polyacrylamide gel electrophoresis (24) showed at least seven distinct cationic components.

**Myelin Basic Proteins (MBP).** Bovine, monkey, guinea pig, and rabbit MBP were prepared and generously provided by Dr. Marian Kies of the National Institutes of Health, Bethesda, Md. (25).

**Human Sera.** Serum samples were obtained and prepared from individuals with elevated levels of CRP as well as from healthy laboratory personnel as described (17).

**Buffers.** Gelatin-veronal-buffered saline (GVB<sup>++</sup>), 5% dextrose (D<sub>5</sub>W<sup>++</sup>), and glucose-GVB<sup>++</sup> (GGVB<sup>++</sup>) with final ionic strengths equivalent to solutions of 0.075 M NaCl and 0.1 M NaCl (2/3 GVB<sup>++</sup>) were prepared with optimum calcium and magnesium chloride (17, 26, 27). EDTA and ethylene glycol bis( $\beta$ -aminoethyl ether)*N*-*N*'-tetraacetic acid (EGTA) were made to 0.01 M in GVB at a final pH of 7.4 (EDTA-GVB and EGTA-GVB, respectively).

**Antisera.** Rabbit antisera against sheep erythrocytes and human CRP were purchased from Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.

**Antrypol (Suramin).** Sodium Suramin U. S. P. was purchased from FBA Pharmaceuticals, Inc. New York.

**C Components.** Fresh frozen guinea pig serum was obtained from Texas Biological Laboratories, Inc., Fort Worth, Texas. C1 (27), C2 (27), C-EDTA (26), hydrazine-C (28), and hydrazine-potassium thiocyanate-treated C (29), were prepared from guinea pig serum. C3, C8, and C9 were purchased from Cordis Laboratories, Miami, Fla.

**Cellular Intermediates.** Sheep erythrocytes (E), antibody-sensitized erythrocytes (EA), EAC<sub>1</sub>, EAC<sub>14</sub>, EAC<sub>4</sub>, and EAC<sub>142</sub> were prepared according to established methods (26, 27, 30) as previously described (31). EAC<sub>1-7</sub> were purchased from Cordis Laboratories. EAC<sub>43</sub> (antrypol) were prepared by a minor modification of the procedure described by Lachmann et al. (29).

**Measurement of CRP.** CRP was measured by radial immunodiffusion as previously described (17).

*Preparation of CRP.* CRP was purified from ascites or pleural fluids by affinity chromatography based on the calcium-dependent binding of CRP to CPS (18). The procedure for this purification will be published in detail.<sup>2</sup>

*Preparation of CRP-Deficient Acute Phase Serum.* Acute phase serum (CRP  $\geq 100 \mu\text{g/ml}$ ) which showed maximum reactivity with poly-L-lysine (PLL) and protamine was centrifuged (10,000 *g*) for 20 min at 4°C, and the lipids were removed. The serum was applied to a CPS-Biogel column<sup>2</sup> (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with veronal-buffered saline with optimum divalent cations (VBS<sup>++</sup>) and  $\text{NaN}_3$  (0.002 M) at 4°C. The serum eluate was monitored by  $A_{280\text{nm}}$  and hemolytic C activity, and was frozen immediately and maintained at -70°C until use.

*Assay for Hemolytic C and C-Component Activities.* The effect of polycations on the hemolytic C activity of normal and acute phase sera was determined by incubation of serum (0.1 ml), buffer (0.35 ml 2/3 GVB<sup>++</sup>), and polycation (0.05 ml) for 60 min at 30°C (17). When CRP was added, the total vol remained 0.5 ml, and the polycation was added last. When agents such as phosphorylcholine, tetra-L-lysine (TLL), or Polybrene were assayed from their ability to inhibit polycation-CRP-induced consumption of C, varying doses of these substances were prereacted with CRP for 30 min at 37°C in a total vol of 0.2 ml; additional buffer, serum, and reactive polycation (e.g., PLL) or CPS were added in that order to bring the final reaction vol to 0.5 ml, and the mixture was preincubated for 60 min at 30°C. Residual hemolytic C and C1, C4, and C2 component activities were determined as previously described (26, 27, 31). Assays for residual hemolytic C3, C5, and C6 activities were performed in tubes according to the methods described by Lachmann et al. (29). C7 was measured by hemolytic radial diffusion also as described by Lachmann et al. (29). C8 and C9 hemolytic activities were assayed according to Vroon et al. (32).

## Results

*Effect of PLL Polymers on Hemolytic C Activity in Acute Phase Serum.* Polymers of L-lysine were found to initiate C consumption selectively in acute phase serum. The reactivity varied markedly according to polymer size. Polymers of 2,000, 4,000, and 8,000 daltons showed maximum reactivity, inducing 40–100% C consumption at concentrations (5–10  $\mu\text{g/ml}$  preincubation mixture) 10-fold lower than those (50–100  $\mu\text{g/ml}$ ) showing anticomplementary activity in normal serum (Fig. 1 and Table I). Polymers of 1,700, 11,000, and 23,000 daltons showed moderate (15–30%) C-consuming activity, while L-lysine, lysyl-L-lysine, TLL, and the largest PLL polymers tested (70,000, 178,000, and 400,000 daltons, respectively) had no (<15%) significant C-consuming activity selectively in acute phase serum.

All the polymers larger than the tetramer, independent of the degree of polymerization, were anticomplementary at concentrations  $>50 \mu\text{g/ml}$  in both normal and acute phase sera. This nonselective anticomplementary effect, which occurred at concentrations 10- to 20-fold higher than those inducing C consumption selectively in acute phase sera, may be analogous to the nonspecific "fixation" of C by PLL in the sera of normal and immunized rabbits reported by Arnon et al. (33), and probably involves direct interaction with certain C components.

*Identification of PLL-Induced C Consumption in Acute Phase Serum as a CRP-Mediated Reaction.* The consumption of C in acute phase serum initiated by PLL, like protamine-initiated C consumption, was found to be mediated via CRP. Absorption of CRP from acute phase serum (250  $\mu\text{g CRP/ml}$ ) by passage through the CPS-Biogel column resulted in loss of ability of PLL (4,000 daltons)

<sup>2</sup> Osmand, A. P., R. F. Mortensen, J. Siegel and H. Gewurz. Interactions of C-reactive protein with the complement system. III. Complement-dependent passive hemolysis initiated by CRP. Manuscript in preparation.

to induce C consumption, and the addition of purified CRP (250  $\mu\text{g/ml}$ ) completely restored this activity (Fig. 2 A); PLL also resulted in C depletion in normal human serum to which purified ascites CRP had been added (Fig. 2 B). In these normal human serum-CRP reaction systems, the amount of PLL optimal for peak CRP-mediated C consumption (2.5  $\mu\text{g/ml}$ ) consistently was less than that required for maximal activity in both acute phase and absorbed-reconstituted acute phase sera (5.0–10.0  $\mu\text{g/ml}$ ). The optimal polycation concentration appeared to be a function of both the individual CRP preparation and the serum in which the activity was assayed. The higher polycation concentration required for optimal activity in acute phase serum may reflect the presence of polyanionic substances known to be elevated during the acute phase. The relationship between polymer size and C-consuming activity observed in acute phase serum also could be identified in normal human serum to which CRP was added. Finally, phosphorylcholine inhibited PLL-initiated consumption of C (Fig. 3) in amounts (e.g.,  $10^{-5}$  M) which had no inhibitory effect on immune complex- or zymosan-induced consumption of C. The CRP-mediated C consumption initiated by PLL, like CRP-mediated C consumption initiated by CPS (16),

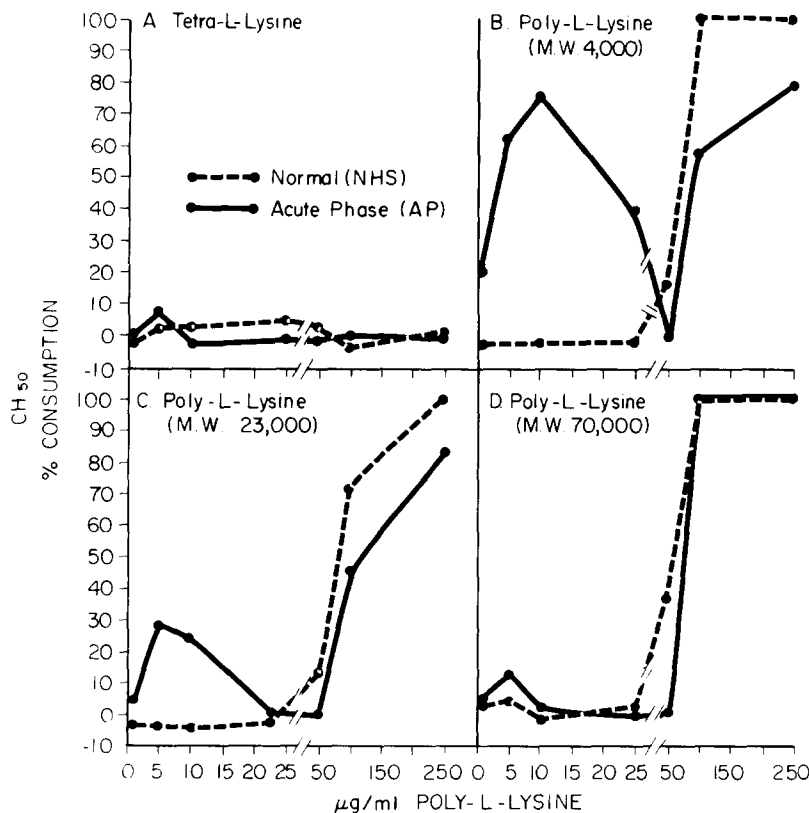


FIG. 1. Effect of representative polymers of L-lysine (PLL) on C activity of normal ( $<3$   $\mu\text{g}$  CRP/ml) and acute phase (75  $\mu\text{g}$  CRP/ml) sera. Residual C activity was measured after 60 min preincubation at 30°C. With small amounts of PLL ( $<50$   $\mu\text{g/ml}$ ; optimal, 10  $\mu\text{g/ml}$ ), C consumption was seen selectively in acute phase sera, and only with polymers of optimal (1,700–23,000 daltons) size. Polymers  $\geq 1,700$  daltons were anti-C in amounts  $>50$   $\mu\text{g/ml}$ .

TABLE I  
*Reactivity of Synthetic and Natural Polycations in CRP-Containing Serum Systems\**

| Polycation                          | Molecular weight | Concentrations tested | Complement consumption |
|-------------------------------------|------------------|-----------------------|------------------------|
|                                     | <i>daltons</i>   | <i>μg/ml</i>          |                        |
| Protamine                           | 4,600            | 1-1,000               | +++                    |
| L-lysine                            | 172              | 1-8,000               | -                      |
| Lysyl-L-lysine                      | 347              | 1-4,000               | -                      |
| Tetra-L-lysine                      | 680              | 1-5,000               | -                      |
| Poly-L-lysine                       | 1,700            | 1-250                 | +                      |
|                                     | 2,000            | 1-500                 | +++                    |
|                                     | 4,000            | 1-500                 | +++                    |
|                                     | 8,000            | 1-500                 | ++                     |
|                                     | 11,000           | 1-500                 | +                      |
|                                     | 23,000           | 1-500                 | +                      |
|                                     | 70,000           | 1-500                 | -                      |
|                                     | 178,000          | 1-500                 | -                      |
|                                     | 400,000          | 1-500                 | -                      |
| Poly-L-ornithine                    | 5,000-20,000     | 0.5-500               | -                      |
|                                     | 165,000          | 0.5-500               | -                      |
| Poly-L-arginine                     | 17,000           | 1-200                 | +                      |
|                                     | 15,000-50,000    | 1-200                 | +                      |
|                                     | 65,000           | 1-200                 | -                      |
| Polybrene®                          | 5,000-10,000     | 1-1,000               | -                      |
| Histone (lysine-rich)               | 21,000-26,000    | 10-1,000              | +++                    |
| Histone (arginine-rich)             | 12,000-16,000    | 10-2,000              | +++                    |
| Myelin basic protein (Bovine)       | 18,400           | 25-1,500              | +++                    |
| Leukocyte cationic protein (rabbit) | 4,000-8,000      | 2.5-100               | ++                     |
| Lysozyme (egg white)                | 11,000           | 5-1,000               | -                      |
| Lysozyme (human)                    | 15,000           | 5-1,000               | -                      |

\* 0.1 ml serum, 0.35 ml buffer (2/3 GVB++), and 0.05 ml polycation were preincubated for 60 min at 37°C, and assayed for residual total hemolytic C activity; +++ represents 60-100%; ++, 30-60%; +, 15-30%; and -, 0-15% complement consumption.

was inhibited by glycerophosphorylcholine and acetylcholine with decreasing efficiency.

*C Component Depletion Profile during CRP-PLL Interactions.* PLL (2,000 or 4,000 daltons) added to normal human serum-CRP mixtures induced efficient depletion of hemolytic C1, C4, and C2 and substantial (50-80%) depletion of C3 and C5 (Fig. 4). C6 activity showed minimal depletion and levels of C7, C8, and C9 were unaffected. Similar depletion profiles were found upon interaction of protamine with CRP.

PLL and CRP alone each consumed C4 at the concentrations used, which were 10- to 20-fold lower than the amounts inducing depletion of total C activity. Higher concentrations of PLL resulted in C1-consuming activity in absence of depletion of C4. The basis of the anti-C4 activity of CRP was not clear; it was dose dependent, occurred also in serum preheated to 56°C for 30 min to inactivate C1, and was not inhibited by phosphorylcholine.

*Effect of Other Synthetic Polycations on C Consumption in CRP-Containing Sera.* Synthetic polycations in addition to PLL were tested for C consumption in both acute phase serum and normal serum to which CRP had been added. Low mol wt polymers of L-arginine (17,000 and 15,000–50,000 daltons) showed intermediate (25%) C consumption which was optimal at 5–10  $\mu\text{g/ml}$  (Table I), an activity equivalent to L-lysine polymers of that size range. A higher mol wt polymer of L-arginine (65,000 daltons), like PLL polymers in this size range, showed no C-consuming activity (Table I). Polymers (5,000–20,000 and 165,000 daltons) of L-ornithine, which differs from L-lysine by a single methylene group, and Polybrene (5,000–10,000 daltons), a synthetic polymer of hexadimethrine bromide, did not consume C in the presence of CRP (Table I).

*Effects of Naturally Occurring Polycations on C Consumption in CRP-Containing Serum.* Several naturally occurring polycations, including histones,

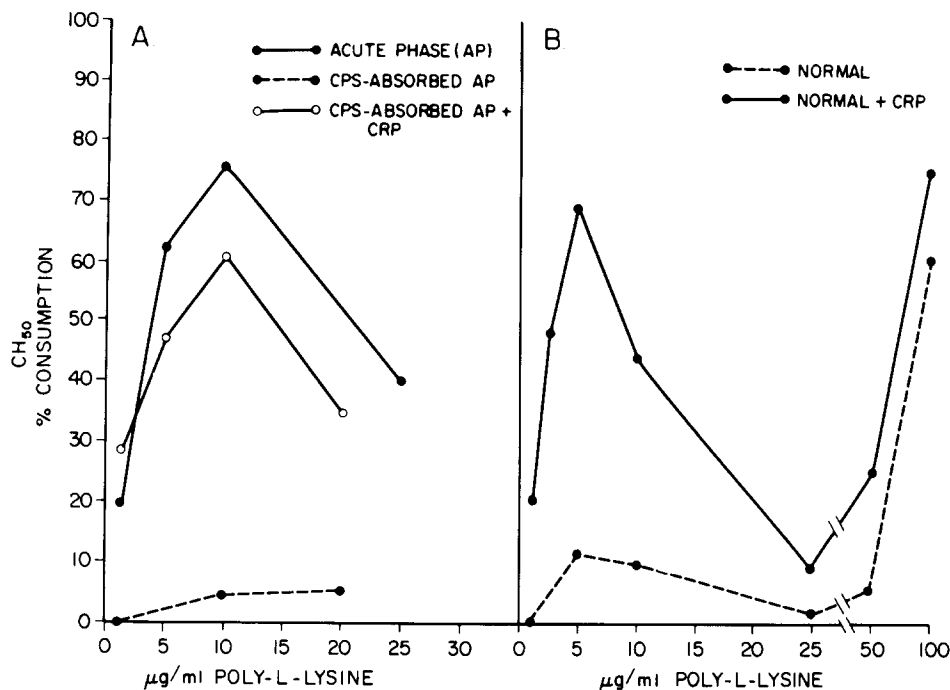


FIG. 2. C consumption by PLL (4,000 daltons) in the presence and absence of CRP (250  $\mu\text{g/ml}$ ). (A) Effect of PLL in acute phase serum, acute phase serum absorbed with CPS by affinity chromatography, and absorbed acute phase serum to which purified CRP had been added; (B) PLL-initiated C consumption in normal human serum in the presence and absence of CRP (250  $\mu\text{g/ml}$ ). C consumption was seen only in the presence of CRP in both normal and acute phase sera.

MBP, and leukocyte cationic proteins, were tested for their ability to consume C in the presence of CRP (Table I and Fig. 5). The histones showed marked (>80%) C-consuming activity when CRP was present, but none in its absence; the concentration of the lysine-rich histone required for 50% consumption was 10-fold lower than that of the arginine-rich histone (50 as compared to 500  $\mu\text{g}/\text{ml}$ ). MBP derived from the cow, monkey, rabbit, and guinea pig likewise had pronounced C-consuming activity in the presence of CRP but none in its absence; 50% C consumption was observed with approximately 100  $\mu\text{g}$  bovine MBP/ml, and maximum reactivity of 80% or greater C consumption was observed with concentrations of 500  $\mu\text{g}/\text{ml}$  or more. LCP showed dose-dependent C-

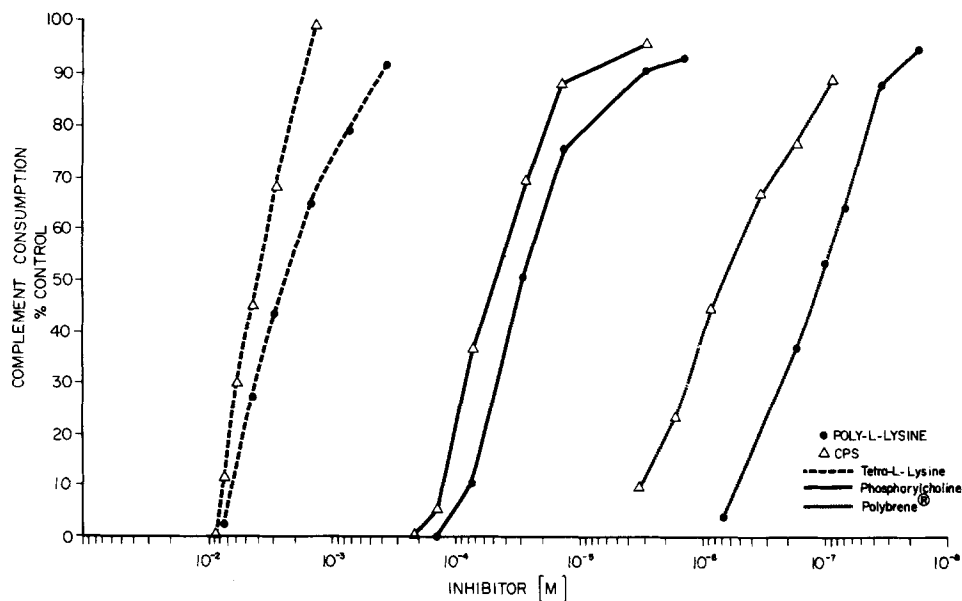


Fig. 3. Comparison of the inhibition of CPS-CRP- and PLL-CRP-induced C consumption by TLL, phosphorylcholine, and Polybrene. The relative inhibitory activity of these agents (Polybrene > phosphorylcholine > TLL) was identical in both systems; larger amounts were required to inhibit C consumption initiated by CPS.

Fig. 4. Effect of PLL (2,000 daltons; 2.5  $\mu\text{g}/\text{ml}$ ) and CRP (250  $\mu\text{g}/\text{ml}$  serum) on C component hemolytic activities in normal human serum. The interaction of PLL and CRP induced marked depletion of C1-C5, but had little or no effect on C6-C9.

consuming activity which was markedly potentiated in the presence of CRP; maximum C-consuming activity (>90%) in the presence of CRP was observed with approximately 300  $\mu\text{g/ml}$  LCP, while only 38% C consumption was observed in its absence. By contrast, lysozymes of both human and egg white origin showed no C-consuming activity.

The C-depletion profiles resulting from the interaction of lysine-rich histones and MBP with CRP in normal serum were essentially identical to that induced by PLL. The C consumption induced by lysine-rich histones and MBP was inhibited by phosphorylcholine; less phosphorylcholine was required for 50% inhibition of C consumption by the lysine-rich histones ( $7.8 \times 10^{-6}$  M) and MBP ( $3 \times 10^{-6}$  M) than by PLL ( $2.4 \times 10^{-5}$  M) or protamine ( $1.8 \times 10^{-5}$  M), indicating that CRP has a lower affinity for the lysine-rich histones and for MBP than it has for PLL or protamine.

*Effect of Inactive Polycations on CRP-Mediated Consumption of C.* TLL in amounts which had no effect on C consumption by immune complexes or zymosan inhibited CRP-polycation-mediated C consumption. Concentrations of

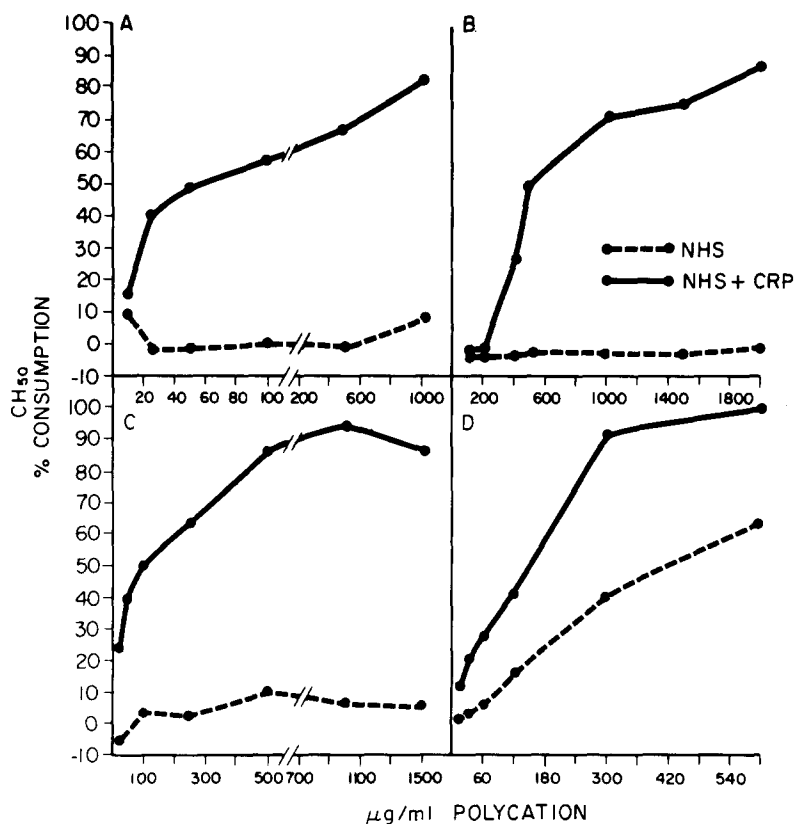


FIG. 5. C consumption by naturally occurring polycations in normal serum to which purified CRP (250  $\mu\text{g/ml}$ ) had been added. (A) Lysine-rich histone, (B) arginine-rich histone, (C) bovine MBP, and (D) rabbit leukocyte cationic protein. The histones and MBP induced significant C consumption selectively in the presence of CRP, which in addition enhanced C consumption by leukocyte cationic protein.



$2.3 \times 10^{-3}$  and  $1.9 \times 10^{-3}$  M resulted in 50% inhibition of PLL- (Fig. 3) and protamine-initiated C consumption, respectively; consumption by histones and MBP was inhibited at concentrations approximately 2- to 4-fold lower. These results are consistent with the relative affinities of CRP for the polycations cited above. The affinity of CRP for TLL was found to be much lower than its affinity for phosphorylcholine, as evidenced by the 100-fold greater relative molar concentration of TLL required for inhibition of C activation. TLL also inhibited C depletion initiated by CPS (Fig. 3); the concentration ( $4 \times 10^{-3}$  M) required was approximately twice that required for inhibition of C consumption by PLL. Twice as much phosphorylcholine also was required to inhibit C consumption induced by CPS ( $4.9 \times 10^{-5}$  M) than that induced by PLL ( $2.4 \times 10^{-5}$  M), indicating that CRP has a greater affinity for CPS than for PLL.

The other polycations which did not induce C consumption in the presence of CRP, including the higher molecular weight PLL (e.g., 400,000 daltons), Polybrene, poly-L-ornithine (5,000–20,000 daltons), and egg white lysozyme also inhibited C consumption by active polycations and CPS; Polybrene was approximately 100-fold more effective than phosphorylcholine in this respect (Fig. 3).

### Discussion

CRP recently was found to be a potent activator of the C system when reacting with CPS and choline phosphatides (16). While investigating polyanion-polycation-induced activation of C, we found that protamine also induced activation of the primary C pathway by interaction with CRP (17). We therefore wondered whether polycations generally and certain naturally occurring cationic proteins specifically could represent significant reactivities for CRP, and in this way lead to initiation or modulation of the inflammatory response.

In the present investigation synthetic and naturally occurring polycations, including homopolymers of L-lysine (1,700–23,000 daltons) and arginine (15,000–50,000 daltons), histones, MBP, and LCP, were found to activate C via CRP. Smaller or larger polymers of L-lysine were inactive, suggesting an optimal size range consistent with the known molecular weights of the histones (34), MBP (35), and LCP (22). The active naturally occurring polycations are rich in lysine and arginine (17–25%) with varying ratios and distributions of these residues; MBP, which has a random distribution of basic residues, showed the weakest reactivity with CRP. Other polycations of similar mol wt, including poly-L-ornithine (5,000–20,000 daltons), Polybrene (5,000–10,000 daltons), and hen egg (14,000 daltons containing 13% lysine and arginine) (36) and human lysozymes were without activity. Thus, while the structural characteristics required for polycations to initiate CRP-mediated C activation are not yet clear, they would seem to include specific basic groups, polycation molecular size, basic residue distribution, tertiary structure, and/or critical intermolecular configuration.

Each of the polycations tested which failed to activate C in the presence of CRP was able to inhibit C activation by the active polycations as well as by CPS. It would seem that reactivity with CRP is a property of polycations generally, and additional alteration or aggregation of CRP is required for initiation of C consumption. Since phosphorylcholine also inhibited C consumption by both polycations and CPS, it appears that these react at an identical or overlapping

combining site or sites on CRP. However, it should be noted that to date binding of polycations to CRP has been inferred only from these indirect studies involving C interactions.

CRP seems to have a lower affinity for polycations than for CPS since higher molar concentrations of both phosphorylcholine and polycation were required to inhibit C consumption by CPS than by the active polycations. Nonetheless, Polybrene was more effective than phosphorylcholine in inhibiting the C-consuming reactions of CRP. Polybrene, a heparin inhibitor, is a synthetic, linear polymeric, quaternary polyamine; its increased capacity for inhibition may be a reflection of its polymeric nature rather than the activity of its monomeric unit, which was calculated to be less than that of phosphorylcholine. This interpretation is supported by the similar efficiency of inhibition of C consumption observed with the inactive 400,000 dalton polymer of L-lysine. Whatever the basis, such polycations may serve a physiological or pharmacological role as a consequence of an ability to modulate reactions of CRP.

Protamine-CRP-mediated C consumption had been reported to involve non-immune activation of the primary C pathway characterized by marked depletion of C1, C4, and C2, with minimal effect on C3-9 (17). However, in the present studies the interactions of CRP with PLL and other active polycations including protamine were shown also to deplete hemolytic C3 and C5. Selective depletion of C1-C5 was seen also during CPS-CRP interactions,<sup>3</sup> supporting earlier observations of significant C1, C4, and C2 depletion, electrophoretic conversion of C3, and distinct but lesser consumption of C3-9 by complexes of CRP and CPS (16). Since recently CRP was found to initiate C-dependent lysis of erythrocytes passively coated with CPS (37), it seems to be a potent activator of the primary C pathway which under suitable conditions can initiate expression of the full biologic potential of the C system. Whether the interaction of CRP with active polycations results in C activation at the level of C1q as it does in CRP-CPS interactions (38), and whether other serum factors are required, is not yet clear.

The naturally occurring polycations with capacity to react with CRP are of interest since these include leukocyte cationic proteins and histones, which are available and may accumulate at sites of injury and tissue destruction. Perhaps reactivity with CRP results in activation of C at inflammatory sites, in modulation of the ability of polycations to initiate mast cell (39) and platelet (40) activation, or in favoring of CRP binding at anionic cell surface sites. The reactivity of CRP with the MBP raises the question of CRP involvement in the pathogenesis of allergic encephalomyelitis, in which MBP has been implicated (41). Further, since the structure of MBP has been extensively defined (35), this protein and its fragments should facilitate definition of the combining site(s) of CRP; such investigations have been initiated with Dr. Marian Kies of the National Institutes of Health, Bethesda, Md.

CRP thus provides an additional route to activation of C-dependent host reactions, supporting the concept that it is a modulator of the inflammatory process. The capacity of CRP to interact with polycations with and without C

<sup>3</sup> Johnson, R., J. Siegel, R. F. Mortensen, A. P. Osmand and H. Gewurz. Interactions of C-reactive protein with the complement system. IV. CPS-initiated consumption of C via CRP and antibody. Manuscript in preparation.

activation introduces a potential for physiologic or pharmacologic manipulation of CRP-initiated C-mediated inflammatory reactions. The interactions between CRP, polycations, and C may be an important component of host responses in health and disease.

### Summary

Cationic homopolymers of poly-L-lysine were found to activate complement (C) via C-reactive protein (CRP) and deplete C3 and C5 as well as early-acting C components. Maximum C consumption was obtained with polymers of 2,000–8,000 daltons; polymers of 1,700, 11,000, and 23,000 daltons were intermediate in reactivity, while L-lysine, lysyl-L-lysine, tetra-L-lysine, and polymers of 70,000–400,000 daltons lacked significant C-consuming activity. Naturally occurring polycations which consumed C in the presence of CRP included myelin basic proteins, cationic proteins of rabbit leukocytes, and both lysine- and arginine-rich histones; poly-L-arginine polymers of 17,000 but not 65,000 daltons also were C-consuming. Polycations without such reactivity included poly-L-ornithine (5,000 and 165,000 daltons), egg white and human lysozymes, and Polybrene. The polycations which failed to induce C consumption via CRP, inhibited its consumption by both active polycations and by C-polysaccharide (CPS). The relative inhibitory capacity of phosphorylcholine and polycations in CPS- and polycations-CRP systems was consistent with the concept that phosphate esters and polycations react at the same or an overlapping combining site. The ability of certain polycations to activate C via CRP increases the potential for initiation of host reactions via C. The capacity of other polycations to inhibit C activation via CRP introduces a potential for physiologic or pharmacologic manipulation. These considerations would seem to expand the potential role of CRP in the initiation and modulation of the inflammatory response.

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