Vi antigen was there evidence of a nonuronic acid component. The conditions used to degrade the Vi antigen were so mild that it is unreasonable to assume that any labile substances could have been destroyed in the process. Therefore, we conclude that the native Vi antigen contains only N- and O-acetylated galacturonic acid residues.

Acknowledgments

We wish to thank Drs. L. M. Corwin and R. E. Whiteside for many helpful discussions during the course of these studies.

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Immunochemical Studies of the Reaction between a Mouse Myeloma Macroglobulin and Dextrans*

M. A. Leon, N. M. Young, and K. R. McIntire

ABSTRACT: The immunoglobulin (IgM) secreted by the mouse plasmacytoma MOPC 104E, reacts with a number of dextrans in the manner characteristic of antibody–antigen reactions, *viz.*, precipitation of soluble dextran, inhibition of precipitation by small oligosaccharides, agglutination of dextran-coated erythrocytes, and fixation of complement. Fixation is more efficient at 37° for 1 hr than at 4° for 18 hr. The order of efficiency for inhibition of dextran–IgM reactions by α-linked glucose oligosaccharides is 1,3 ≫ 1,6 > 1,2 > 1,4. Nigerotetraose and nigeropentaose are the best inhibitors of the nigerodextrin series.

Affinity between IgM and various dextrans is determined by measuring the amount of nigerose required to inhibit precipitation of a constant amount of IgM. This affinity is directly proportional to activity of the dextran in microcomplement fixation.

Myeloma proteins have proved valuable in studies of the primary amino acid sequence, subunit structure, and molecular morphology of immunoglobulin molecules. The myeloma proteins which display antibody activity toward known antigens are of great potential value in studying antibody functions and the nature of the binding site.

The first reported mouse myeloma with antibody activity showed specificity for the C-polysaccharide of the pneumococcus (Cohn, 1967). Shortly thereafter activity of other mouse myeloma proteins was demonstrated for the ligands dinitrophenol and trinitrophenol (Eisen *et al.*, 1968) and 5-AcU, purine, and AMP (Schubert *et al.*, 1968).

We undertook (with Dr. M. Potter) a program of screening murine myeloma sera for reactivity with polysaccharides,

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TABLE I: Properties and Reactivities of Dextrans.

Dextran	Linkage Analysis[a] 1,6 %	1,4 + 1,2 %	1,3 %	Passive Hemagglutination Inhibition[b] B1254L Cells (μg/ml)	B1355S1,3 Cells (μg/ml)	Pptn of IgM[c] (%)	50% Inhibn of Pptn by Nigerose[d] (μmoles)	50% Complement Fixation[e] (μg)
B1355S1,3	57	8	35	1.6	32	100	2.8	0.0035
B742	67	21	12	6.2	1000	100	0.82	0.015
B1398	70	11	19	12.5	nr[g] at 500	100	0.35	0.05
B1299S	50	50	0	25	nr at 500	100	0.13	5.0[f]
B1254L	69	31	0	25	nr at 500	100	0.115	nr[g]
B512	95	5	0	nr[g] at 100	nr at 500	0		nr
B640	95	5	0	nr at 100	nr at 500	0		nr
B1142	63	8	29	nr at 100		6		25.0
B1141	79	3	18	100	nr at 500	85		2.0
B1192	78	4	18	nr at 100		11		10.0
B742L	81	19	0	nr at 100		6		
B1396	81	19	0			100		nr
B1526	79	21	0			6		nr
B1383	84	16	0			33		nr
B1399	65	35	0			80		nr

[a] Data taken from Jeanes *et al.* (1954). [b] Lowest concentration of dextran solution added giving complete inhibition of agglutination. [c] Percentage of IgM precipitated from 104E serum (0.2 ml containing approximately 170 μg of IgM) by dextran (0.2 ml containing 800 μg of dextran) assuming B1355S1,3 precipitated 100% of the IgM. [d] Nigerose required for 50% inhibition of precipitation using amount of antigen required to give slight antigen excess with diluted serum containing approximately 400 μg of IgM, in final volume of 0.8 ml. [e] Amount of dextran giving 50% fixation with diluted serum containing approximately 10 μg of MOPC 104E IgM. Dextrans which were not reactive were tested with up to 25 μg. [f] The strain producing B1299S also yields a fraction, B1299L, containing 6% α-1,3 linkages. [g] nr = not reactive.

using double diffusion in gel (Ouchterlony technique) to detect reactions. Polysaccharides were chosen because their mono- and oligosaccharide constituents and derivatives frequently are available. These compounds are useful for experimental investigation of the binding sites of any reactive myeloma protein by techniques such as inhibition of precipitation, equilibrium dialysis, and affinity labeling. Seven mouse myelomas were found to produce immunoglobulins which react with polysaccharides (Potter and Leon, 1968). Six of these seven "antibodies" are of the IgA class as are the other mouse myeloma proteins with known antibody activity noted above. The seventh reactive protein is a macroglobulin, MOPC 104E, for which physicochemical data and antigenic analysis have already been reported (McIntire *et al.*, 1965). This paper presents experimental data on the reaction of MOPC 104E IgM with dextrans and a description of the specificity of the reaction.

Materials and Methods

Sera were obtained from BALB/c mice bearing the MOPC 104E tumor and were stored at −60°. The IgM was purified as described by McIntire *et al.* (1965) and had an $E_{280}^{1\%}$ value of 16.0. Subunits (IgMs) were prepared with dithiothreitol according to Miller and Metzger (1965).

Dextrans were obtained through the courtesy of Dr. Allene Jeanes. Characteristics of these dextrans are presented in Table I. The α-1,3-linked series of glucose oligosaccharides (nigerodextrins) was provided by Dr. I. R. Johnston (Johnston, 1965), and the α-1,2-linked disaccharide, kojibiose, by Drs. I. Goldstein and E. A. Kabat. Other oligosaccharides were commercial products (Koch-Light Ltd. and Pierce Chemical Co.). Traces of higher oligosaccharides in the isomaltose and maltose were removed by chromatography on Sephadex G-15. The Sephadex G-15 column was exhaustively washed with water to remove traces of soluble dextran before use.

Buffers employed in these studies were a sodium chloride–phosphate buffer, pH 7.2 (0.15 M in sodium chloride–0.01 M in phosphate),[1] and a barbital buffer (pH 7.4) containing 5×10^{-4} M Mg^{2+} and 1.5×10^{-4} M Ca^{2+} (Mayer *et al.*, 1946) with 8 ml of 6% gelatin added per l. Immunoelectrophoresis and double diffusion in agar were carried out as previously described (Potter, 1967).

Passive hemagglutination inhibition was used for preliminary investigation of the reaction of MOPC 104E IgM with dextrans and oligosaccharides. Sheep erythrocytes were sensitized with palmitoyl dextrans prepared by a modification of the method of Tsumita and Ohashi (1964). Optimal conditions for sensitization with dextran B1355S1,3 were as follows. To 40 mg of dextran suspended in 4 ml of pyridine was added 25 μl of palmitoyl chloride. The suspension was mixed by

[1] Abbreviations used are: PBS, phosphate-buffered saline; GBB, barbital buffer containing gelatin. Immunoglobulin nomenclature as recommended by the World Health Organization (*Bull. World Health Org. 30*, 447 (1964)).

rotation for 2 days at room temperature, then poured into 20 ml of isopropyl alcohol. The precipitate was centrifuged, washed twice with isopropyl alcohol, and dried with ether. Washed sheep erythrocytes were sensitized by incubating one volume of 2.5% cells with one volume of a 100-μg/ml solution of palmitoyl B1355S1,3 in PBS for 1 hr; the cells were then washed three times with two volumes of PBS and finally resuspended in two volumes of PBS containing fetal calf serum (2%). Optimal conditions for dextran B1254L were found to be 40 mg of dextran to 50 μl of palmitoyl chloride, and a 200-μg/ml solution for sensitization.

The tests were carried out in disposable trays (Linbro Chemical Co., Inc.) using microtiter equipment (Cooke Engineering Co.). Each test was performed in duplicate using 0.025 ml each of serum, diluted in GBB, sensitized sheep erythrocytes (1.25%), and either GBB or test substance in GBB. Controls with unsensitized erythrocytes were also included. Titers were read after 2 hr at room temperature.

Precipitin reactions were incubated for 1 hr at 20° and then left over night at 4°. Washing with saline was performed in a 4° cold room to avoid solubilizing the precipitates (Heidelberger and Rebers, 1958). The supernatants were tested for the presence of excess MOPC 104E IgM by the hemagglutination procedure described above, using B1355S1,3-sensitized erythrocytes, and for excess antigen by addition of a further portion of serum. The precipitates were dissolved in 2 ml of 0.02 M sodium hydroxide, and protein concentrations were measured by the method of Heidelberger and MacPherson (1943a,b) on a reduced scale.

Hapten inhibition tests were carried out similarly except that the hapten was preincubated for 30 min at 20° with the serum before addition of dextran. Protein concentration was measured as above. Further details are included in the legends of tables and figures.

Microcomplement fixation tests using guinea pig serum (Wasserman and Levine, 1960) were carried out in GBB at either 37° for 70 min or for 16 hr at 4°, as indicated, in a total volume of 2.0 ml. Sheep erythrocytes (2.8×10^7) sensitized by hemolysin (Difco Laboratories) were then added in a volume of 0.5 ml and the mixture was incubated at 37° for 60 min. After diluting with 1.5 ml of GBB, intact cells were removed by centrifugation and the optical densities of the supernatants were measured at 413 mμ.

Results

The screening of over 100 mouse myeloma sera by double diffusion in gel demonstrated that MOPC 104E serum was the only one which reacted with the dextran B1355S1,3. The fact that the tumor-secreted IgM was responsible for the precipitation of dextran was demonstrated by immunoelectrophoresis (Figure 1). Rabbit anti-IgM and dextran B1355-S1,3 react with the same component of MOPC 104E serum. Absorption of MOPC 104E serum with B1355S1,3 removed essentially all of the IgM (Figure 1b). MOPC 104E IgM was unreactive with other antigens including lipopolysaccharides of *Salmonella*, *Escherichia coli*, and *Bacillus proteus*, dinitro- or trinitrophenylated human serum albumin, pneumococcal polysaccharides, levans, horse ferritin, keyhole limpet hemocyanin, mouse liver DNA, and mouse plasmacytoma glycogen.

Using 4-mg/ml solutions, the 15 dextrans listed in Table I were tested for reactivity with diluted MOPC 104E serum

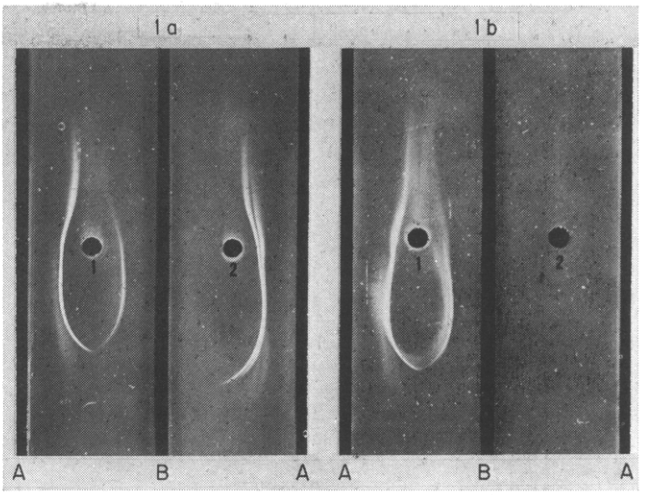

FIGURE 1: Immunoelectrophoresis studies (a) Of MOPC 104E serum (well 1) showing reaction with rabbit anti-MOPC 104E IgM 77 (trenches A). The IgM reacts as an antibody against B1355S1,3 (5 mg/ml, trench B). Another BALB/c IgM myeloma (MOPC 410, well 2) does not react with dextran but is identified by the anti-IgM. (b) Comparison of the reaction with anti-MOPC 104E IgM 77 (trenches A) of MOPC 104E serum before (well 1) and after (well 2) absorption with B1355S1,3 (final dilution of serum in both wells is 1:2).

(containing approximately 1.2 mg/ml of IgM) by double diffusion in gel (Ouchterlony procedure). Only four of the dextrans gave lines: B1355S1,3, B742, B1398, and B1141. No spurring occurred between the lines formed by the reactive dextrans.

Passive Hemagglutination. Sheep erythrocytes, sensitized with palmitoyl dextrans B1355S1,3 or B1254L as described, were readily agglutinated by dilutions of MOPC 104E serum containing 0.2–1.0 μg of IgM/ml. Cells coated with B1355S1,3 gave superior patterns and were agglutinated at lower serum concentrations than B1254L-coated cells. Inhibition experiments were carried out with dextrans and oligosaccharides and the data are recorded in Tables I and II. Hemagglutination inhibition of B1355S1,3-coated cells by dextrans required much more of the test substances than did inhibition of B1254L-coated cells. Agglutination of the latter was inhibited by several dextrans which failed to inhibit when B1355S1,3-coated cells were used (Table I). Similarly, the oligosaccharides isomaltose, kojibiose, panose, and nigerodextrins inhibited agglutination of B1254L-coated cells, whereas only nigerodextrins inhibited B1355S1,3-coated cells (Table II). The results show that dextrans and oligosaccharides containing α-1,3-linked glucose inhibit hemagglutination by MOPC 104E IgM best. However, the myeloma protein also reacts with glucose residues in other linkages as shown by inhibition of the B1254L system by isomaltose and kojibiose, as well as the following quantitative hapten inhibition data.

Precipitin Reaction. Solutions of the fifteen dextrans (4 mg/ml) were tested at a single level (800 μg) to determine whether or not they precipitated MOPC 104E IgM from a serum pool. The results are shown in Table I. Seven dextrans with different percentages of the various linkages were further tested over a range of concentrations for their ability to precipitate MOPC 104E IgM. The data are presented in Figure 2. The five active dextrans precipitated essentially the

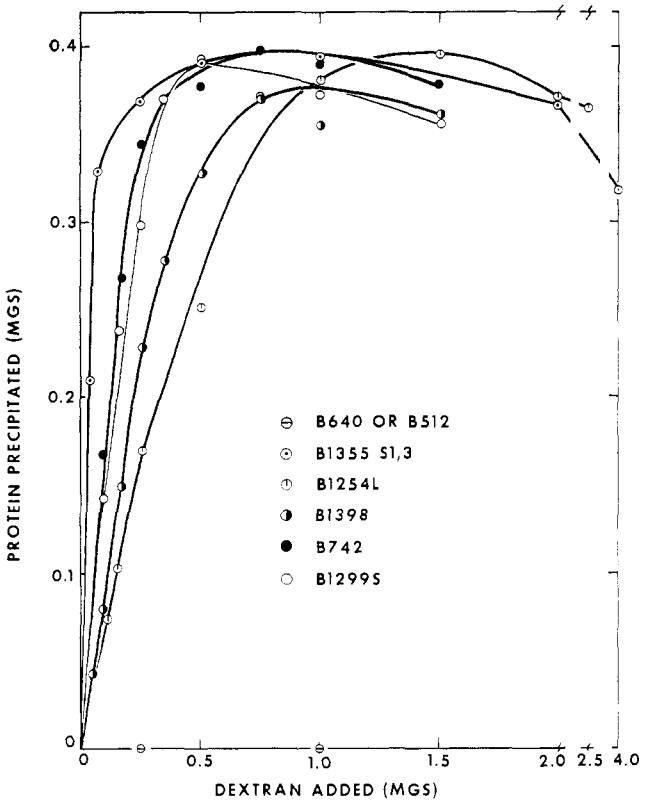

FIGURE 2: Precipitin curves obtained with diluted MOPC 104E serum (approximately 400 μg of IgM/test) and dextrans. Final volume was 0.8 ml.

same quantity of protein from the serum although different amounts of the various dextrans were required for maximal precipitation. The two dextrans, B512 and B640, did not precipitate with MOPC 104E serum and in other tests did not inhibit precipitation by B1254L. As in the passive hemagglutination tests, B1355S1,3 was the most active dextran, on a weight basis, of those tested. When tested with a purified preparation of MOPC 104E IgM, B1355S1,3 precipitated 85% of the total protein.

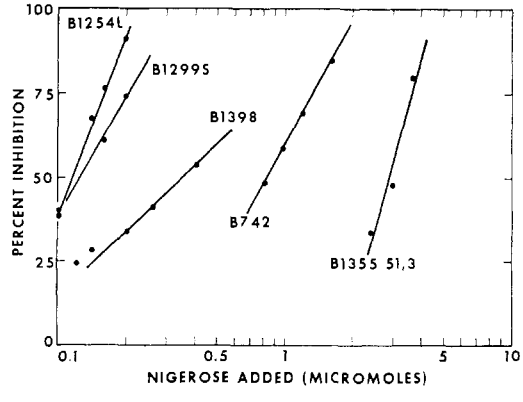

FIGURE 3: Inhibition by nigerose of the precipitation of MOPC 104E serum with various dextrans in the region of slight antigen excess. Final volume was 0.8 ml, with approximately 400 μg of IgM (diluted serum).

TABLE II: Passive Hemagglutination Inhibition by Oligosaccharides.[a]

Oligosaccharide	B1254L-Coated Cells	B1355S1,3-Coated Cells
Nigerotetraose	4×10^{-5}	1×10^{-2}
Nigerotriose	4×10^{-5}	1×10^{-2}
Nigerose	8×10^{-5}	2.5×10^{-2}
Panose	1.2×10^{-3}	No inhibition at 0.01 M
Isomaltose	2.5×10^{-3}	No inhibition at 0.01 M
Kojibiose	6×10^{-3}	No inhibition at 0.01 M
Maltose	No inhibition at 0.01 M	No inhibition at 0.01 M
α,α-Trehalose	No inhibition at 0.01 M	No inhibition at 0.01 M
Laminaribiose	No inhibition at 0.01 M	No inhibition at 0.01 M
Cellobiose	No inhibition at 0.01 M	No inhibition at 0.01 M

[a] Values quoted are the lowest molarities (calculated from the twofold dilutions used) giving complete inhibition of 1 → 2000 MOPC 104E serum for B1254L cells or 1 → 10,000 MOPC 104E serum for B1355S1,3 cells. Isomaltotriose could not be tested as, at a concentration of 0.01 M, a control of unsensitized cells was nonspecifically agglutinated.

Inhibition of the reaction of the five dextrans precipitating with MOPC 104E serum was studied with the potent inhibitor nigerose. The data in Figure 3 and Table I show the great difference in concentration of nigerose required to give 50% inhibition between B1254L and B1355S1,3. Moreover, the amount of nigerose required for inhibition of the remaining dextrans is not in direct relation to linkage compositions as measured by periodate oxidation methods.

Inhibition, by members of the nigerodextrin series, of the precipitin reaction of B1254L with MOPC 104E serum is shown in Figure 4. Nigeropentaose and nigerotetraose were equally effective inhibitors and slightly better than nigerotriose. Far more nigerodextrin was required to inhibit the B1355S1,3 system than the B1254L but similar relative inhibitory powers between nigerose, nigerotriose, and nigerotetraose (0.37:0.9:1) were observed.

Inhibition of the precipitin reaction of B1254L with MOPC 104E serum by various α-linked glucose oligosaccharides gave the results shown in Figure 5 and summarized in Table III. Here, as was shown above in the hemagglutination system, α-linked oligosaccharides other than nigerodextrins inhibit, albeit poorly, when compared with the nigerodextrins. It can be seen in Figure 5 that the poorer the inhibitor, the less steep is the slope of the line.

Microcomplement Fixation. Purified MOPC 104E IgM or MOPC 104E serum and a number of dextrans combined to fix complement. Again, the most reactive dextran of those tested was B1355S1,3. Using approximately 0.2 μg of MOPC

TABLE III: Inhibition of B1254L Precipitation with MOPC 104E by Various Sugars.

Sugar	Molarity for Inhibn[a]	Rel Inhibitory Power[b]
Nigeropentaose	0.000066	1
Nigerotetraose	0.000066	1
Nigerotriose	0.000070	0.94
Nigerose	0.00017	0.39
Panose	0.0016	0.041
Isomaltotriose	0.0020	0.033
Isomaltose	0.0044	0.015
Kojibiose	0.0094	0.0070
Maltose	0.016	0.0041
Methyl α-D-glucoside	0.037	0.0018

[a] Final molarity of inhibitor required for 50% inhibition of precipitation between 1 mg of B1254L and approximately 0.170 mg of MOPC 104E IgM (diluted serum) in a final volume of 0.5 ml. [b] Expressed with respect to nigeropentaose.

104E IgM (either diluted serum or purified protein) at 37°, complement was fixed at levels of B1355S1,3 ranging from 0.0005 to 1 μg (Figure 6). Complement fixation was better at 37° for 1 hr than at 4° overnight. To compare B1355S1,3 with less reactive dextrans, complement fixation tests were performed using approximately 10 μg of MOPC 104E IgM. The results are given in Table I. Dextrans were not tested at levels greater than 25 μg because of the high frequency of anticomplementary effects.

Efficiency of a dextran in complement fixation can be measured as the amount of dextran required for 50% fixation in the presence of a constant amount of MOPC 104E IgM (Table I). The strength of binding between dextran and MOPC 104E IgM can be measured as the amount of nigerose required to inhibit precipitation by 50% (Figure 3 and Table I). The relation, for four dextrans, between efficiency in complement fixation and strength of binding with MOPC 104E IgM is shown in Figure 7. The greater the affinity of a dextran for MOPC 104E IgM, as measured by the amount of nigerose required for inhibition, the smaller was the quantity of that dextran required for complement fixation.

In the microcomplement fixation test the soluble complexes formed by reaction of B1254L with MOPC 104E IgM did not fix guinea pig complement. However, washed specific precipitates formed by reaction of larger quantities of B1254L and MOPC 104E IgM fixed guinea pig complement. Details of these experiments will be reported elsewhere.

It was not possible to test nigerodextrins as inhibitors in the complement fixation system as they were anticomplementary even at final concentrations of 0.005 M.

Discussion

The evidence that the reactive species in MOPC 104E serum is the IgM myeloma protein is threefold: (1) Immunoelectrophoresis of MOPC 104E serum, using specific anti-IgM and B1355S1,3 as developing agents, shows that the IgM is the reactive protein (Figure 1a). (2) Absorption of the serum with B1355S1,3 removes essentially all of the myeloma protein (Figure 1b). (3) A purified MOPC 104E IgM preparation was 85% precipitable by dextran B1355S1,3.

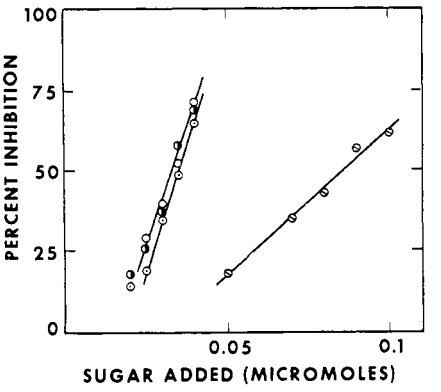

FIGURE 4: Inhibition by nigerose (⊘), nigerotriose (⊙), nigerotetraose (◑), and nigeropentaose (○) of precipitation, at equivalence, of MOPC 104E serum with B1254L. The final volume was 0.5 ml containing approximately 170 μg of IgM (diluted serum) and 1 mg of B1254L.

In reacting with dextrans, MOPC 104E IgM shows properties typical of many antibodies, namely, specificity (Tables II and III), complement fixation (Figure 6), agglutination of antigen-coated cells, and precipitation with soluble antigen (Figure 2). The binding between MOPC 104E IgM and nigerodextrins also represents the first reported reaction of a myeloma protein with an uncharged, nonhydrophobic ligand.

Comparison of our findings for the mouse IgM with the data for a human anti-dextran, whose major specificity is also directed toward α-1,3-linked glucosyl units (Allen and Kabat, 1959), shows the following similarities and differences. (1) The molar concentration of nigerose required for 50% inhibition of the most reactive dextran in the MOPC 104E system is of a similar order to the concentration required to inhibit the most reactive dextran in the human antibody system. (2) The relative efficiencies of the disaccharides tested are significantly different in the two systems: nigerose > isomaltose > kojibiose > maltose for the MOPC 104E

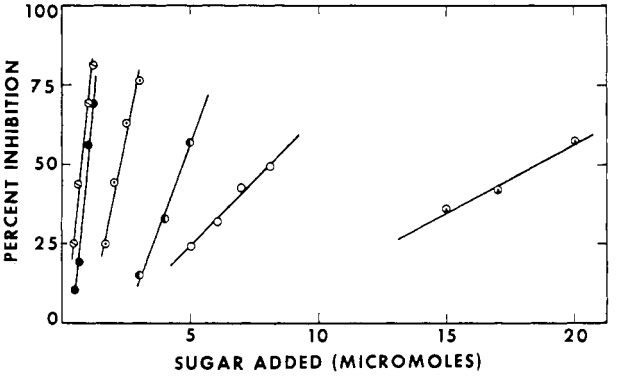

FIGURE 5: Inhibition by panose (⊘), isomaltotriose (●), isomaltose (⊙), kojibiose (◐), maltose (○), and methyl α-D-glucoside (◒) of precipitation of MOPC 104E serum with B1254L. Conditions as Figure 4.

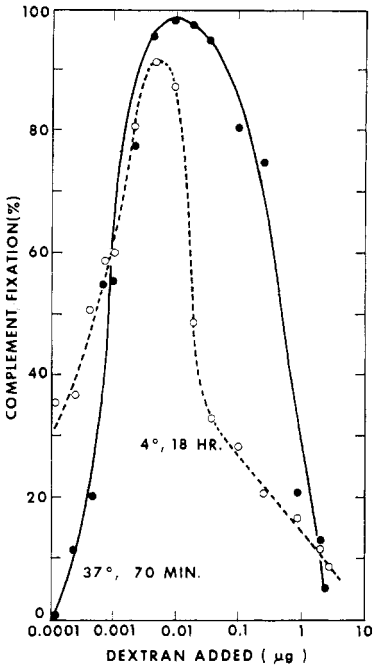

FIGURE 6: Complement fixation, at 4° for 18 hr or 37° for 70 min by the system B1355S1,3 and MOPC 104E serum. Test volume of 2 ml (before addition of erythrocytes) contained approximately 0.2 μg of IgM (diluted heat inactivated MOPC 104E serum) diluted guinea pig serum as source of complement and the indicated amount of dextran.

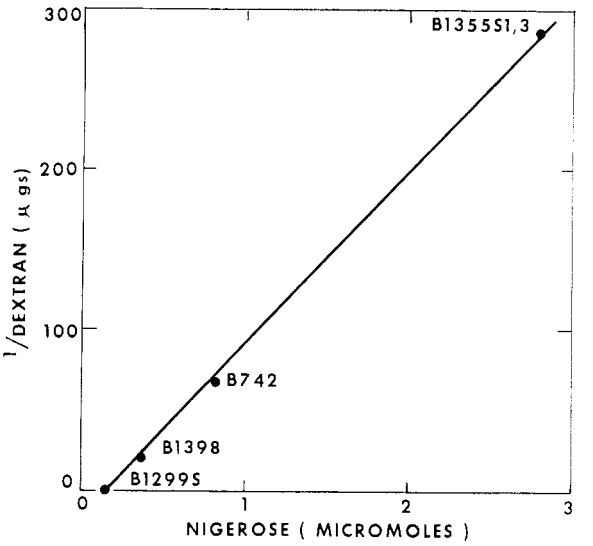

FIGURE 7: Relationship between the amount of nigerose required for 50% inhibition of precipitation and the reciprocal of the quantity of dextran required for 50% complement fixation. Data taken from Table I.

IgM and nigerose > kojibiose ≅ maltose ≫ isomaltose for the human antidextran. The finding that nigerose is 100 times more potent than maltose, and 50 times more potent than kojibiose, in inhibiting reaction of MOPC 104E IgM, and only about 6 times more potent than maltose and kojibiose in inhibiting the human antiserum, may be due to intrinsic differences in the combining sites, or to the presence of some antibody specific for α-1,4- and/or α-1,2-linked glucose in the human antiserum. The immunogen used in the human antidextran studies, B1355S-4, contains 57% 1,6-, 8% 1,4- and 1,2-, and 35% 1,3-like linkages and the antiserum used for inhibition studies was absorbed to remove only antibody directed against 1,6-linked glucose. (3) Despite its content of 29% α-1,3-like linkages, dextran B1142 does not precipitate efficiently with MOPC 104E IgM and precipitates only small amounts of antibody from the unabsorbed human anti-dextran sera. (4) The ratio of myeloma protein precipitated to milligram of dextran added, in the region of maximal precipitation is about 0.8 for the most reactive dextran (B1355S1,3) in the MOPC 104E system whereas it is about 2 for the best dextran (B1355S-4) in the human anti-dextran system. The fact that the mouse protein is IgM and the human antibody, by analogy with other human anti-dextrans is probably IgG (Kabat, 1954; Schlossman and Kabat, 1962; Edelman and Kabat, 1964; Allen *et al.*, 1964) may not explain this difference since purified anti-A antibodies of IgG and IgM type show almost identical precipitin curves (Kaplan and Kabat, 1966). More germane is the fact that we are approaching the specificity of MOPC 104E IgM by empirical methods. While our data on the relative effectiveness of various inhibitors (Table III) show that the IgM has specificity for α-1,3-linked glucose units, the possibility exists that additional sugar residues (not necessarily glucose), substituents such as phosphate, or an aglycone could increase the inhibitory effectiveness of the nigerodextrins. Macromolecules containing such hypothetical subunits should precipitate significantly more MOPC 104E IgM per milligram added than B1355S1,3.

Comparison of the relative effectiveness of the α-1,3-linked oligosaccharides in inhibiting precipitation (Table III) shows that nigerotriose is markedly better than nigerose. Maximal effectiveness is reached by the tetraose which is only slightly better than nigerotriose. These data suggest that the maximal size of the combining sites on MOPC 104E IgM are complementary to the triose or tetraose provided the sites do not have additional regions complementary to other as yet unidentified units (*vide supra*). Alternatively, the reactive groups in the hapten may attain the conformation most complementary to the combining site (Goodman, 1969) by the trimer-tetramer stage in the nigerodextrin series. While there are no data on inhibition of conventional antibody of α-1,3-glucosyl specificity by a nigerodextrin series, extensive studies of inhibition of human anti-dextran of α-1,6-specificity with the isomaltodextrin series have been made (Kabat, 1960; Schlossman and Kabat, 1962). These studies show that the hexa- and heptasaccharides are the best inhibitors of the native antisera.

The heterogeneity of the antibodies in these human anti-dextran sera was shown clearly by preparing antibody fractions which differed markedly in their capacity to bind small or large oligosaccharides (Schlossman and Kabat, 1962). Although one IgA myeloma protein with antihapten antibody activity proved to have binding sites which were homogeneous (Eisen *et al.*, 1968) no data are as yet available on the homogeneity of the MOPC 104E IgM sites. Preliminary studies of precipitation of MOPC 104E sera by increasing amounts of dextrans B1383 and B1399 show that these dextrans do not precipitate all of the MOPC 104E IgM. Whether these

results are due to heterogeneity of MOPC 104E IgM, or to other factors, is under investigation.

Although dextran B1299S yields no 1,3-like linkages by periodate oxidation methods, 1.0% of the total dextran was recovered as nigerose following partial acetolysis (Suzuki and Hehre, 1964). Similar small amounts of nigerose were recovered from B1254L and all other dextrans tested which yield no 1,3-like linkages by periodate. Do B1299S and B1254L precipitate MOPC 104E (Figure 2) solely by virtue of their small content of α-1,3-glucosyl linkages, or do the α-1,2-, α-1,6- and α-1,4-glucosyl linkages, which have weak affinities for MOPC 104E, also contribute? A 1% content of nigerose in the B1299S is sufficient to provide an excess of nigerose per IgM combining site, at equivalence, even assuming decavalence for the IgM. Whether any of the α-1,3-linked glucose residues in B1299S are suitably disposed for reactivity with MOPC 104E is not known. However, the essentially identical dextran B1299S-3 reacts poorly with human anti-dextran of α-1,3 specificity (Allen and Kabat, 1959) but as strongly as B1299S with MOPC 104E IgM (unpublished experiments). These findings suggest that glucose in linkages other than α-1,3 may participate in precipitation in the latter system. It must be emphasized that the data in Tables II and III, showing weak affinity for 1,6-, 1,4-, and 1,2-linked disaccharides, refer to affinity of a disaccharide for *an individual binding site on the IgM*. Strong binding between dextrans such as B1299S and the IgM is possible from the cumulative effect produced by combination of, for example, several α-1,6-linked residues, appropriately spaced on the flexible dextran molecule, with several binding sites *on the same IgM molecule* (Klinman and Karush, 1967). Against this explanation, is the finding that dextran B512 neither precipitates with MOPC 104E IgM nor inhibits precipitation of B1254L by MOPC 104E IgM. Dextran B512 has side chains terminating in α-1,6-glucosyl residues and precipitates well with human antidextran sera of α-1,6-glucosyl specificity showing that these residues are available for reaction (summarized in Kabat, 1960). Dextran B512 might therefore be expected to react with MOPC 104E IgM if the cumulative bonding hypothesis were correct. Studies of the precipitin behavior of the reduced and alkylated subunit, IgMs, should yield information relative to this argument.

The relative strengths of binding between MOPC 104E IgM and five reactive dextrans are shown by the inhibition data of Figure 3 and Table I. Dextrans B1299S and B1254L, which yield no 1,3-like linkages on periodate oxidation are less firmly bound than those dextrans which yield α-1,3-like linkages. As discussed above, the differences in binding strengths of the dextrans can be ascribed to quantitative differences in amount of α-1,3-linked glucose (as nigerosyl, nigerotriosyl, etc.) available for reactivity, and/or to differences in the kinds of residues (α-1,3-, α-1,4-, α-1,6-, α-1,2-linked glucose units) reacting with MOPC 104E IgM.

MOPC 104E IgM-fixed complement more efficiently at 37° than at 4° (Figure 6), a result in accord with other data on fixation by IgM anti-DNA antibodies (Sandberg and Stollar, 1966). Complement fixation, on the microscale is related to the strength of binding between a given dextran and MOPC 104E IgM as deduced from comparative inhibition data (Table I and Figure 3). For the few dextrans studied, the amount of a dextran required for 50% complement fixation is inversely proportional to the ease of inhibition of the dextran–IgM reaction (Figure 7). A certain minimal affinity between a dextran and MOPC 104E IgM, expressed in Figure 7 as micromoles of nigerose required for 50% inhibition, appears necessary for microcomplement fixation. Dextran B1254L, which is only slightly more readily dissociated than B1299S falls just below this minimal affinity. Microcomplement fixation and nigerose inhibition data for more dextrans is currently being gathered.

Failure to fix complement at a low-affinity binding site can have either a structural or kinetic basis. Thus, imperfect conformation of the complement binding site(s) on the IgM may result from poor fit of an antigenic determinant with the antibody combining site. Alternatively, low-affinity bonds may form and break with such rapidity that the probability of reaction with the first component of complement becomes negligible. This situation might well occur for a dextran with many weak antigenic determinants constantly interchanging with each other at an antibody combining site.

The concept that a minimal affinity antibody combining site and antigenic determinant is necessary for complement fixation may be of significance in studies of immune complex diseases. The capacity of antigen–antibody complexes to fix complement and initiate the lesions of serum sickness or glomerulonephritis may be a function of affinity as well as other factors.

Although a consistent order of reactivity of dextrans in the various tests employed was found, the reactivities did not always correlate with linkage analyses by periodate oxidation. Similar observations were made previously with bovine anti-dextran antibodies (Sage *et al.*, 1963). This lack of correlation might be expected since these methods have a lower limit of 2% for detection of 1,3-like units, and units linked through C-2 and C-4, or C-2 and C-3 cannot be distinguished from 1,3-linked units (Rankin and Jeanes, 1954). Further, the linkage analyses do not indicate the accessibility of the glucosyl units for reaction with MOPC 104E IgM.

The delineation of the specificity of MOPC 104E IgM makes possible investigations of its valence and heterogeneity. In preliminary experiments the subunit IgMs agglutinated B1355S1,3-coated erythrocytes and precipitated with soluble antigen suggesting that the subunit is at least divalent.

Glucans, linked α-1,3, have been found in the cell walls of a number of yeasts and fungi (Johnston, 1965; Bacon *et al.*, 1968). Such organisms, growing as part of the normal mouse flora, may have originally stimulated the clone of cells producing MOPC 104E IgM.

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