[CONTRIBUTION FROM THE DEPARTMENTS OF MICROBIOLOGY AND NEUROLOGY, COLLEGE OF PHYSICIANS AND SURGEONS AND THE NEUROLOGICAL INSTITUTE, PRESBYTERIAN HOSPITAL]

Immunochemical Studies on Dextrans[1,2]

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The ability of several dextrans to precipitate human anti-dextran from four different sera of 1 → 6 specificity has been found to be correlated with the proportion of 1 → 6 linkages present. Differences in immunochemical reactivity of two preparations, B1299 S-3 and B742 LR, provide new evidence of the unusual chemical structure of these dextrans. Two anti-dextran sera prepared against the same dextran, S-5-A-1.0, show differences in their antibody specificities. One serum contains only anti-dextran of 1 → 6 specificity while the other has in addition antibody with specificities involving 1 → 4 like and 1 → 3 like linkages.

It has been clearly shown that injection of small amounts (1 mg.) of dextran into humans leads to the formation of precipitating antibodies to dextran[3a,3b,4] which persist for long periods of time.[5] Most of the antisera previously reported[3b] contained anti-dextran with a specificity directed toward some multiple of the 1 → 6 anhydroglucopyranose unit present in the immunizing dextran. That anti-dextran might vary in its specificity was shown[3b] by the production of a serum with two types of anti-dextran. One type showed a specificity directed against units in 1 → 6 glucosidic linkage and the other of a different specificity involving units linked other than or in addition to the 1 → 6 positions. Inhibition studies[6] with oligosaccharides of known structure have confirmed this and have provided evidence for other anti-dextrans of specificity directed toward the 1 → 4 like and 1 → 3 like linkages.

Precipitin reactions of several human anti-dextrans of 1 → 6 specificity[3,6] were studied, employing as antigens a series of native dextrans and native dextran fractions whose relative proportions of 1 → 6, 1 → 4 like and 1 → 3 like linkages were known.[7-9] Since the formic acid liberated in periodate oxidation comes from terminal non-reducing units linked only through C1 as well as from units linked only through C1 and C6, in accordance with the convention of Rankin and Jeanes[7] and Jeanes, *et al.*,[8] the sum of these two values permits calculation of the proportion of 1 → 6 linkages, but gives no information about the number of 1 → 6 linked units. The findings that oligosaccharides of the isomaltose series specifically inhibited precipitation of certain anti-dextran sera by dextran while those of the maltose series and other oligosaccharides did not[6] served to characterize the specificity of this type of antidextran as being directed toward a terminal non-reducing chain of at least three α-D-glucopyranoses with only 1 → 6 linked units; this antibody is referred to as antibody of 1 → 6 specificity.[6,3b] It was also shown that the second type of antidextran had some antibody with a specificity directed toward a terminal non-reducing chain of at least three α-D-glucopyranoses with only 1 → 4 linked units since precipitation of this portion of the anti-dextran by NRRL dextran B 1299S-3 was inhibited by much lower concentrations of 1 → 4 linked oligosaccharides such as maltotriose, maltotetraose and maltopentaose than by maltose or by 1 → 6 linked oligosaccharides. For such inhibition to take place it was inferred that dextran B 1299S-3 possessed a number of terminal non-reducing sequences of at least three glucopyranoses linked only by 1 → 4 units which were reacting to precipitate this antibody. Evidence for this is given in detail in reference 6. The immunochemical behavior of all but two of the various dextrans with different antidextrans of 1 → 6 specificity could be correlated with the proportion of 1 → 6 linkages determined by periodate oxidation. Explanations for the atypical behavior of these two dextrans are suggested.

Experimental

Dextrans.—Native dextran samples CSC 236 and 279 were obtained from Drs. F. Schulz and M. E. Bachmann of Commercial Solvents Corporation. Clinical fraction S-5-A-1.0 was prepared at the National Bureau of Standards[10] and supplied by Drs. S. G. Weissberg and H. S. Isbell. The authors are indebted to Dr. Allene Jeanes of the Northern Regional Research Laboratory who supplied samples of all the other dextrans and dextran fractions employed in this study as well as periodate oxidation data.[7-9,11,12]

Dextran B1299 S-3 was produced by strain "K" of Neill, *et al.*[13] At the request of Drs. Hehre and Neill, dextran from strain "K" was prepared and characterized at the Northern Regional Research Laboratory because of the unusual immunological properties observed for it by Hehre.[14] These investigations have been continued by Hehre on dextran B1299 S-3.[14b]

The two native dextran fractions designated B742 LR and B742 C3R were prepared and characterized as described by Wilham, *et al.*,[15] and represent discrete fractions of differ-

(1) This investigation was carried out under the Office of Naval Research, Navy Department, Washington, D. C. and the William J. Matheson Commission. Reproduction in whole or in part is permitted for any purpose of the United States Government.

(2) These findings were presented at the meeting of the Division of Carbohydrate Chemistry of the American Chemical Society, New York, N. Y., September 12–17, 1954.

(3) (a) E. A. Kabat and D. Berg, *Ann. N. Y. Acad. Sci.*, **55**, 471 (1952); (b) *J. Immunol.*, **70**, 514 (1953).

(4) E. A. Kabat, D. Berg, D. Rittenberg, L. Ponticorvo, M. L. Eidinoff and L. Hellman, THIS JOURNAL, **76**, 564 (1954).

(5) P. H. Maurer, *Proc. Soc. Exp. Biol. Med.*, **83**, 879 (1953).

(6) E. A. Kabat, THIS JOURNAL, **76**, 3709 (1954).

(7) J. C. Rankin and A. Jeanes, *ibid.*, **76**, 4435 (1954).

(8) (a) A. Jeanes, W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, M. J. Austin, J. E. Cluskey, B. E. Fisher, H. M. Tsuchiya and C. E. Rist, *ibid.*, **76**, 5041 (1954); (b) A. Jeanes, personal communication.

(9) J. W. Sloan, B. H. Alexander, R. L. Lohmar, I. A. Wolf and C. E. Rist, THIS JOURNAL, **76**, 4429 (1954).

(10) S. G. Weissberg and H. S. Isbell, National Bureau of Standards Report 1160 (1951); Report 1713 (1952).

(11) (a) A. Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950); (b) **74**, 5339 (1952).

(12) A. Jeanes, C. A. Wilham and C. E. Hilbert, *ibid.*, **75**, 3667 (1953).

(13) J. M. Neill, J. Y. Sugg, E. J. Hehre and E. Jaffe, *Proc. Soc. Exp. Biol. Med.*, **47**, 339 (1941).

(14) (a) E. J. Hehre, *ibid.*, **54**, 18 (1943); (b) E. J. Hehre, unpublished data.

(15) C. A. Wilham, B. H. Alexander and A. Jeanes, *Arch. Biochem. Biophysics*, in press.

ent size distribution and ratios of 1 → 6 to non 1 → 6 linkages.[16]

The proportions of 1 → 6, 1 → 4 like and 1 → 3 like linkages in most of these dextrans may be found in reference 8a. B 742 LR gave values identical[8b] with those of B 742 L; B 742 C3R, however, differs slightly from B 742-S[8a] in that it contains 61% 1 → 6, 18% 1 → 4 like and 21% 1 → 3 like linkages.[8b] Clinical dextran fraction S-5-A-1.3 contains 86% 1 → 6, 4% 1 → 4 like, and 10% 1 → 3 like linkages[8b] and these values are assumed to hold for the comparable fraction S-5-A-1.0 used in this study. The convention of Jeanes and co-workers[7-9] has been followed with respect to the designation of the non 1 → 6 linkages and their limitations in the structural interpretation of their periodate data apply to the present study.

Antisera.—Five human anti-dextran sera ID_7 (anti-B 1255), $9D_3$ (anti-S-5-A-1.0), $30D_2$ (anti-OP163), $36D_2$ (anti-CSC236), $116D_2$ (anti-S-5-A-1.0) were employed. Earlier bleedings of antisera 1, 9 and 30 have been reported in detail.[3b]

Antidextrans $1D_7$, $30D_2$, $36D_2$ and $116D_2$ contained antibody of 1 → 6 specificity as defined above since precipitation of their antidextran by dextran was specifically inhibited by 1 → 6 linked oligosaccharides, with isomaltotriose being more effective than isomaltose, and not by 1 → 4 linked oligosaccharides.[6]

Sera $9D_3$ and $116D_2$ were produced by individuals who were immunized with comparable amounts (1 mg.) of the same preparation (S-5-A-1.0) by the same route. Antidextran present in these sera, however, is markedly different. While both antisera contain antibody of 1 → 6 specificity, antiserum $9D_3$ contains in addition antidextran of non 1 → 6 specificity.[6]

Precipitin Studies.—Quantitative precipitin curves were set up with four 1 → 6 specific sera ($1D_7$, $30D_2$, $36D_2$, $116D_2$) and serum $9D_3$ containing antidextran of 1 → 6 and non 1 → 6 specificity. A measured volume of antiserum was added to analytically measured amounts of the various dextrans, kept at 37° for one hour, in the refrigerator for 6 to 8 days, centrifuged and washed twice with chilled saline. Specific precipitates were analyzed for antibody N by the Folin–Ciocalteu tyrosine method,[17,18] using reagents standardized against known amounts of normal human γ-globulin[19] and antibody.[20]

Results

From the quantitative precipitin curves (Fig. 1) obtained with antidextrans $116D_2$ and $36D_2$ of 1 → 6 specificity, it can be seen, except for B1299 with $36D_2$, that all dextran preparations tested, despite varying proportions of 1 → 6, 1 → 4 like and 1 → 3 like linkages, precipitate the bulk of antibody N from 1.0 ml. of serum. Dextrans precipitated 26.3–33.2 μg. and 22.4–28.3 μg. antibody N from 1.0 ml. of sera 116 and 36. B1299 S-3, however, precipitated only about 10 μg. antibody N from antiserum $36D_2$. In the region of antibody excess, native dextrans and native dextran fractions with higher proportions of 1 → 6 linkages are more effective per unit weight in precipitating antibody N. Data on individuals 1 and 30 are not given since they were essentially similar to $36D_2$ and $116D_2$ and to the earlier serum samples from these individuals previously reported.[3b]

Precipitin curves obtained with antiserum $9D_3$ are in striking contrast to those obtained with $116D_2$ although both individuals were injected with the same dextran. The presence of several peaks in the precipitin curve with homologous antigen (S-5-A-1.0) indicates at least two types of antibody, one of 1 → 6 specificity present in small amounts and the other of non 1 → 6 specificity present in larger amounts.[3b] The maximum antibody N precipitated from 1.0 ml. of serum by the different dextrans varies considerably from 11.1–46.2 μg. antibody N. Dextrans seem to fall into three groups, those precipitating about 12 μg. antibody N (B1377, B1375, B1424, B742LR and B1399), those precipitating about 30 μg. (S-5-A-1.0, B1299 S-3) and others about 40 μg. antibody N (B1355 S-4, B742 C3R). Preparations which remove greater amounts of antibody N from antiserum 9 have a higher proportion of 1 → 4 like and 1 → 3 like linkages. The greatest amount of antibody N is precipitated by dextrans B742 C3R and B1355 S-4 with the highest proportion of 1 → 3 like linkages 26 and 35%, respectively.

A quantitative estimate of the ability of each dextran to precipitate anti-dextran of 1 → 6 specificity from sera 1, 30, 36 and 116 was obtained from the amount of antigen required to precipitate 50% of the maximum antibody N precipitable by the given dextran. This was determined directly from precipitin curves (Fig. 1). The amount of antigen required to precipitate 50% of the antibody N rather than the least amount required to precipitate the maximum was determined since in some systems the precipitin curve rises gradually over such a wide antigen concentration that the smallest quantity of antigen required for maximal precipitation could not be accurately determined.

Figure 2 shows the amount of each dextran required to precipitate 50% of the antibody N plotted against the proportion of 1 → 6 linkages present in the preparation. With all four 1 → 6 specific sera (1, 30, 36 and 116), as the proportion of 1 → 6 linkages decreases, the dextran preparations are less effective in their ability per unit weight to precipitate antibody N in the region of antibody excess. Dextrans with 70% or more 1 → 6 linkages appear to be equally effective on a weight basis, while those with lower proportions of 1 → 6 linkages are less effective in precipitating anti-dextran of 1 → 6 specificity. Data obtained with antiserum $9D_3$ show no definite correlation of precipitating power and proportion of 1 → 6 linkages. This behavior is to be expected in view of the large amount of antibody N of non 1 → 6 specificity present in the antiserum.

The significant difference in behavior of B 742 LR from other preparations employing four 1 → 6 specific anti-dextrans would tend to indicate dissimilarities between B 742 LR and other dextrans with comparable proportions of 1 → 6 linkages. Dextran fraction B 742 LR possessing 81% 1 → 6 linkages is significantly less effective per unit weight in its precipitating ability. Native dextrans B 1375 and B 1385 possessing 81% 1 → 6 linkages are more effective as is dextran B 1355-IC with 88% 1 → 6 linkages. B 742 LR is more comparable to preparations with lower proportions of 1 → 6 linkages. Native dextran B1299 S-3 appears to be more effective than would be expected from Fig. 2 for a dextran possessing only 50% 1 → 6 linkages. While this dextran precipitates the bulk of antibody N from $116D_2$ and $1D_7$ (26.3 and 27.5 μg., respectively, as compared with ranges for the other dextrans of 26.3–33.2 μg. per 1.0 ml. $116D_2$ and 25.7–29.8 μg.

(16) R. Lohmar, THIS JOURNAL, **74**, 4974 (1952).

(17) M. Heidelberger and C. F. C. MacPherson, *Science*, **97**, 405 (1943); **98**, 63 (1943).

(18) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," Chas. C. Thomas, Springfield, Ill., 1948.

(19) E. A. Kabat and A. E. Bezer, *J. Exper. Med.*, **82**, 207 (1945).

(20) S. M. Beiser and E. A. Kabat, THIS JOURNAL, **73**, 501 (1951).

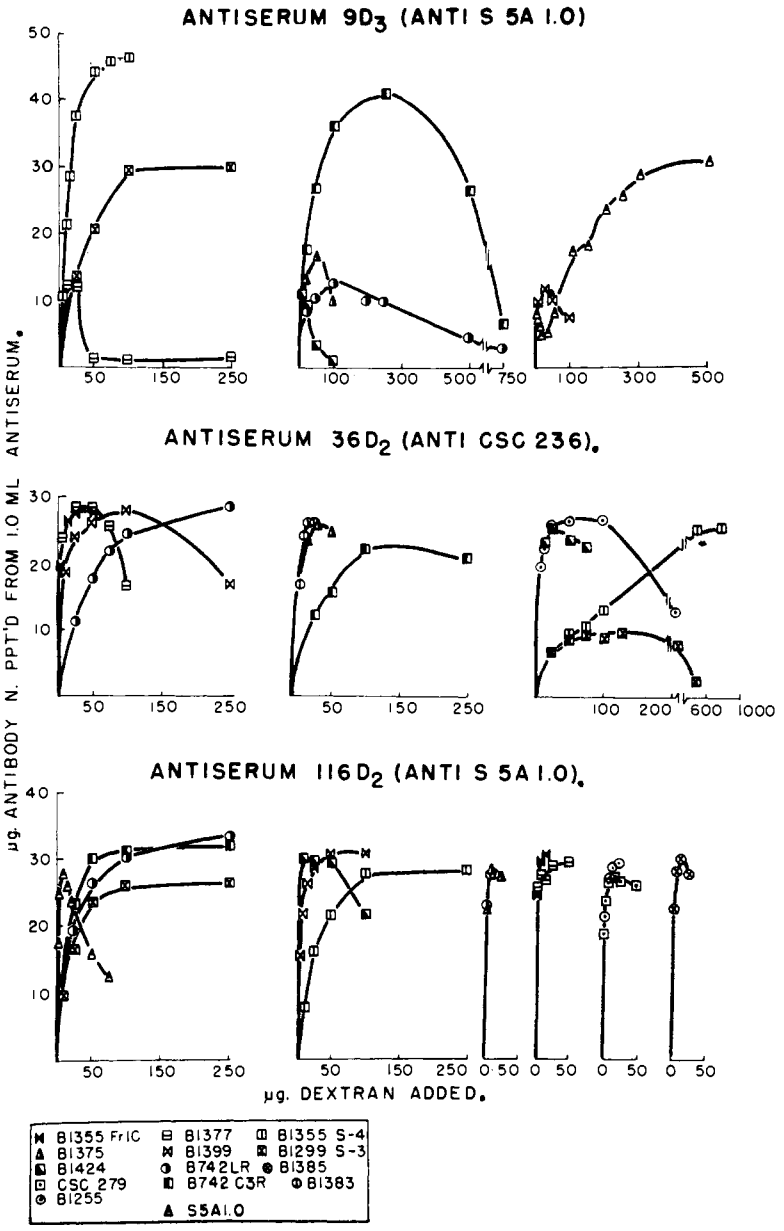

Fig. 1.—Quantitative precipitin curves for three antidextrans with various dextrans.

antibody N per 1.5 ml. $1D_7$), it precipitates only a portion of the total antibody N from sera $36D_2$ and $30D_2$ (9.9 and 31.4 μg. as compared with 22.4–28.3 μg. per 1.0 ml. $36D_2$ and 41.9–47.8 μg. antibody N per 0.5 ml. $30D_2$, respectively).

Discussion

The data presented in Figs. 1 and 2 support and extend earlier interpretations of the relationship between reactivity with antibody and the structure of dextrans insofar as can be determined by periodate oxidation. The major type of antibody produced in humans on injection of dextran is antibody with a specificity directed against some multiple of the 1 → 6 anhydroglucopyranose ring[3b,6] as indicated for antiserum $36D_2$ and $116D_2$ in Fig. 1. Occasional individuals may produce antibody with specificities involving the 1 → 4 like and the 1 → 3 like linkages as well. This is seen in the precipitin curves of serum $9D_3$ in which dextrans with larger numbers of 1 → 4 like and 1 → 3 like linkages precipitate more antibody N than can be precipitated by other dextrans. The factors responsible for the production of antibodies with different specificities are not known. It is of especial interest that of the two individuals who gave good antibody responses to dextran S 5A1.0, one should have produced antibody only of the 1 → 6 type ($116D_2$) while the other reacted to the same antigen by the production of antibody with specificities involving the 1 → 4 like and the 1 → 3 like linkages as well.

The plots of the precipitating capacities of the various dextrans with four anti-dextrans of 1 → 6 specificity show, with one exception, that native dextrans with 70% or more 1 → 6 linkages are about equally effective in precipitating anti-dextran. As the proportion of 1 → 6 linkages decreases below 70% the capacity to precipitate antibody per given weight of the dextran decreases. This is in keeping with the earlier inference[3b] that increasing numbers of non 1 → 6 linkages either reduce the number of combining sites per dextran molecule or interfere with the close approach of the reactive grouping to the combining site on the antibody molecule. As a consequence larger numbers of dextran molecules with such non 1 → 6 linkages are required to precipitate the antibody.

It is noteworthy that these findings are independent of the uncertainties as to the nature of the non 1 → 6 linkages in the various dextrans since they vary only with the percentage of 1 → 6 linkages.

From studies[6] on the capacities of various 1 → 6 linked oligosaccharides to inhibit precipitation of anti-dextran of 1 → 6 specificity in antiserum from individual 30, it has been inferred that the antibody molecules have combining sites with configurations complementary on an average to at least three and probably four 1 → 6 anhydroglucopyranose units with a terminal non-reducing end. This is in accord with a large body of immunochemical data showing that terminal groups play a dominant role in determining specificity in antigen–antibody reactions. Landsteiner and van der Scheer[21] prepared azoprotein antigens in which peptides of known structure were coupled to the protein and found the specificity to depend primarily on the structure of the terminal amino acid with a free

(21) (a) K. Landsteiner and J. van der Scheer, *J. Exp. Med.*, **55**, 781 (1932); (b) **59**, 769 (1934).

carboxyl. With artificial carbohydrate–protein antigens, the specificity of antisera prepared against glycosides of maltose, gentiobiose, cellobiose and lactose were shown by Goebel, *et al.*,[22] to be determined primarily by the configuration of the terminal non-reducing hexose.

Cross reactivity of lung galactan and tamarind seed polysaccharide with type XIV antipneumococcal serum was attributed by Heidelberger, *et al.*,[23] to the presence of terminal non-reducing galactose residues in these materials. Similarly the inhibiting effects of various sugars on the precipitation of anti-B by blood group B substances was found to be related to a terminal non-reducing 1 → 6 -α-galactosidic linkage.[24]

Thus, the reactivity of the various dextrans with this 1 → 6 antibody would be determined by the number of terminal sequences of four or more units which they possess. The reduced reactivity of the dextrans with less than 70% 1 → 6 linkages falling on the curves in Fig. 2 would suggest that the distribution of the non 1 → 6 linkages is essentially random and only when the number of such non 1 → 6 linkages increases to the point at which the number of terminal reactive groupings of three or four anhydroglucopyranose units is reduced appreciably, does lower reactivity with antibody result. The studies of Jones, Dimler, Jeanes, Wilham and Rist have shown that at least 80% of the branches in B512 are only one unit long.[25] The high molecular weights of native dextrans would provide large numbers of reactive groupings per molecule even with a relatively small percentage of non 1 → 6 linkages to provide branches so that the capacity to precipitate antibody would be only slightly affected by differences in molecular weight. It has been shown[3b] already that only a relatively slight increase in capacity to precipitate antibody occurs when clinical dextrans with molecular weights ranging from 10,000 to 100,000 are prepared from native dextrans with molecular weights of many millions.

Two of the preparations, B1299 S-3 and B742 LR, showed a striking difference in behavior from all of the other dextrans (Fig. 2). With all four anti-dextrans of 1 → 6 specificity, B1299 S-3 had a greater capacity to precipitate anti-dextran than would be anticipated from the proportion of 1 → 6 linkages. Precipitation of anti-dextran of 1 → 4 specificity from individual 9 by this dextran was inhibited by oligosaccharides of the 1 → 4 series from maltose to maltopentaose[6] and not by Schardinger dextrins. The combining site of this anti-dextran of individual 9 was found to be complementary to an open chain of at least three and probably four 1 → 4 linked anhydroglucopyranose units and hence it was inferred that dextran B1299 S-3 had such three or four unit chains of 1 → 4 linked units in its

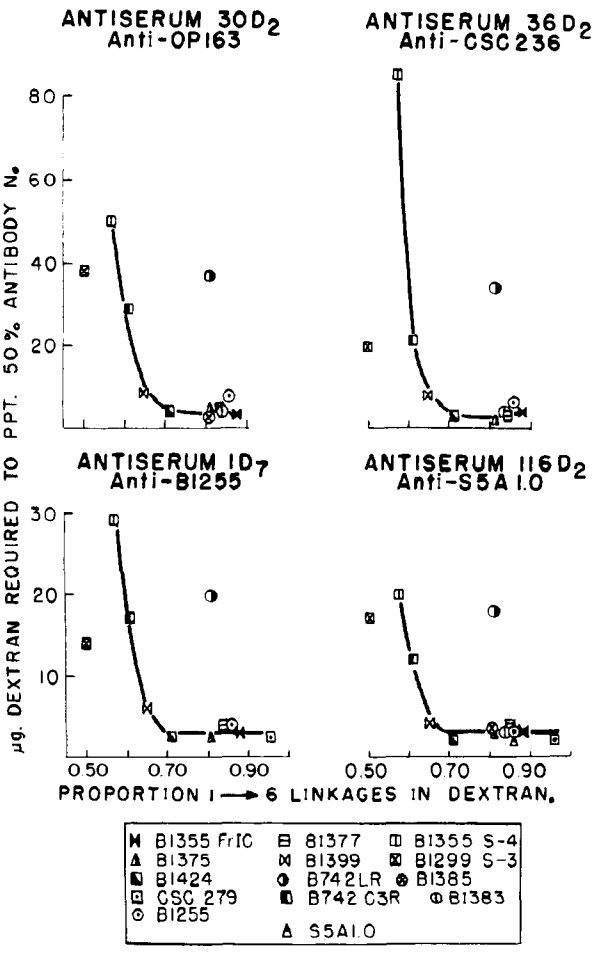

Fig. 2.—Relation between precipitating power of dextran for antidextran and the proportion of 1→6 linkages.

structure. The existence of chains of 1 → 4 units would reduce the number of such units capable of interrupting sequences of 1 → 6 linked units giving rise to longer sequences of terminal 1 → 6 residues and result in increased reactivity with the 1 → 6 antibody relative to that expected from the number of non 1 → 6 linkages determined.

This interpretation would be subject to modification should further studies reveal, contrary to present immunochemical expectations, that α 1 → 3 or α 1 → 2 linked oligosaccharides have a specific inhibitory effect on the reaction of antibody of 1 → 6 specificity with dextran B1299 S-3. There is also some uncertainty, however, as to the proportion of 1 → 6 linkages in this dextran because of difficulties encountered in periodate oxidation.[8b] For this to account for the displacement of this point from the curves in Fig. 2, however, would require that the true proportion of 1 → 6 linkages was 0.59 to 0.62 instead of the reported value of 0.50 which is not too likely. The predicted existence of sequences of 1 → 4 like linkages with terminal non-reducing ends in this dextran[6] must await definitive structural studies.

In the case of dextran B742 LR the converse situation was noted. This dextran with 81% 1 → 6 linkages has very much lower capacity to precipitate anti-dextran than other dextrans with compar-

(22) W. F. Goebel, O. T. Avery and F. M. Babers, *J. Exp. Med.*, **60**, 599 (1934).

(23) M. Heidelberger, Z. Dische, W. Brock Neely and M. L. Wolfrom, THIS JOURNAL, **77**, 3511 (1955).

(24) E. A. Kabat and S Leskowitz, *Federation Proc.*, **15**, 467 (1955); THIS JOURNAL, **77**, 5159 (1955).

(25) R. W. Jones, R. J. Dimler, A. Jeanes, C. A. Wilham and C. E. Rist, Division of Carbohydrate Chemistry of the American Chemical Society, Abstracts of Papers presented at New York, N. Y., Sept. 12–17, 1954.

able proportions of 1 → 6 linkages, such as B1375, B1385, B1383, etc. Assuming that the 19% of 1 → 4 like units present in this dextran were mostly at branch points and that chains of 1 → 4 like units did not occur, as appears likely from the failure of this dextran to precipitate more than about 12 μg. of antibody N from antiserum $9D_3$ (*cf.* Fig. 1), a branching ratio of 4.3 can be calculated for B742 LR and for B1385 and B1375. Despite the finding that such a ratio allows the latter two dextrans to be effective in precipitating antibody and permits the existence of substantial numbers of terminal sequences of four 1 → 6 glucopyranose units, B742 LR was only $^1/_6$ to $^1/_7$ as effective per unit weight in precipitating anti-dextran. Accordingly one would predict that the structure of B742 LR would differ from those of the other dextrans in that many of the 1 → 4 branches are so distributed as to reduce disproportionately the number of terminal non-reducing sequences of four or more glucose units and increase the length of the inner chains of 1 → 6 units. A 1 → 4 branch occurring on the second or third glucose unit from a non-reducing end group would substantially reduce the capacity of that group to combine with 1 → 6 anti-dextran, since it has been shown that the capacity of isomaltose to bind anti-dextran was only about $^1/_{60}$ that of isomaltotriose.

Recent inhibition studies (E. A. K. in preparation)[26] have shown that the antibodies are heterogeneous with combining sites ranging in size from units complementary to four to six (1 → 6) anhydroglucopyranose units. The capacities of the tetrasaccharide and hexasaccharide to inhibit precipitation of anti-dextran by dextran are sufficiently close so that for the present discussion branching would probably have to occur nearer to the non-reducing end than the fourth or third ring to cause the striking decrease in reactivity noted. The correctness of this prediction almost must necessarily await elucidation of the structure of this dextran. It has been shown previously[27] that a preparation of B742 highly comparable with B742 LR[8b] but having only 75% 1 → 6 linkages was more resistant to the action of dextranase from *Penicillium funiculosum* NRRL strain 1768 than was another dextran having the same content of 1 → 6 linkages. When treated with a comparable preparation of 1768 dextranase in this Laboratory, dextran B742 LR likewise showed a lower conversion to reducing sugars than did dextrans B1375 and B1385 which, like B742 LR, have 81% 1 → 6 linkages.

The finding that B742 LR differs in its susceptibility to enzymatic degradation from other dextrans with comparable proportions of 1 → 6 linkages is in agreement with the immunochemical findings and provides additional evidence of the unusual structure of this dextran.

Certain minor differences in specificity of the 1 → 6 antibodies remain to be explained, especially the ability of B1299 S3 to precipitate almost all of the antidextran from antiserum $116D_2$ and $1D_7$, whereas it could only precipitate about $^1/_3$ of the antibody from antiserum $36D_2$ and about $^2/_3$ from antiserum $30D_2$. Such variations are quite common to other cross reacting antigen–antibody systems and may be dependent on the heterogeneity in size of the 1 → 6 antibody combining sites.[26]

Acknowledgment.—The authors wish to express their sincere appreciation and thanks to Drs. Allene Jeanes and H. M. Tsuchiya for their numerous suggestions in reviewing the manuscript and to Miss Gloria Petrilli for technical assistance.

(26) E. A. Kabat, in preparation.

(27) H. M. Tsuchiya, A. Jeanes, H. M. Bricker and C. A. Wilham, *J. Bact.*, **64**, 513 (1952).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

The Effect of Alkali on Carbohydrates. I. Saccharinic Anilides Derived from D-Glucose, L-Arabinose and Cellobiose[1]

BY JOHN W. GREEN

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The behavior of seven crystalline saccharinic anilides on the paper chromatogram has been observed. The R_f values vary inversely with the molecular weight, and are greater for the α-metasaccharinic anilides than for the β-isomers. Periodate oxidation of milligram quantities of anilides and subsequent paper chromatography of the fragments identify the "metasaccharinic acid" type of structure. Treatment of cellobiose with hot 8 *N* NaOH leads to the formation of both iso- and metasaccharinic acids, identified as the anilides.

In a previous communication[2] a method of paper chromatography of saccharinic acids as the anilides was reported. This paper deals with the isolation of five crystalline saccharinic anilides, and a method of structural determination of metasaccharinic anilides by periodate oxidation.

In the original method the anilide mixtures derived from various sugars were resolved on the paper chromatogram into a series of four spots, by use of a 9:1:2 v./v. mixture of acetone, water and benzene. Now it has been shown that these spots represent, in order of decreasing R_f values, the anilides of C_3 (lactic), C_4, C_5 and C_6 saccharinic acids. Hence, the rate of movement of the various anilides on a paper chromatogram is inversely proportional to the molecular weight (see Table I).

The fastest moving spot is D,L-lactic anilide,[3] as

(1) Presented before the Division of Carbohydrate Chemistry at the Minneapolis Meeting of the American Chemical Society, September 12, 1955.

(2) J. W. Green, THIS JOURNAL, **76**, 5791 (1954).

(3) Leipen, *Monatsh.*, **9**, 45 (1888); C. A. Bischoff and P. Walden, *Ann.*, **279**, 71 (1894).