

INTERACTIONS OF C-REACTIVE PROTEIN WITH THE COMPLEMENT SYSTEM

I. PROTAMINE-INDUCED CONSUMPTION OF COMPLEMENT IN ACUTE PHASE SERA*· †

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Recent discoveries have reinforced the concept of the essential role of complement (C)¹ in host defense and inflammation. These include elucidation of the chemistry of the C system and the multiple biologic functions which it mediates, including noncytotoxic release of histamine, chemotactic attraction of neutrophils and other leukocytes, immune adherence and binding to B lymphocytes, promotion of phagocytosis, and reactions of membrane damage (3-5). Although it previously had been thought that only immune complexes could activate the C system, in recent years numerous additional ways of activating single or groups of components have been defined. These include direct or indirect activation of C1s, C3, and C5 by bacterial or mammalian enzymes (6-10), and activation of C3-9 via the alternate or properdin pathway perhaps even independent of antibody (3-5, 11, 12). The primary C pathway also can be activated nonenzymatically by materials other than antigen-antibody complexes: among the active substances are IgG aggregated by heat, carbowax, staphylococcal protein A, or reduced insulin (13-17); certain polyanions such as polyinosinic acid (18), DNA (19), and dextran sulfate (19) as well as complexes of DNA and lysozyme (20, 21), and C-reactive protein (CRP) and C-polysaccharide (22). The ubiquitous nature of these nonimmune activating agents suggests that "nonspecific" modes of C activation have real biologic importance.

We recently have been investigating interactions between certain polycations (e.g., protamine) and polyanions (e.g., heparin) which in normal human serum lead to dramatic activation of the early-acting components of the C system (23, 24). In the course of these experiments we observed that protamine sulfate, even in the absence

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¹ *Abbreviations used in this paper:* C, complement the nomenclature for classical complement components conforms to that agreed upon under the auspices of the World Health Organization [1968. *Bull. W. H. O.* **39**:395]; AHGG, aggregated human gamma globulin; CRP, C-reactive protein; EGTA, ethylene glycol bis(β -aminoethyl ether) *N-N'*-tetraacetic acid; GGVB⁺⁺, glucose plus GVB⁺⁺; GVB⁺⁺, gelatin veronal-buffered saline; RSC, relative salt concentration.

of heparin, was a potent activator of C in the sera of certain patients in the acute phase of inflammation. The studies presented herein initially were directed to defining the basis of this protamine-induced consumption of C in acute phase serum.

It is well known that characteristic changes occur in the blood during acute inflammation. These include an increased erythrocyte sedimentation rate (ESR); the appearance or elevation of serum concentrations of CRP, orosomucoid, haptoglobin, alpha-1 antitrypsin, antichymotrypsin, ceruloplasmin, certain C and clotting components, hexosamines, and mucopolysaccharides such as chondroitin sulfate; and depressed levels of certain other serum proteins including transferrin, alpha-1 and beta lipoproteins, albumin, and prealbumin (25-27). The ability of protamine to induce C consumption selectively in acute phase sera suggested that one or more of these "acute phase reactants" could serve as an activator of the C system.

In this study we have identified the protamine-reactive factor as a non-dialyzable, heat-stable serum fraction which partitions with CRP during its separation and purification, and have reproduced the protamine-induced activation of C in normal serum by addition of purified CRP. This CRP-protamine interaction results in dramatic consumption of C1, C4, and C2. Thus, CRP, when reacting with certain polycations such as protamine sulfate, is a potent activator of the primary C pathway. Interactions between CRP and its substrates, via an effect on the C system, may well have an important role in reactions of host defense, inflammation, and repair.

Materials and Methods

Protamine Sulfate.—Protamine sulfate was purchased from Calbiochem, Los Angeles, Calif.

Barium Sulfate (BaSO₄).—BaSO₄ was purchased from Fisher Scientific Co., Fair Lawn, N. J.

Phosphoryl choline.—Phosphoryl choline was purchased from Sigma Chemical Co., St. Louis, Mo.

Sephadex G-200.—Sephadex G-200 was purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

Agarose.—Seakem agarose was purchased from Bio-Medical Systems, Rockland, Maine.

Ethylene Glycol Bis(β-Aminoethyl Ether) (EGTA).—EGTA was obtained from Sigma Chemical Co.

Ethylenediaminetetraacetic acid (EDTA).—EDTA was purchased from Fisher Scientific Co.

Buffers.—Gelatin-veronal-buffered saline with optimum (0.15 mM) calcium chloride and (1.0 mM) magnesium chloride (GVB⁺⁺) was prepared according to Mayer (28); GGVB⁺⁺ was prepared by mixing equal amounts of GVB⁺⁺ and 5% glucose containing optimum calcium chloride and magnesium chloride (29); EDTA and EGTA were made to 0.01 M in gelatin veronal-buffered saline at a final pH of 7.4 (EDTA-GVB and EGTA-GVB, respectively).

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

C Components.—Fresh frozen guinea pig serum was obtained from Texas Biological Laboratories, Inc., Fort Worth, Texas. C1 (29), C2 (29), and C-EDTA (28) were prepared from guinea pig serum.

Cellular Intermediates.—Sheep erythrocytes (E), antibody sensitized erythrocytes (EA), EAC1, EAC14, and EAC4 were prepared according to established methods (28-30) as previously described (31).

Human Sera.—Serum samples were prepared from individuals with elevated levels of CRP and/or elevated erythrocyte sedimentation rates, as well as from both healthy laboratory personnel and patients without detectable acute phase alterations, by allowing blood samples to stand at room temperature for up to 1 h and at 4°C for up to 3 h before centrifugation; serum samples were kept at -70°C before assay. For certain preparations of CRP, serum samples from several individuals with elevated CRP levels were pooled.

Human Ascites Fluid.—Ascites fluids rich in CRP, made to 0.001 M with sodium azide, were maintained at -20°C until time of purification.

Assay for Hemolytic C and C Component Activities.—The effect of protamine on serum hemolytic C activities was tested by incubation of 0.2 ml of serum, 0.7 ml GVB⁺⁺, and 0.1 ml protamine (usually 25 µg) at 30°C for 60 min. Titrations of residual total hemolytic C activity and classical hemolytic C component activities were performed, and the percent C consumption determined in the usual way (28, 29, 31). In certain experiments, heated acute phase serum, purified CRP, test serum fractions or phosphoryl choline were added to the preincubation mixtures as indicated in the text; the total vol was usually 1.0 ml, and protamine always was added last.

Variations of Ionic Strength and pH.—Ionic strength was varied by mixing appropriate amounts of GVB⁺⁺ and 5% glucose with optimum divalent cations to make solutions of 0.05, 0.075, 0.10, 0.125, and 0.15 M relative salt concentration (RSC). The pH was varied by adding appropriate amounts of NaOH or HCl (0.1 M) to the serum-buffer mixture immediately before assay; the pH was redetermined immediately at the end of the preincubation period. The pH of acute phase sera, in contrast to that of normal sera, always returned to levels of pH 6.5 or higher during the preincubation intervals. Experimental results are expressed in terms of the initial pH.

Measurement of CRP.—CRP was measured by the radial immunodiffusion method of Mancini et al. (32). An optimum dilution of rabbit-anti-CRP (0.15 ml antiserum in a final vol of 3.5 ml) was incorporated into the Seakem agarose mixture prepared in phosphate-buffered saline, pH 7.6, containing 0.01 M EDTA and 0.001 M NaN₃. Test samples were reacted to endpoint by overnight diffusion at 26°C, and recorded in the usual way.

Preparation of CRP.—Purified CRP was prepared from pools of human sera and from individual ascites fluids both by the method of Osmand (33), involving chromatography on DEAE-cellulose and BioGel A, and by a modification of the method of Ganrot and Kindmark (34), involving precipitation with barium sulfate.

Fractionation of Acute Phase Sera.—To define the protamine-reactive factor(s) in acute phase sera, serum pools containing high levels of CRP were precipitated with anhydrous Na₂SO₄ (20% wt/vol), pH 6.0. The mixture was incubated for 3 h at 27°C. The supernate, which contained the protamine-reactive material, was dialyzed against 0.1 M tris, 0.5 M NaCl, 0.01 M EDTA, and 0.01 M NaN₃, pH 7.2, and applied to a Sephadex G-200 column (120 x 5 cm) equilibrated in the same buffer. The column was run at 30 ml/h by reverse flow.

RESULTS

Effect of Protamine on Hemolytic C and C Component Activities in Acute Phase Sera.—We earlier had observed that small amounts of protamine sulfate consumed C when preincubated with the sera of certain individuals with acute inflammatory diseases. We determined the effect of protamine (100 µg/ml in these preliminary experiments) on total hemolytic C activity during incubation with sera of 40 normal individuals and 58 patients who were in the acute phase of inflammation as indicated by erythrocyte sedimentation rates greater than 35 mm/h and CRP levels greater than 50 µg/ml. The results are shown in Fig. 1. 49 of the 58 acute phase sera showed C depletion of at least 50%; the group

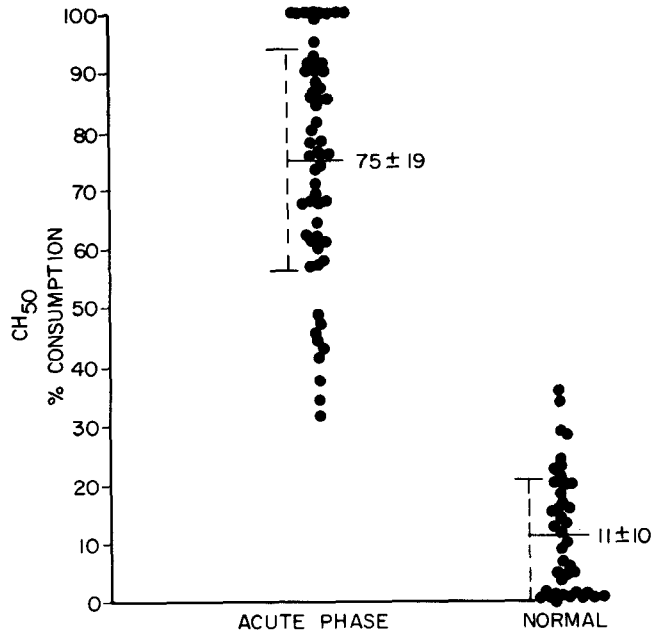


FIG. 1. The effect of protamine on C activity (CH_{50}) in normal and acute phase sera. Sera were designated "normal" on the basis of clinical history, undetectable CRP ($<3 \mu\text{g/ml}$), and an erythrocyte sedimentation rate (ESR) of $<20 \text{ mm/h}$; "acute phase" sera each had CRP levels greater than $50 \mu\text{g/ml}$ and an ESR greater than 35 mm/h . The percent CH_{50} consumed was determined after preincubation of 0.2-ml serum with $100 \mu\text{g}$ protamine in a final vol of 1.0-ml buffered saline (GVB⁺⁺) at 37°C for 60 min. Protamine induced substantial consumption of C selectively in the acute phase sera.

mean value was $75 \pm 19\%$ C consumed. By contrast, none of the normal individuals displayed as much as 50% depletion of C, with a group mean value of only $11 \pm 10\%$ C consumed. Hence, protamine-induced consumption of C regularly could be demonstrated in individuals in the acute phase.

We next determined the classical hemolytic C component depletion profile (Fig. 2). The predominant component depleted was C1, levels of which were consistently reduced by at least 90%; large amounts of C4 and C2 also were consumed. Only minimal depletion of C3-9 was observed, reflecting markedly inefficient fluid phase formation of the C3 convertase. Thus, protamine apparently induced activation of the classical primary pathway at the level of C1 during incubation in acute phase sera.

Conditions Optimal for Protamine-Induced Consumption of C.—Varying doses of protamine sulfate ($5\text{--}1,000 \mu\text{g/ml}$) were compared for their ability to consume C in acute phase and normal sera, respectively (Fig. 3). Substantial C consumption was seen even when preincubations were performed with $10 \mu\text{g}$ protamine/ml, and $25\text{--}100 \mu\text{g}$ protamine/ml induced maximum depletion of C.

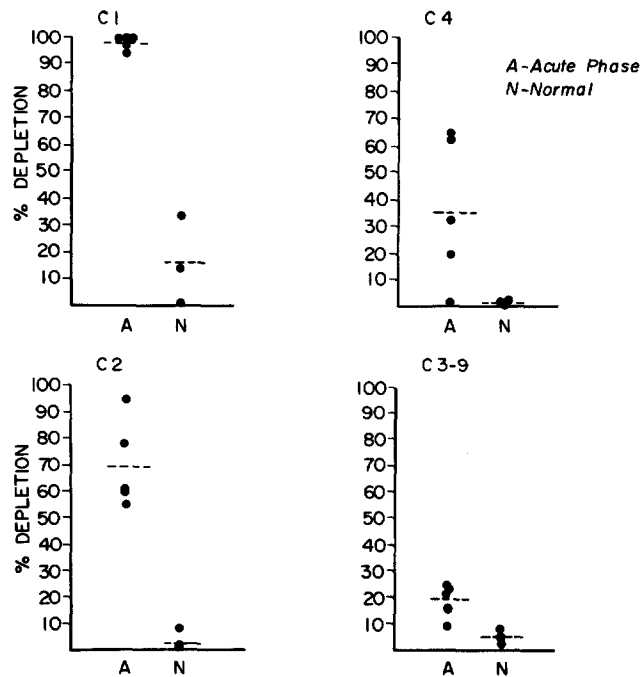


FIG. 2. The effect of preincubation with protamine on classical C component activities in acute phase and normal sera preincubated as in Fig. 1. Acute phase sera, selected from patients who showed total CH_{50} consumption of 70% or greater, were used for this and subsequent experiments unless otherwise noted. Dramatic consumption of C1, C4, and C2 was seen in the acute phase sera.

These amounts of protamine had no effect upon hemolytic C activity in normal sera. Somewhat larger amounts of protamine ($250 \mu\text{g/ml}$) resulted in reduced amounts of C consumption in acute phase sera and were slightly "anticomplementary" in normal sera, while still larger amounts of protamine ($500\text{--}1,000 \mu\text{g/ml}$) were C consuming both in acute phase and normal sera. A concentration of $25 \mu\text{g/ml}$ protamine, 10 times lower than the $250 \mu\text{g/ml}$ dose which was C consuming in normal sera, therefore was used in all subsequent experiments unless otherwise noted.

Protamine-induced consumption of C was markedly dependent upon time and temperature (Fig. 4). When protamine was preincubated in acute phase serum at 37°C , 22°C , 14°C , and 0°C , for varying intervals up to 180 min, hemolytic C activity was most rapidly depleted at 37°C ; 80% C consumption was seen within 15 min and there was no observable residual hemolytic activity after 60 min. C was consumed more slowly (55% at 15 min) at room temperature (22°C) but also was completely depleted after 60 min of preincubation; the reaction proceeded less efficiently, though distinctly, when preincubations were

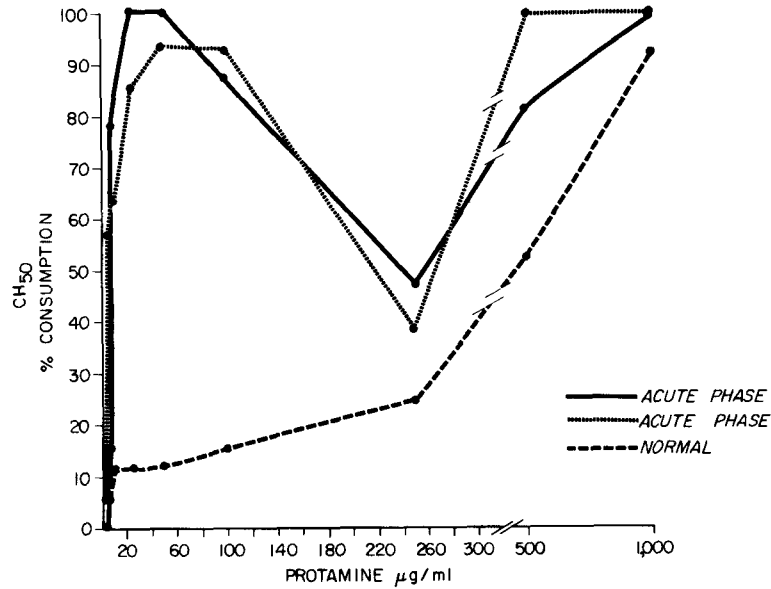


FIG. 3. The effect of preincubation with varying concentrations of protamine on C activity in acute phase and normal sera. The percent CH_{50} consumed was determined after 60 min preincubation at $30^{\circ}C$ in mixtures containing 0.2-ml serum in a final vol of 1.0 ml; the reaction was performed at pH 7.5 and 0.10 M RSC. Low dosages of protamine (10–100 μg) induced significant C consumption preferentially in the acute phase sera. Dosages greater than 250 μg protamine were C consuming in both normal and acute phase sera.

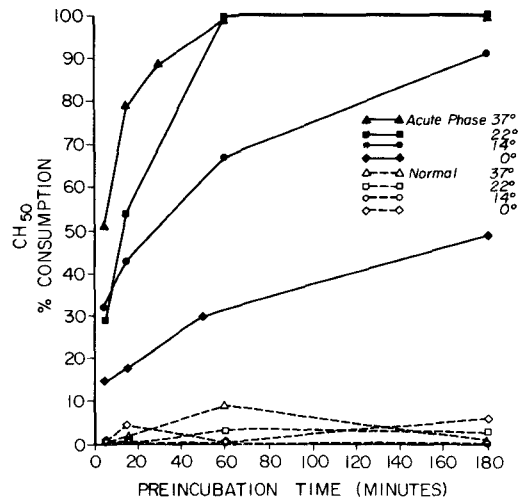


FIG. 4. The effect of preincubation time and temperature on the consumption of C by 25 μg protamine in acute phase serum. Depletion of C, observed at all temperatures, was most rapid and extensive at $37^{\circ}C$ and $30^{\circ}C$.

carried out at 14°C and 0°C, respectively. No consumption of C was observed in the normal control sera under these preincubation conditions. Further experimentation indicated that a 60 min preincubation period at 30°C offered the preferable reaction system, since maximum total hemolytic C activity was consumed with minimal "spontaneous" depletion of C activity in sera incubated in the absence of protamine. This condition was used in all subsequent experiments.

Protamine-induced consumption of C was maximal between 0.05–0.10 M relative salt concentration (RSC). Reduced C consumption was seen during preincubations at higher ionic strengths, while preincubation at lower ionic strengths (including those at 0.05 M RSC) resulted in spontaneous depression of hemolytic C activity. An RSC of 0.10 M proved to be optimal for the preincubation time and temperature selected. The reaction was favored at neutral pH levels with optimum consumption seen between pH 7.5 and 8.0; marked reduction of protamine-induced C consumption was observed at pH 9.5. Protamine did not induce consumption of C in acute phase sera in the presence of either 0.01 M EDTA or 0.01 M EGTA, indicating a requirement for calcium ions. The reaction was not affected by the presence of 0.1% gelatin in the buffer system. The optimal preincubation conditions, adopted for all subsequent experiments, consisted of a final serum concentration of 20% in veronal-buffered NaCl-glucose (RSC 0.10 M; pH 7.5) with gelatin and divalent cations as described (28); all preincubations were carried out for 60 min at 30°C.

Isolation of the Factor in Acute Phase Serum Responsible for Protamine-Induced C Consumption.—The protamine-reactive substance(s) in acute phase sera was found to be heat stable and nondialyzable. Small amounts of acute phase serum (e.g., 25 μ l), heated to 56°C for 30 min and dialyzed for 2 h against multiple changes of buffer in the cold, when added to 100 μ l normal control serum resulted in maximal consumption of C upon preincubation with protamine; heated, dialyzed normal serum, even in much higher amounts, had no such effect.

To begin separation of the active substance(s), a pool of acute phase sera was precipitated by adjusting the pH to 6.0 with 1 N HCl and adding 20% Na₂SO₄; substantial protamine-reactive material was recovered in the supernatant fraction, while none was observed in the redissolved precipitate. All of the recoverable CRP was in the supernatant fraction. The active supernate was applied to a Sephadex G-200 column; the elution profile (Fig. 5) shows that the peak protamine-reactive material eluted between the IgG and albumin markers, along with the elution peak of CRP, and corresponded well with the known CRP mol wt of approximately 120,000–140,000 daltons (33, 35). Our attention therefore was directed to the possible identity of the protamine-reactive factor with CRP. Further purifications were performed according to previously established procedures for isolation of CRP (see below).

Association of the Acute Phase Reactivity with CRP.—The level of CRP in over 90 sera taken from both normal individuals and patients in the acute phase

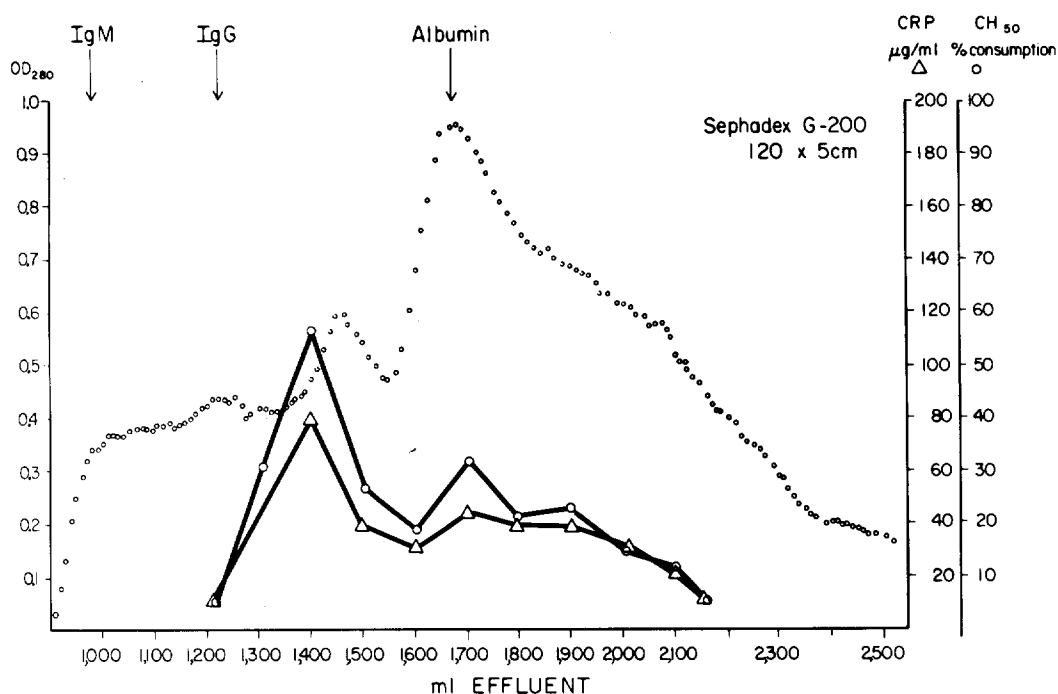


FIG. 5. Partition of the protamine-reactive serum factor(s). A pool of acute phase sera was precipitated with 20% Na_2SO_4 at pH 6.0; the dialyzed supernate (42 ml), which contained 66 $\mu\text{g}/\text{ml}$ CRP, was applied to a 120 x 5 cm Sephadex G-200 column prepared in 0.1 M tris 0.5 M NaCl, 0.01 M EDTA, and 0.01 M NaN_3 , pH 7.2; the protein concentration of the fractions expressed as OD_{280} is shown with open unconnected circles. Fractions were pooled, concentrated 10-fold, dialyzed against buffer, and tested for protamine-induced complement consumption (connected circles) and CRP concentration (triangles). The majority of the protamine-reactive material eluted with the peak of CRP between the IgG and albumin markers.

was compared with the percent C consumed when these sera were preincubated with protamine (Fig. 6). A significant correlation was observed between the level of CRP and the percent of protamine-induced C consumption. An analysis of this association by the arc sine transformation showed a correlation coefficient of 0.78. No serum with less than 50 $\mu\text{g}/\text{ml}$ CRP showed as much as 40% C consumption when preincubated with protamine, while all sera with greater than 200 $\mu\text{g}/\text{ml}$ CRP exhibited C consumption of at least this degree.

The participation of CRP in protamine-induced consumption of C in acute phase sera was substantiated in yet another way. Phosphoryl choline is known to be a major substrate for CRP, and is known to inhibit its reactions with C-polysaccharide as well as with certain other choline-containing compounds with which CRP can interact (36). We tested the ability of small amounts of this substance to inhibit the protamine-induced C consumption during pre-

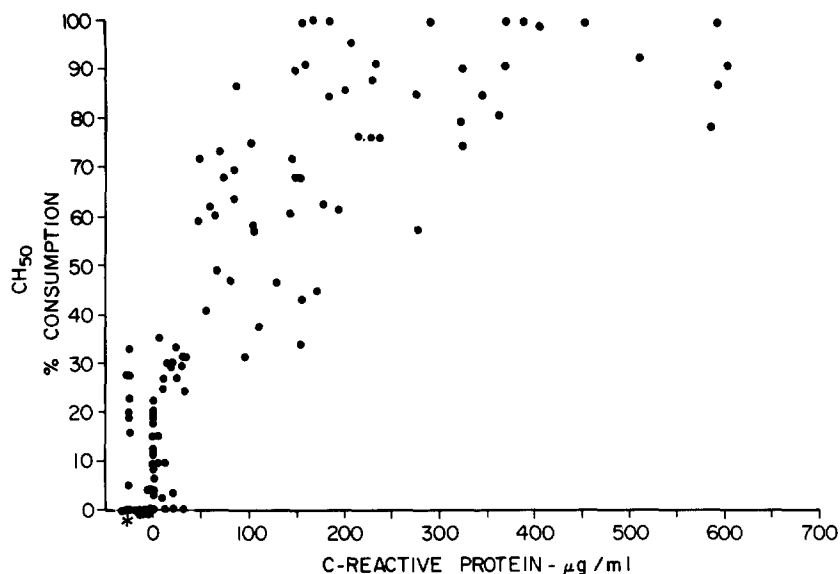


FIG. 6. Correlation of serum CRP levels with protamine-induced consumption of C. The column at the left, denoted by the asterisk, represents normal laboratory personnel; all other points represent randomly selected hospitalized patients. The arc sine transformation gives a correlation coefficient of 0.78.

incubation in acute phase sera (Fig. 7). Substantial inhibition was seen with amounts of phosphoryl choline as low as $5 \mu\text{g/ml}$; $1\text{--}200 \mu\text{g/ml}$ of phosphoryl choline had no inhibitory effect either upon hemolytic C activity, or C consumption by aggregated human gamma globulin (AHGG) or zymosan, in these normal or acute phase sera. Therefore, it appeared most likely that protamine was reacting with CRP to produce the observed consumption of C in acute phase serum.

Addition of Purified CRP to Normal Sera.—The effects of adding purified preparations of serum or ascites fluid CRP to normal sera was investigated. The supernate of CRP-rich serum precipitated in Na_2SO_4 (20% wt/vol, pH 6.0) was adsorbed to BaSO_4 (34) and resolubilized; it contained all the antigenically identifiable CRP and was free of other serum proteins and acute phase reactants as indicated by immunoelectrophoresis and polyacrylamide gel electrophoresis. Ascites CRP was purified by chromatography on DEAE-cellulose and BioGel A (33). These CRP preparations, when added to normal human serum, were found to induce substantial consumption of C when pre-incubated with small amounts of protamine (Fig. 8). $100 \mu\text{g}$ of CRP under these conditions induced consumption of approximately 50% of the available C, whereas $500 \mu\text{g}$ of CRP led to a C consumption of 80–90%. This degree of C consumption was remarkably similar to that produced by heat-inactivated

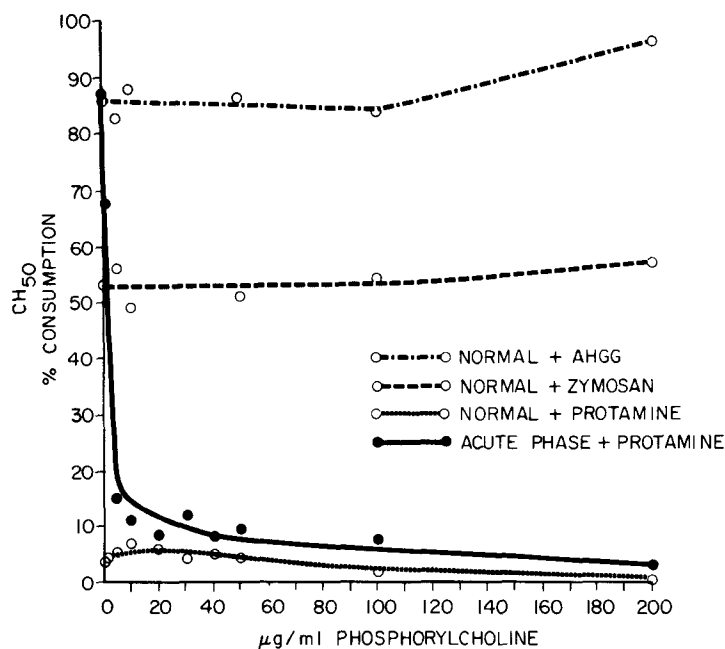


FIG. 7. Effect of phosphoryl choline on protamine-induced consumption of C in acute phase serum. Small amounts of phosphoryl choline ($10 \mu\text{g}/\text{ml}$) completely inhibited protamine-induced consumption of C in acute phase serum, while even much larger amounts (up to $200 \mu\text{g}/\text{ml}$) had no effect upon C consumption induced by either AHGG or zymosan.

acute phase serum with comparable amounts of CRP, and also was equivalent to the average reactivity of the patient groups at corresponding levels of CRP (Fig. 8).

The classical C component depletion profile was determined when protamine was preincubated with these normal serum-CRP mixtures (Fig. 9). Marked protamine-induced consumption of C1, C4, and C2 was observed in reaction mixtures containing either serum or ascites CRP; the CRP preparations themselves showed minimal but distinct C4- and C2-consuming activities, respectively. Only minimal, if any, consumption of C3-9 could be observed. Hence, the C component depletion profile induced by protamine in normal sera to which CRP was added was similar to that seen when the parent acute phase sera were preincubated with protamine. We therefore conclude that protamine induces consumption of C in acute phase serum by interaction with CRP, and further, that this interaction involves activation of the primary C pathway at the level of C1.

DISCUSSION

We had previously observed that interactions between certain polyanions and polycations, like interactions between antigen and antibody, can result in

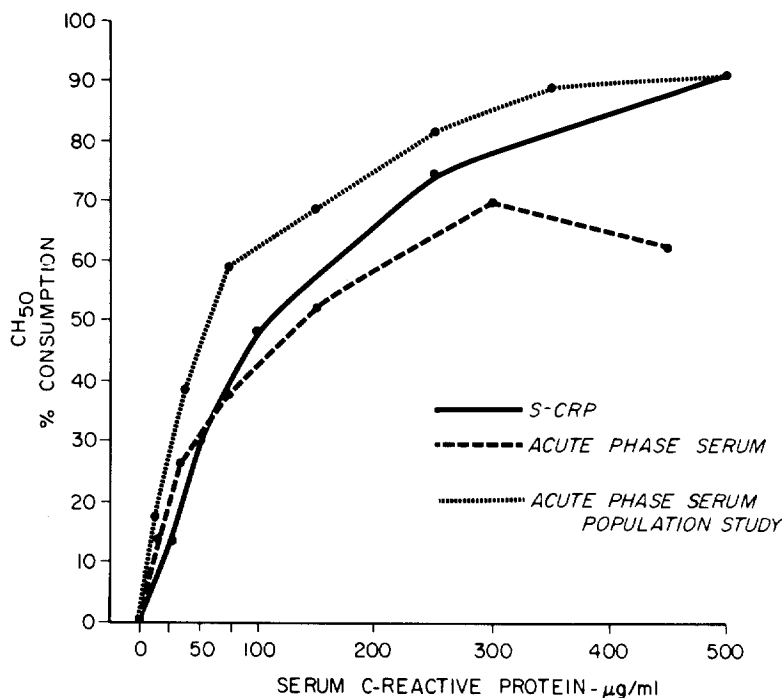


FIG. 8. Effect of protamine on C consumption in normal serum to which heat-inactivated acute phase serum or purified CRP was added. In both instances C consumption in proportion to the amount of CRP added was observed. A quantitatively similar relationship was seen between C consumption and CRP concentration when the patients in the population study cited in Fig. 6 were grouped according to CRP level.

consumption of large amounts of C when they occur in fresh normal serum (23, 24). In the course of these studies we had noted that whereas small amounts of protamine had no effect upon C activity during preincubations in normal serum, the addition of protamine to sera from individuals with acute inflammatory diseases frequently resulted in consumption of large amounts of C. Since it is well known that numerous highly charged substances are found in the blood stream during the "acute phase", including mucopolysaccharides such as chondroitin sulfate, and perhaps even heparin (25), we thought it not unlikely that one of these materials could be reacting with protamine to initiate the consumption of C which we had observed. Indeed, it seemed possible that one or more of the acute phase reactants could in this way serve as an entry point to the potential for inflammation and repair which resides in the C system. We therefore initiated these studies to determine the underlying basis for protamine-induced consumption of C in acute phase sera.

We found, to our surprise, that the predominant acute phase reactant capable of reacting with this polycation to result in consumption of C was CRP. CRP was present in all sera in which protamine-induced consumption of C was ob-

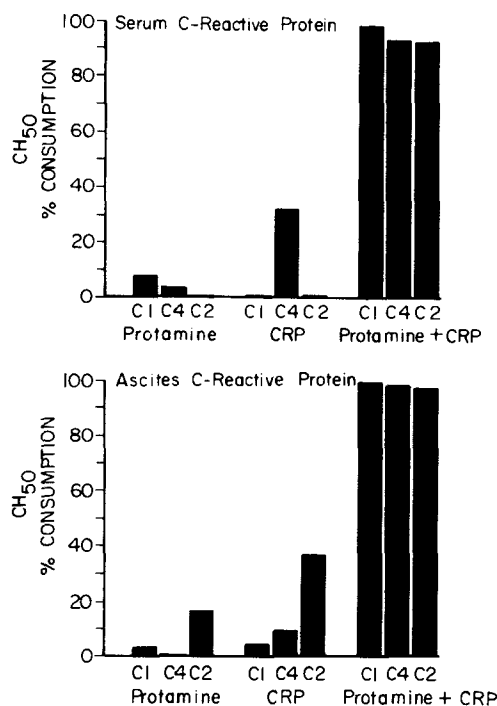


FIG. 9. Effect of protamine on consumption of classical C components in normal serum to which purified serum or ascites CRP was added. Substantial consumption (>90%) of C1, C4, and C2 was observed in the presence of CRP (200 $\mu\text{g}/\text{ml}$).

served, and the degree of C consumption correlated remarkably well ($\rho = 0.78$) with the amount of CRP which was present. When acute phase sera were partitioned, the protamine-reactive material was found only in fractions which contained CRP. Addition of highly purified CRP prepared either from serum or ascites fluid rendered normal serum capable of displaying C depletion upon preincubation with protamine, and to a degree which correlated well with the degree of C consumption observed in acute phase sera containing similar amounts of CRP. Finally, phosphoryl choline, a known substrate of CRP (36), could inhibit protamine-induced consumption of C in acute phase sera as well as in normal sera to which CRP had been added, while it had no effect upon consumption of C by other agents such as AHGG or zymosan. We concluded that the C consumption observed indeed was mediated by CRP.

This reaction appeared to involve an activation of the primary C pathway. Marked consumption of C components C1, C4, and C2, with minimal, if any, consumption of C3-9 was observed both in acute phase sera and in normal sera to which CRP had been added. The reaction was markedly dependent upon time, temperature, and calcium ions, and was inhibited when large amounts of protamine were added. The ionic strength optimum for maximal C consumption

(0.10 M RSC) is optimum for activation of C1, while the optimal pH was 7.5–8.0. Very small amounts of protamine, as little as 5 $\mu\text{g}/\text{ml}$, sufficed to activate the primary C pathway via CRP. Thus, CRP, like IgG and IgM, can react with certain extrinsically derived substances to result in activation of the C system, and it seems not unlikely that this is one of the major functions of CRP (22).

Kaplan and Volanakis had reported that choline phosphatides (e.g., lecithin and sphingomyelin) and C-polysaccharide reacted with CRP to result in consumption of C (22). We were surprised to find that protamine and certain (but not all) other polycations including polymers of poly-L-lysine and lysine-rich and arginine-rich histones also could initiate C consumption via CRP.² The major substrates of CRP are considered to be phosphoryl choline, phosphate esters, and related compounds; substances rich in these groups, such as C-polysaccharide, lecithin, and membrane-derived choline-containing compounds, have been observed to be strongly reactive with CRP (36, 37). Zymosan (38) and certain sulphated polysaccharides including agar (34), and heparin and dextran sulfate under conditions of low ionic strength (37), also have been reported to be reactive with CRP. It appears that polycations represent an additional group of substrates reactive with CRP. Perhaps CRP can react with other polycationic materials such as various leukocyte-derived cationic proteins in the fluid phase, or with cationic substrates exposed on or attached to cell surfaces under given conditions, to result in activation and/or attachment of the C components and initiation or extension of the inflammatory process.

Therefore, CRP can be considered among the agents which can lead to “nonimmune” activation of the first component of C. Nonimmune activation of C1 has been known to occur in at least three distinct ways. Firstly, gamma globulins aggregated or coprecipitated by heating or chemical coupling (13, 14), carbowax (15), staphylococcal protein A (16), or insulin (17) can activate C1 independently of an antigen-antibody interaction, presumably via an interaction between gamma globulin and C1q. A second way involves direct interaction with C1q, and has been seen with agents such as polyinosinic acid (18), and polyanions such as DNA (19) and dextran sulfate (19). Thirdly, plasmin, trypsin, and lysosomal enzymes (6) have been found to activate C1 directly at the level of C1s. It has not yet been established by which of these routes polyanion-polycation complexes such as DNA and lysozyme (20, 21) or heparin and protamine (23, 24) activate C1; since certain polyanions can activate C1q directly, it seems likely that aggregates involving these substances can do so at least as well. We would speculate that the CRP-protamine (polycation) interaction also activates C at the level of C1q. Whatever the underlying mechanism, CRP serves as a route of access to C1 which is selectively available during acute inflammation. Thus, CRP seems to provide to

² Siegel, J., A. P. Osmand, and H. Gewurz. Interactions of C-reactive protein with the complement system. II. CRP-mediated consumption of complement by poly-L-lysine polymers and other polycations. Manuscript in preparation.

its substrates nonimmune entry into the primary C pathway analogous to that which the early-acting factor(s) in the properdin system seem to provide to the alternate C pathway for certain of the substances with which it interacts (11). The biologic significance of this avenue of C1 activation remains to be defined.

Most but not all of the known biologic effects of C are mediated via the biologically potent C3-9 components (3-5); however, the protamine-CRP interaction resulted in minimal, if any, depletion of C3-9. It is not yet clear whether polycations simply cannot support the development of the C3 convertase, or whether larger or membrane-associated polycationic materials are necessary to display this effect. The CRP-mediated activation of C does involve substantial consumption of C1, C4, and C2. Perhaps utilization of these components in itself reflects an important event in inflammation, defense or repair, such as release of a kinin-like peptide (39), deposition of early-acting C components in preparation for immune adherence (40), immunoconglutination (41), viral neutralization (42), binding of B lymphocytes (43, 44), or other functions not yet appreciated. In any case, since CRP, like immunoglobulins, can activate the C system, these studies serve to extend the analogies between CRP and antibody which already include similarity of amino acid composition (33) and ability to induce reactions of precipitation (45), agglutination (46), opsonization (47), mitogenesis (48), and capsular swelling (49). Whether CRP shares with antibody other of its biologic functions, such as the ability to bind to B cells, platelets, and rheumatoid factor, remains to be seen.

A role of CRP as an agent which consumes C raises the possibility that other reactants of the acute phase may also have this capacity. The ubiquitous appearance of the "acute phase response" in vertebrate and subvertebrate species may have an underlying survival advantage in the delivery into the circulation of materials with a capacity to enhance, limit, or modulate inflammatory and/or repair reactions via the C system.

SUMMARY

Protamine sulfate was found to consume large amounts of C selectively during preincubation with sera of individuals in the "acute phase". Marked depletion of C1, C4, and C2 with minimal, if any, depletion of C3-9, was observed. The consumption was time and temperature dependent, occurring most rapidly and extensively at 37°C, 0.10 M relative salt concentration and pH 7.5-8.0; it required calcium ions. It was mediated by a heat-stable nondialyzable factor which separated with C-reactive protein (CRP) during fractionation and purification, correlated with serum CRP levels, and, like other known reactivities of CRP, was inhibited by phosphoryl choline. Preparations of CRP purified either from serum or ascites resulted in consumption of large amounts of C1, C4, and C2 when preincubated with normal serum and protamine. We conclude that CRP is a potent activator of the C system at the level of C1, and that polycat-

ions such as protamine sulfate are substrates of CRP which can bring about this activation. It seems not unlikely that one role of CRP in health and disease involves its ability to interact with the C system.

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Note Added in Proof.—Studies by Kaplan and Volanakis concerning their discovery and elucidation of the interaction between CRP, C-polysaccharide and the C system have recently been published 1974. *J. Immunol.* **112**:2135 and **113**:9.

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