IMMUNOLOGICAL REACTIONS BETWEEN DEXTRAN POLYSACCHARIDE AND SOME BACTERIAL ANTISERA

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(Received for publication December 21, 1931)

The name dextran was given by Scheibler (1) to the mucous fermentation products present in beet juices. He found that this substance on hydrolysis yielded considerable quantities of glucose and when oxidized with nitric acid gave oxalic acid. He was led to regard it as an anhydride of glucose.

Many other descriptions of this and similar substances are given in literature, but at present it is generally accepted that the production of the gum is due to the action of bacteria on rich sugar media. Depending on the kind of sugar and the type of organism used, different polysaccharides are obtained. In the case of gum-producing bacteria, large amounts of polysaccharides are produced because the nutrient medium used is rich in sugar. Boas (2) recommended a culture medium containing from 5 to 10 per cent of carbohydrate. According to the commonly accepted theory, it is assumed that most, if not all, carbohydrate-decomposing microorganisms possess synthesizing properties which, during the early stages of their development give rise under favorable conditions to the condensation of hexoses and other simple sugars to complex polysaccharides. In cases in which these synthetic polysaccharides readily imbibe water, a colloid, *i.e.*, a mucus, is formed.

The most important of the bacteria capable of producing dextran from saccharose is *Leuconostoc mesenterioides*. The method of preparation, purification and structure of the dextran produced by this organism will be found described in forthcoming papers (3).

The material used in our experiments was kindly supplied by Prof. Hibbert and shown by analysis to be nitrogen- and ash-free.

An antidextran serum has been prepared by the author by adsorbing the dextran on collodion particles utilizing a method reported in a recent paper (4). The relation between the dextran polysaccharide

TABLE I

Precipitin Test with Dextran Polysaccharide and Various Antibacterial Sera

Serum	Dextran 1-5,000	Serum	Dextran 1-5,000	Serum	Dextran 1-5,000
S. paratyphi A	4*	Pneumococcus Type III	3	*Strep. faecalis* (1914)	1
S. suipestifer	4	Pneumococcus Type II	4	Enterococcus (1917)	4
Strep. haemolyticus	0	Pneumococcus Type I	4	Strep. ulcer. colitis type (1838)	0
S. morgani	4	Strep. ulcer. colitis type (1915)	0	Strep. ulcer. colitis type (1916)	4
E. typhi	4	*B. dysenteriae* (Shiga)	0	*S. aertrycke*	4
S. schottmuelleri	0	*B. dysenteriae* (Hiss)	4	*B. proteus*	0
Strep. haemolyticus (S.F.)	2—	*B. anthracis*	0	*B. tuberculosis*	0
Strep. haemolyticus (erysipelas)	0	Meningococcus polysaccharide	0	*S. gallinarum*	4
Strep. viridans	0	Anti-R I pneumococcus	4	Strep. ulcer. colitis (1918)	3
Strep. cardioarthritidis	0	Anti-R II pneumococcus	4	*Strep. poliomyelitis*	0

Read after 5 hours at 37°C.—overnight in ice box.

* 4 = definite large floccules settling in a disc; 3 = smaller floccules settling; 2 = definite fine precipitate; 1 = very slight fine precipitate; 0 = negative.

and various bacterial antisera has now been investigated and various interesting cross-reactions have been obtained while testing the specificity of this polysaccharide. The bacterial antisera utilized in the work were all prepared by injecting rabbits with whole formalinized culture of the organisms. The injections were given intravenously and treatment continued until tests on trial bleeding showed a definite, specific polysaccharide precipitate. In a few cases antisera produced in horses by a similar technique were employed.

TABLE II

Titration with Dextran Polysaccharide against Several Antibacterial Sera (Compared with Homologous Serum R 12)

Dilution of polysaccharide	1/4,000	1/8,000	1/16,000	1/32,000	1/64,000	1/128,000	1/256,000	1/512,000	1/1,024,000
Antidextran R12	4	4	4	4	4	4	4	3	1
Antityphoid	4	4	3	2	1	0	0	0	0
Antimorgani	4	4	4	2	1	0	0	0	0
Antisuipestifer	4	4	4	3	2	1	0	0	0
Antigallinarum	4	4	4	3	0	0	0	0	0
Antiparatyphi A	4	4	3	2	0	0	0	0	0
Antiaertrycke	4	4	4	3	2	0	0	0	0
Anti- R pneumococcus	4	4	3	2	0	0	0	0	0
Antipneumococcus I	4	3	0	0	0	0	0	0	0
Antipneumococcus II	4	4	2	0	0	0	0	0	0
Antipneumococcus III	4	3	2	0	0	0	0	0	0
Antienterococcus (1917)	4	4	2	2	1	0	0	0	0
Antistrep. ulcer. colitis (1916)	4	4	3	2	1	0	0	0	0
Antistrep. ulcer. colitis (1918)	3	2	1	0	0	0	0	0	0

Read after 5 hours at 37°C. and overnight in ice box.

More than thirty different antisera were investigated in order to select the ones capable of reacting with the dextran at a dilution of 1–5,000. The results of these tests are shown in Table I.

The dextran was titrated with the reacting sera in various dilutions, using for comparison the specific antidextran serum. The results of this test are shown in Table II. It is of interest to notice the approximate constancy in the titration of the majority of the antisera, the value lying between 32,000 and 64,000. The variation can doubtless

be accounted for by variation in the potency of the sera when tested against their own specific antibody The high titer of the specific antidextran serum is conspicuous.

Having observed the groupings of these antisera into species each group was then studied separately. The first group is the Salmonella and it included *E. typhi.* The results of the titration of the sera, with the dextran at 1–5,000 dilution, are shown in Table III. It is of interest to note that *S. schottmuelleri* (Para B) does not give a reaction with dextran, but this exception can be explained by the fact that this serum does not give specific carbohydrate precipitates. Perhaps the variations with the sera are related to the differences in amount of antidextran antibodies which may parallel those of the specific anticarbohydrate antibodies.

TABLE III

Titration of Antisera of the Salmonella Group and E. typhi with Dextran Polysaccharide (1–5,000)

Antiserum..	*Morgani* 9	*Suipestifer* 1	*Gallinarum* 1	*Aertrycke* 5	Para A 52028	Para B 2	Typhoid	Control + R 12	Control
1/2	4	4	4	4	4	0	4	4	0
1/4	3	4	3	4	3	0	3	4	0
1/8	1	4	3	4	2	0	2	4	0
1/16	0	4	3	4	2	0	1	2	0

Read after 5 hours at 37°C. and overnight in ice box.

To ascertain whether the antidextran antibody was absorbed by the specific polysaccharide, absorption tests were made with each sera. On one sample the specific carbohydrate was absorbed and on the other the dextran. Each sample was then titrated with the homologous polysaccharide and with the dextran. The results are found in Table IV. No difference is to be observed in any of the absorbed sera, demonstrating that the antidextran antibody is not associated with the specific carbohydrate antibody, but is a definite entity.

The second group studied was the pneumococcus. In Table V the results are given of the titration of different antipneumococcic sera, type-specific and anti-"R" from Types I and II. The type-specific sera gave weaker reactions than the anti-R sera. To ascertain whe-

ther the precipitate obtained in the type-specific sera was due to the "C" substance, absorption tests were made with the specific and C carbohydrates. The absorbed sera were then precipitated with the dextran, giving the same results as on the control sera that were not absorbed This eliminates the type-specific or the group antibodies (anti-C) as the source of the precipitate with dextran. These results

TABLE IV

Results of Precipitin Test with Homologous Polysaccharide (1–1,000) and Dextran on Sera Absorbed with Same

Test...	Titration of sera with homologous polysaccharide 1–1,000					Titration of sera after absorption with homologous SSS with its own polysaccharide 1–1,000					Titration of sera with dextran 1–5,000 after being absorbed with homologous polysaccharide				
Serum...	*Morgani*	*Suipestifer*	*Gallinarum*	*Aertrycke*	Para A	*Morgani*	*Suipestifer*	*Gallinarum*	*Aertrycke*	Para A	*Morgani*	*Suipestifer*	*Gallinarum*	*Aertrycke*	Para A
1/2	3	4	3	4	3	—	—	—	—	—	4	4	4	4	4
1/4	3	4	3	4	2	0	0	0	0	0	4	4	4	4	3
1/8	1	3	0	4	0	0	0	0	0	0	3	4	3	4	0
1/16	0	0	0	2	0	0	0	0	0	0	1	2	0	3	0

Test...	Titration of sera with dextran 1–5,000					Titration of sera with homologous polysaccharide 1–1,000 after absorption with dextran					Titration of sera with dextran after absorption with dextran				
Serum...	*Morgani*	*Suipestifer*	*Gallinarum*	*Aertrycke*	Para A	*Morgani*	*Suipestifer*	*Gallinarum*	*Aertrycke*	Para A	*Morgani*	*Suipestifer*	*Gallinarum*	*Aertrycke*	Para A
1/2	4	4	4	4	4	—	—	—	—	—	—	—	—	—	—
1/4	3	4	3	4	3	2	4	3	4	2	2	2	3	4	0
1/8	1	4	3	4	2	0	3	2	4	0	0	1	0	2	0
1/16	0	4	3	4	2	0	2	0	3	0	0	0	0	2	0

Read after 5 hours at 37°C. and overnight in ice box.

are in accord with those observed with the Salmonella group as reported above.

The third group studied was the *Streptococcus viridans* (Bargen) of different types (Strains 1915, 1916, 1918 and 1838); a strain of *Streptococcus faecalis* (1914), and another of enterococcus (1917). These organisms are closely related. Table VI shows the results of the precipitin test with dextran. It is of great interest to note that

Antisera 1914 and 1916 show a distinct difference in their reaction towards dextran, and as is known they are extremely difficult to differentiate by any other method (except absorption of specific anti-carbohydrate antibodies).

All of these strains are immunologically different, their polysaccharides are characteristic, yet while Strains 1916, 1917 and 1918 have

TABLE V

Titration of Antipneumococcus Sera (S and R Types) with Dextran Polysaccharide (1–5,000)

Serum.....	Pneumo-coccus I	Pneumo-coccus II	Pneumo-coccus III	Pneumo-coccus R I	Pneumo-coccus R II	Control + R 12	Control
1/2	3	3	3	4	4	4	0
1/4	2	1	1	4	4	4	0
1/8	1	0	0	3	3	4	0
1/16	1	0	0	2	1	2	0

Read after 5 hours at 37°C. and overnight in ice box.

TABLE VI

Titration of Sera from Streptococcus viridans (Bargen) and Similar Organisms, with Dextran Polysaccharide (1–5,000)

Strain No.....	1914	1915	1916	1917	1918	1838
1/2	2	1–	4	4	4	1–
1/4	0	0	4	4	4	0
1/8	0	0	4	4	1	0
1/16	0	0	2	4	0	0

Read after 5 hours at 37°C. and overnight in ice box.

antidextran antibodies, the other three have not. This would appear to suggest fundamental differences in molecular composition of the polysaccharides.

To determine if the antidextran antibody could be absorbed by the specific carbohydrate, Sera 1916, 1917 and 1918 were absorbed with their homologous polysaccharide and then the dextran precipitin test repeated. There was no noticeable difference, this being in agreement with the results obtained with the Salmonella and the pneumococcus groups.

DISCUSSION

Avery, Heidelberger and Goebel (5) have already described the cross-reactions between the Pneumococcus Type II antiserum and the *B. friedlaenderi* polysaccharide and *vice versa*. This crossing they attribute to a similarity in a portion of the complex molecule or a very similar configuration of atoms. Immunological similarity between *B. anthrax*, meningococci, *B. subtilis* and *B. mesentericus* has also been shown (6) and the same explanation offered as that by the above authors.

These experiments suggest that some of the bacterial polysaccharides may contain several active antigenic groups in their molecular structure. Certain of these may be more active in stimulating antibody formation or be acted upon by antibodies of a simpler nature. As immunization progresses, increasing amounts of the complex specific antibodies would be formed in response to the complex bacterial polysaccharide. In a study of the polysaccharides of the Salmonella group their action has been found to be a specific one in agreement with Furth and Landsteiner (7). At the same time it has been possible to show (unpublished work) that the polysaccharides from *S. aertrycke* and *S. suipestifer* cross mutually with the respective antisera.

The results in the absorption tests with all the groups studied suggest that the dextran antibody is a distinct entity, for in all cases it remains after absorption of the specific carbohydrate antibodies. It may be that this is a simpler antibody, the primitive or immature stage of the more complex one which is constantly being formed in process of immunization to the bacterial polysaccharides. This work suggests a possible application of the immunological method to the study of the chemical structure of complex carbohydrates.

SUMMARY

Dextran, the synthetic polysaccharide produced by *Leuconostoc mesenterioides* from saccharose, reacts immunologically with antisera from pneumococci, some of the Salmonella and some of the types of *Streptococcus viridans* (Bargen).

This immunological relationship is independent of the specific

antipolysaccharide antibodies of these sera, suggesting the existence of a distinct antibody produced by an active group of the specific bacterial polysaccharide, which is similar or identical to the active group of the dextran polysaccharide.

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