clearly establishes that Woolley (1951) was correct when he proposed that the biosyntheses of riboflavin and of the DBI moiety of vitamin $B_{12}$ were intimately connected. The common precursor to the two structures, however, is 6,7-dimethyl-8-ribityllumazine rather than the 1,2-diamino-4,5-dimethylbenzene originally proposed.

Previously it has been found that the C-2 carbon atom of the DBI moiety of $B_{12}$ is biosynthetically derived from the C-1 position of ribose (Alworth *et al.*, 1969). The present results indicate that all of the carbon atoms of DBI may be derived from 6,7-dimethyl-8-ribityllumazine. The C-6,7-dimethyl portion of the lumazine serves as the precursor of the 4,5-dimethyl-1,2-phenylene unit of DBI, while C-1′ of the ribityl chain of the lumazine is probably the immediate precursor of the C-2 carbon of the DBI.

Since 6,7-dimethyl-$^{14}C$-8-ribityllumazine is an established precursor of specifically labeled riboflavin-$^{14}C$ (Plaut, 1963), the results reported here are consistent with Renz's observation (1970) that randomly labeled riboflavin-$^{14}C$ is converted into the DBI moiety of vitamin $B_{12}$ by broken *P. shermanii* cell preparations. It remains to be definitively established, however, that riboflavin is an obligatory intermediate in the biosynthesis of DBI in *P. shermanii* as proposed by Renz. It seems possible that related but branching pathways could lead to the formation of both riboflavin and DBI directly from the common 6,7-dimethyl-8-ribityllumazine precursor.

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# Specificity for Phosphorylcholine of Six Murine Myeloma Proteins Reactive with *Pneumococcus* C Polysaccharide and β-Lipoprotein*

Myron A. Leon† and N. Martin Young

ABSTRACT: Six murine IgA myeloma proteins, precipitating with the C-polysaccharide of *Pneumococcus*, recognize the same chemical determinant, phosphorylcholine. The myeloma proteins may be divided into three classes on the basis of inhibition of precipitation by the compounds choline, phosphorylcholine, glycerophosphorylcholine, and phosphonocholine.

The characteristic inhibition patterns are: class I, phosphorylcholine = glycerophosphorylcholine > choline > phosphonocholine; class II, phosphorylcholine > glycerophosphorylcholine = phosphonocholine ≫ choline; and class III, phosphorylcholine > glycerophosphorylcholine > phosphonocholine ≫ choline. Although choline is a poor inhibitor of the proteins in classes II and III, the quaternary nitrogen group appears to be a prerequisite for binding of the phosphate ester, since phosphorylethanolamine and glycerophosphate are not inhibitors of precipitation. All six myeloma proteins agglutinate erythrocytes coated with human β-lipoprotein. Representative proteins from each of the three classes precipitated human β-lipoprotein.

Investigations of the structure of antibody binding sites have been limited by the heterogeneity of the antibody population present in a normal antiserum. General properties of the binding site regions of normal heterogeneous antibodies, such as the presence of basic amino acids in or near sites directed toward negatively charged antigens (Freedman *et al.*, 1968),

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and of tyrosine residues near anti-lactosyl (Wofsy *et al.*, 1967) and anti-dinitrophenyl (Wofsy *et al.*, 1962), sites have been reported. The position of such tyrosine residues on the light and heavy chains may be deduced by nearest neighbor analyses (Thorpe and Singer, 1969). More detailed knowledge of the binding site may be expected from studies of those homogeneous myeloma proteins which have been reported to possess antibody-like activity (Metzger *et al.*, 1969). Further, if several myeloma proteins which bind the same chemical determinant are found, analysis would show whether peptide segments of unique sequence are an absolute requirement for such specificity, and in addition would show the range of structural diversity that is possible around such segments in the region of the binding site. Similar data may be obtainable using antibodies of restricted heterogeneity (Krause, 1970; Haber, 1970).

This paper reports on the determinant specificity of six myeloma proteins reacting with the C polysaccharide of *Pneumococcus* (PnC),[1] *viz.*, the IgA proteins secreted by the murine plasmacytomas MOPC 167, MOPC 299, MOPC 603 (Potter and Leon, 1968), S63 and S107 (Cohn *et al.*, 1969), and TEPC 15 (M. Potter, personal communication). The nature of the specific determinant recognized by S63 IgA in its reaction with PnC had previously been sought (Cohn *et al.*, 1969), by testing as inhibitors compounds suggested by compositional data for PnC (Gotschlich and Liu, 1967; Brundish and Baddiley, 1967). Choline inhibited, but at such a high concentration that the inhibition was regarded to be of doubtful significance. A later study of PnC structure (Brundish and Baddiley, 1968) prompted us to examine phosphorylcholine and other choline compounds for inhibition. In this paper we demonstrate that all six murine myeloma proteins show specificity for phosphorylcholine. Furthermore, they react with lipoproteins present in serum presumably by virtue of the presence of phosphorylcholine or related compounds in these lipoproteins.

## Materials and Methods

Sera from BALB/c mice bearing the tumors MOPC 299 and TEPC 15 were given to us by Dr. M. Potter, National Cancer Institute, Bethesda, Md. Tumors MOPC 167, MOPC 603, S63, and S107 are maintained in BALB/c mice in our laboratory and were transplanted from mice bearing the appropriate tumors, provided by Drs. M. Potter and M. Cohn, Salk Institute for Biologic Studies, San Diego, Calif. The work described here was carried out with serum pools stored at −60° until used.

Drs. E. Gotschlich, Rockefeller University, New York, N. Y., and M. Heidelberger, New York University Medical School New York, N. Y., provided PnC. Dr. Lena Lewis, Cleveland Clinic, Cleveland, Ohio, provided human β-lipoprotein prepared from normal plasma. Phosphonocholine (*N,N,N*-trimethylaminoethylphosphonate) was given to us by Drs. J. S. Kittredge, City of Hope National Medical Center, Duarte, Calif., and A. F. Rosenthal, Long Island Jewish Medical Center, New Hyde Park, N. Y. Other chemicals used were the best grade obtainable from commercial sources. The cadmium and calcium ions associated with glycerophosphorylcholine and phosphorylcholine, respectively, were removed by precipitation with phosphate. Dilutions of sera and solutions were made with 0.9% NaCl–0.01 M in phosphate (pH 7.3) (PBS).

*Precipitin Reactions.* Mixtures of 0.2 ml of a dilution of mouse myeloma serum, 0.2 ml of a solution of PnC, and 0.1 ml of PBS were incubated at 20° for 1 hr, then 4° for 18 hr. The precipitates were centrifuged, washed twice with cold 0.9% NaCl in a 4° cold room (Heidelberger and Rebers, 1958), and analyzed for protein by a modified Folin procedure (Heidelberger and MacPherson, 1943a,b). Folin values for PnC–IgA precipitates were converted into protein using as standard a purified murine IgM myeloma protein from plasmacytoma MOPC 104E (Leon *et al.*, 1970). For the quantitative precipitin reaction between MOPC 167 serum and lipoprotein, 0.2-ml aliquots of diluted serum were reacted with different volumes of lipoprotein ($OD^{1\,cm}_{280\,nm}$ 0.083) solution, adding PBS to bring each mixture to a final volume of 0.6 ml. Incubation and analysis were then carried out in the manner described above, except that Folin values were not converted into protein.

For inhibition studies, 0.2 ml of serum dilution was preincubated with 0.1 ml of a solution of the test compound for 30 min at 20°; 0.2 ml of PnC (50 μg/ml) or β-lipoprotein ($OD_{280}$ 0.083) was then added. Incubation and subsequent procedures were carried out as above.

Expreiments with MOPC 299 serum were performed on half the above scale as only small amounts of this serum were available; the tumor line no longer secretes IgA.

*Passive Hemagglutination.* Sheep erythrocytes were sensitized with human β-lipoprotein using concanavalin A as a coupling agent (Leon and Young, 1970). The hemagglutination experiments were carried out in disposable plastic trays (Linbro Chemical Co.) using 0.025 ml scale microtiter equipment (Cooke Engineering Co.). For inhibition experiments, myeloma serum and test substances were preincubated for 30 min at room temperature prior to addition of the sensitized cells. Controls with unsensitized erythrocytes and concanavalin A treated erythrocytes were included where appropriate.

## Results

*Precipitin Reactions.* Representative precipitin curves obtained with dilutions of the six myeloma sera and PnC are shown in Figure 1. The modified Folin assay used does not detect PnC; hence these curves represent the protein precipitated and not the total mass of precipitate. Since IgA is present in murine sera as a mixture of oligomers (7 S, 10 S, etc.) in different proportions (Fahey, 1961), the shape of the curves may vary from serum pool to serum pool. In particular, since the monomer obtained by mild reduction of either MOPC 167 or MOPC 603 IgA gave soluble complexes, rather than specific precipitates with PnC (Potter and Leon, 1968), the proportion of monomer IgA in a given serum sample may affect the precipitin curve. A preponderance of monomer in a serum may inhibit precipitation of the oligomers. Additionally, coprecipitation of β-lipoprotein may occur (*vide infra*). Differences between the six precipitin curves, therefore, should not be regarded as necessarily due to variation in the intrinsic reactivity toward PnC of the combining sites in the six proteins.

Inhibition of precipitation by choline and a number of its derivatives yielded data of the type shown in Figure 2. Concentrations of inhibitors required for 50% inhibition of precipitation, derived from experiments with a single serum pool

[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: PBS, phosphate-buffered saline; PnC, *Pneumococcus* C polysaccharide; CRP, c-reactive protein. Immunoglobulin nomenclature as recommended by the World Health Organization (*Bull. W. H. O. 30*, 447 (1964)).

TABLE I: Inhibition of IgA–PnC Systems by Choline and Derivatives.[a]

| Compound | Class I MOPC 167 | | Class II MOPC 603 | | Class III S63 | | S107 | | MOPC 299 | | TEPC 15 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Choline | 0.00088 | 10 | 0.013 | 870 | 0.023 | 560 | 0.020 | 620 | 0.0072 | 430 | 0.023 | 550 |
| Acetylcholine | 0.00088 | 10 | 0.015 | 1000 | 0.020 | 500 | 0.014 | 440 | 0.0064 | 380 | 0.016 | 380 |
| Phosphorylcholine | 0.000090 | 1 | 0.000015 | 1 | 0.000040 | 1 | 0.000032 | 1 | 0.0000168 | 1 | 0.000042 | 1 |
| L-α-Glycerophosphoryl-choline | 0.000072 | 0.8 | 0.00015 | 10 | 0.00040 | 10 | 0.00028 | 8.8 | 0.000126 | 7.5 | 0.00046 | 11 |
| Phosphonocholine | 0.0030 | 33 | 0.00016 | 11 | 0.0035 | 90 | 0.0030 | 93 | 0.00084 | 50 | 0.0040 | 95 |
| *O*-Phosphorylethanol-amine | Noninhibitory at final molarity of 0.04 M in all systems. | | | | | | | | | | | |
| L-α-Glycerophosphate | | | | | | | | | | | | |
| EDTA | Noninhibitory at final molarity of 0.008 M in all systems. | | | | | | | | | | | |

[a] Values of final molarity for 50% inhibition, left columns, and ratio of molarity of test compound relative to phosphorylcholine, right columns.

for each IgA protein, are shown in Table I. The absolute concentration of an inhibitor required for 50% inhibition may vary from sample to sample of any one IgA serum depending on the relative proportions of the various IgA oligomers in the sample. The ratios of efficiencies of the different haptens in inhibiting reaction of PnC with a given IgA have not varied from sample to sample and these ratios have therefore been included to permit comparisons between the six myeloma sera.

Consideration of the inhibition patterns of the six IgA-PnC systems with the ligands tested (Table I) permits division of these myeloma proteins into three classes. The MOPC 167 system is unique in that glycerophosphorycholine is as good if not slightly better an inhibitor than phosphorylcholine. Strong inhibition by choline and acetylcholine is another feature distinguishing this class from all the others. A second pattern is shown by the MOPC 603 system which is distinguished from all the others in that glycerophosphorylcholine and phosphonocholine are essentially equally potent inhibitors. The TEPC 15, S63, S107, and MOPC 299 systems share a third pattern of reactivity with the limited number of compounds examined, *viz.*, choline << phosphorylcholine > glycerophosphorylcholine > phosphonocholine.

*β-Lipoprotein Reactions.* Since choline and phosphorylcholine are constituents of lipoproteins, we examined the reactivity of the myeloma proteins with lipoproteins. Passive hemagglutination tests using cells sensitized with purified human β-lipoprotein or hyperlipemic sera showed that all six myeloma sera agglutinated such cells (Table II). The agglutination reactions were readily inhibited by choline and its derivatives. Reaction of human β-lipoprotein with a representative myeloma serum from each class gave diffuse precipitin lines in double diffusion tests (Figure 3). MOPC 315, a murine IgA myeloma protein reactive with dinitrophenyllysine (Eisen *et al.*, 1968), included as a control, did not react with either β-lipoprotein or PnC. The quantitative precipitin behavior of MOPC 167 serum with human β-lipoprotein is shown in Figure 4. The precipitin reaction (at equivalence) was 50% inhibited by 0.00012 M acetylcholine.

## Discussion

The behavior of the murine IgA myeloma sera with PnC and β-lipoprotein is that typical of antigen–antibody systems. Thus, the sera precipitate PnC (Figure 1) and agglutinate β-lipoprotein-coated cells (Table II). The reactions are spe-

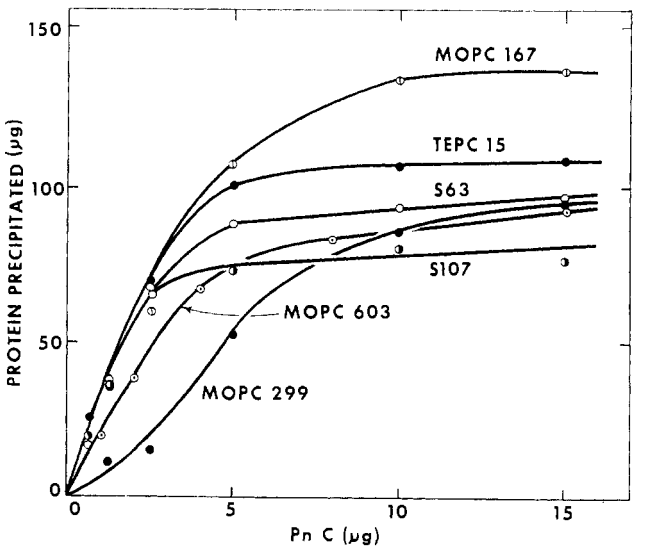


FIGURE 1: Quantitative precipitin reactions between murine myeloma sera and PnC.

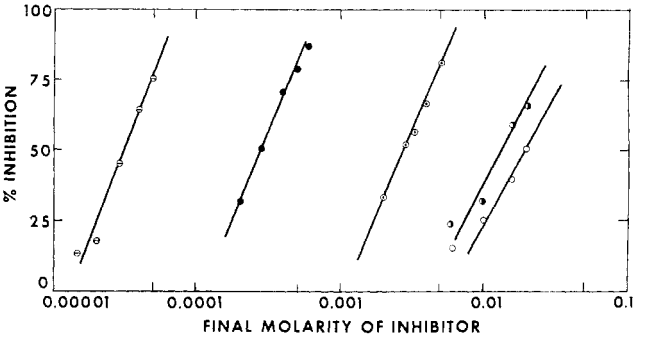


FIGURE 2: Inhibition of the reaction of S107 serum and PnC by choline and derivatives: ⊖ phosphorylcholine; ● glycerophosphorylcholine; ⊙ phosphonocholine; ◑ acetylcholine; ○ choline.

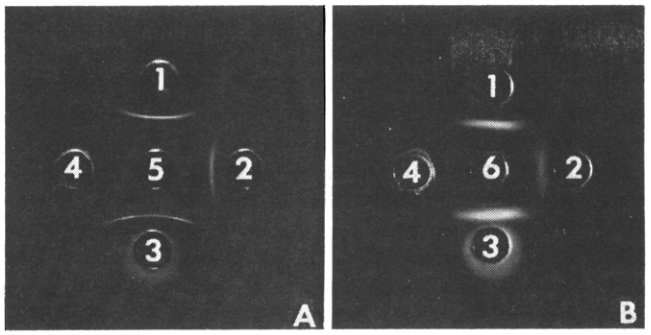


FIGURE 3: Double diffusion in agarose gel between murine myeloma sera and PnC or human β-lipoprotein. Wells 1, MOPC 167 serum; wells 2, MOPC 603 serum; wells 3, TEPC 15 serum; wells 4, MOPC 315 serum; well 5, PnC; well 6, β-lipoprotein.

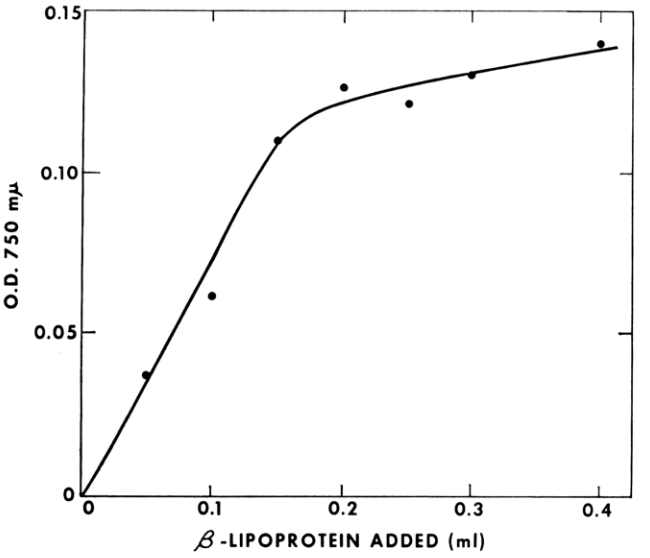


FIGURE 4: Quantitative precipitin reaction between MOPC 167 serum and human β-lipoprotein. The β-lipoprotein was diluted to an $OD_{280}^{1\,cm}$ value of 0.083.

cifically inhibited by small molecules structurally related to PnC (Table I). The binding site has been shown previously to be in the Fab region of the immunoglobulins from MOPC 167, MOPC 603 (Potter and Leon, 1968), and S63 (Cohn *et al.*, 1969). In unpublished experiments conducted with Dr. J. J. Munoz these IgA proteins gave passive cutaneous anaphylaxis in mice when tested with PnC.

In view of recent findings (Parker and Osterland, 1970) caution is warranted in interpreting the binding of ligands by myeloma immunoglobulins even though they may resemble antigen–antibody reactions. These workers have shown that there is a hydrophobic region on Fab pieces of a variety of immunoglobulins, capable of binding ligands such as 8-anilino-1-naphthalenesulfonic acid. The reaction parallels the binding of hapten by specific antibody, with association constants of $8 \times 10^2$–$10^4$ l. mole$^{-1}$. Phosphorylcholine is not hydrophobic and is bound by the IgA myeloma proteins with higher association constants (N. M. Young and M. A. Leon, unpublished observations). Moreover, the identity of idiotypic determinants of the S63 IgA with those of some mouse antibodies in the population induced by PnC (Cohn *et al.*, 1969) is a powerful argument for a close analogy between this IgA and conventional antibodies.

All six IgA proteins recognize the same determinant of PnC, phosphorylcholine. However, the data on relative efficiencies of the inhibitors of precipitation (Table I) show that the proteins may be arranged in three groups. Class I consists of MOPC 167 IgA, class II of MOPC 603 IgA, and class III of the IgA proteins from MOPC 299, S63, S107, and TEPC 15. It is possible that tests with other inhibitors may differentiate the proteins of class III. Indeed MOPC 603 IgA would have been included in this class had not its behavior with phosphonocholine been examined. At present, this classification into three groups is supported by other findings, particularly Potter and Lieberman (1970) using specific mouse antisera to idiotypic determinants on myeloma proteins studied in the present experiments. These workers have shown that MOPC 167 and MOPC 603 each have unique *antigenic* specificities whereas S63, S107, MOPC 299, and TEPC 15 share a third antigenic specificity. Cohn *et al.* (1969) had previously found S63 and S107 to be indistinguishable serologically, though some other differences may exist between them. Peptide maps of MOPC 167 IgA and MOPC 603 IgA light chains show several differences between these two proteins (Potter and Leon, 1968). They also react differently with PnC isolated from Type XIV *Pneumococcus*, MOPC 167 IgA forming soluble complexes whereas MOPC 603 IgA precipitates (Potter and Leon, 1968).

Within the limits imposed by the presence of mixtures of oligomers of IgA in these sera, all six IgA myeloma proteins show similar affinity for phosphorylcholine as measured by inhibiton of precipitation with PnC. Our preliminary studiies of binding of phosphorylcholine by MOPC 167 IgA, MOPC 603 IgA, and S107 IgA, using the method of equilibrium dialysis, yield association constants ranging from $2 \times 10^4$ to $1 \times 10^5$ l. mole$^{-1}$. Studies of a comparable normal system, by equilibrium dialysis, showed that rabbit antibodies to phenoxycholine had association constants between $6.9 \times 10^3$ and $3.2 \times 10^5$ l. mole$^{-1}$ (Marlow *et al.*, 1969).

In comparison to many haptens, phosphorylcholine is relatively small, with a maximum dimension of about 8 Å. The data of Kabat (1966) and Goodman *et al.* (1968) suggest that the combining site of an antibody may accommodate a hapten as large as 34 Å × 12 Å × 7 Å (the size of isomaltohexaose in an extended configuration) though the portion of the hapten contributing most of the binding energy may be much smaller. There would be room within a combining site of this size for specific binding of some additional grouping attached to phosphorylcholine. Thus, as yet untested derivatives of phosphorylcholine may represent closer approxi-

TABLE II: Agglutination of β-Lipoprotein-Coated Erythrocytes by Murine Myeloma Sera.

| Serum | Titer |
|---|---|
| MOPC 167 | 10,000[a] |
| MOPC 603 | 800 |
| MOPC 299 | 320 |
| TEPC 15 | 6,4000[a] |
| S63 | 800 |
| S107 | 800 |

[a] The higher titers in these two instances are reflections of the greater amounts of IgA polymers rather than greater intrinsic reactivities.

mations to the specificities of these proteins, *i.e.*, have a higher association constant than phosphorylcholine.

Despite the fact that addition of a phosphate group to choline, forming phosphorylcholine, produces a more than 400-fold increase in inhibitory power for five of the IgA proteins (Table I), neither phosphorylethanolamine nor glycerophosphate were inhibitory at concentrations up to 0.04 M. The structural features of phosphorylcholine present in phosphorylethanolamine are presumably capable of adopting any conformation phosphorylcholine can, since it is the latter that has a more restricted range of conformations available to it (Zull and Hopfinger, 1969). The lack of reactivity of phosphorylethanolamine is therefore unlikely to be a result of conformational restrictions in the ligand. The data (Table I) emphasize the importance of the quaternary nitrogen when a good inhibitor, such as phosphorylcholine, is bound by these five myeloma proteins, even though choline itself is a very poor inhibitor.

An explanation for these observations might be some form of concerted mechanism in which binding of the quaternary nitrogen leads to production of the phosphate binding site. Traditionally, hapten and antibody have been considered in terms of preexistent structures interacting with each other (the lock and key analogy of Ehrlich). Enzymes, however, are now regarded as capable of altering in conformation when binding small molecules (Koshland and Neet, 1968). Direct evidence of conformation change has been provided by an X-ray crystallographic study of carboxypeptidase (Ludwig *et al.*, 1967), which showed that the side chain of a tyrosine residue in an enzyme–substrate complex was 15 Å away from its position in the free enzyme. An analogous conformational change in the IgA could explain the role of the choline moiety in promoting phosphate binding, *i.e.*, initial binding of the quaternary nitrogen of phosphorylcholine might alter the conformation of the IgA to bring about binding of the phosphate group.

However achieved, the cooperative effect of the choline moiety permits strong binding of specific ligand containing phosphate, without the immunoglobulin being able to bind superficially similar phosphate esters such as nucleotides. Thus, determinant specificity is achieved without reactivity toward important self-constituents. Conformation effects may not play a significant role in binding of all antigens by antibody, but may occur when fine discrimination between important self-constituents and the antigenic determinant is necessary. Thus, the lack of convincing physicochemical evidence of conformational changes in the Fab region on reaction with hapten may be due to restriction of such studies to the dinitrophenyl hapten (Warner and Schumaker, 1970; Cathou and Haber, 1967; Froese, 1968).

The PnC was an efficient precipitant in all six systems, as shown by the approximately 1:10 weight ratios, at equivalence, of PnC added to myeloma protein precipitated. This 1:10 ratio may be contrasted with the 1:1 ratio observed with dextran B1355S1,3 and the IgM murine myeloma MOPC 104E (Leon *et al.*, 1970) and suggests that many repeating units of PnC are available for reaction. That these repeating units have side chains of phosphorylcholine is indicated by both the inhibition data presented in this paper and the structural data for PnC (Brundish and Baddiley, 1968). These side chains may also be responsible for the reaction of PnC with human C-reactive protein (CRP). Phosphate esters inhibit the CRP–PnC system (Gotschlich and Edelman, 1967), and in preliminary experiments with Dr. M. Kaplan we have found that phosphorylcholine is a good inhibitor of the CRP–PnC reaction while acetylcholine was noninhibitory. The six IgA proteins differ from CRP, however, in that the latter's reactivity with PnC is abolished by EDTA (Gotschlich and Edelman, 1967). This reagent was not inhibitory at 0.008 M in any of the IgA systems (Table I).

Our finding of reaction of myeloma proteins with lipoproteins is not unique. Several instances of human myeloma proteins reacting with serum lipoproteins have also been reported (for example, Lewis and Page, 1965; Beaumont *et al.*, 1967; Killander *et al.*, 1967; Spikes *et al.*, 1968). Two of these myeloma proteins react with derivatives of phosphorylcholine, the passive hemagglutination reaction of one, an IgA, being inhibited by lysolecithin (Beaumont and Baudet, 1968) and the other, an IgM, fixing complement when reacted with lecithin (Killander *et al.*, 1967). Such human and murine myeloma proteins may thus be capable of forming complexes with phospholipids and lipoproteins of the cells that synthesize them. In an attempt to mimic this reaction *in vitro*, small amounts of MOPC 167, MOPC 603, and S107 were added to lymphocyte cultures. Stimulation of the lymphocytes resulted, as shown by incorporation of tritiated thymidine (Leon and Takahashi, 1970), demonstrating that these proteins are indeed capable of reaction with cell membranes.

The most significant experimental finding deriving from these studies is that a group of homogeneous immunoglobulins of the IgA class has specificity for the same determinant, phosphorylcholine. Provided that these myeloma proteins are analogous to the immunoglobulin products of individual clones in a conventional heterogeneous antibody response (whether or not they are the products of clones stimulated by one antigen) study of the binding site and animo acid sequence of these proteins should yield valuable information as to the ways in which similar antibody specificities can be generated.

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# Affinity Labeling of Antibodies to the *p*-Azophenyltrimethylammonium Hapten, and a Comparison of Affinity-Labeled Antibodies of Two Different Specificities*

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ABSTRACT: The active sites of rabbit and mouse antibodies to the *p*-azophenyltrimethylammonium (TMA) antigenic determinant were affinity labeled with the reagent, *p*-(trimethylammonium)benzenediazonium difluoroborate. This reagent specifically labels tyrosyl residues on both the heavy and light polypeptide chains, and by the criteria of affinity labeling these residues and their respective polypeptide chains therefore contribute to the formation of the active sites. The marked similarity of the labeling of tyrosyl residues in the active sites of antibodies directed to the positively charged TMA group, to the neutral 2,4-dinitrophenyl (DNP) group, and to the negatively charged *p*-azobenzenearsonate group, in several species, suggests that a common structural relationship exists among these active sites independent of their specificities and species of origin. Support for this suggestion has been obtained from studies of tryptic peptide fragments prepared from both the heavy and light chains of rabbit and mouse anti-DNP and anti-TMA antibodies. These peptides have been fractionated on calibrated columns of Sephadex in 8 M urea, and the distribution of the radioactive affinity label among the different size peptides has been determined. For a given type of chain and species of origin, the distribution of affinity label among the peptides was characteristic and closely similar for the two antibody specificities. These results suggest that the same local region of the antibody molecule is utilized to form active sites of different specificities.

The method of affinity labeling[1] provides a technique for selectively labeling contact residues within enzyme and antibody (Ab)[2] active sites (Wofsy *et al.*, 1962; Singer, 1967). This method has been applied to a number of Ab with different specificities (Singer and Doolittle, 1966; Singer *et al.*, 1967; Wofsy *et al.*, 1967, 1970). Up to the present time, the

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[1] Similar labeling methods were independently developed by several investigators (Baker *et al.*, 1961; Lawson and Schramm, 1962; Schoellmann and Shaw, 1963; Wofsy *et al.*, 1962). The subject of active site labeling has recently been reviewed (Baker, 1967; Singer, 1967).

[2] Abbreviations used are: Ab, antibodies; anti-DNP, anti-2,4-dinitrophenyl; anti-TMA, anti-*p*-azophenyltrimethylammonium; BSA, bovine serum albumin; H, heavy and L, light polypeptide chains; PTBDF, *p*-(trimethylammonium)benzenediazonium difluoroborate; MNBDF, *m*-nitrobenzenediazonium fluoroborate.