STUDIES ON MENINGOCOCCAL INFECTION

XIII. CORRELATION BETWEEN ANTIPOLYSACCHARIDE AND THE ANTIBODY WHICH PROTECTS MICE AGAINST INFECTION WITH TYPE I MENINGOCOCCI

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There is abundant evidence of the type-specific nature of the antibodies that protect mice against infection with the meningococcus. Miller and Castles (1), in describing the use of gastric mucin as an adjuvant for the production of experimental meningococcal infection in mice, noted that monovalent antimeningococcal rabbit sera afforded to mice passive protection against organisms of the homologous type only. Rake (2) observed that the protective power of antimeningococcal horse sera against Type I organisms was correlated with their content of antibody precipitable by a polysaccharide isolated from Type I meningococci. Little (3) found that the titer of Type I protective antibody in antimeningococcal sera ran parallel to the agglutinative titer, as determined by testing with encapsulated organisms. Pittman, Branham, and Sockrider (4) reported that the protective potency of antimeningococcal horse sera was correlated with their content of type-specific precipitins as measured by the formation of halos around colonies of meningococci cultivated upon solid media containing the immune serum. Menzel and Rake (5) showed that the Type II protective antibody in Type II antimeningococcal rabbit sera was identical with the antibody to the type-specific polypeptide hapten isolated by them.

The present report concerns antibody absorption experiments, which were similar to those of Menzel and Rake and which demonstrated that the Type I protective antibody in four antimeningococcal horse sera corresponded almost entirely to one component of the polysaccharide preparations (these preparations were designated "SI") previously isolated from Type I meningococci (6).

Materials and Methods

The four sera employed in the present experiments were selected because of the diversity of their origins, which, it was felt, would broaden the significance of the results. Serum "A" was a sample of the fourth bleeding in the series of monovalent sera previously studied by the authors (7). The bleeding date was February 1, 1934, and the serum contained 0.01 per cent merthiolate (Lilly). Serum "B" was the polyvalent serum "B" discussed previously (8), a commercial preparation not acceptable for marketing because of a deficiency of antibodies other than Type I. It contained a phenolic preservative. Serum "C" was a sample from a freshly prepared batch of polyvalent serum, from a different commercial source than "B." Serum "D" was of considerable historical interest, since it was prepared at The Rockefeller Institute for Medical Research in 1918, when an intensive effort was made to perfect antimeningococcal sera, especially for use by the armed forces. It had been stored meanwhile in refrigerators without the addition of preservatives and still possessed a quite satisfactory content of antibody to SI.
The course of the reactions between SI and sera B, C, and D was analyzed by the quantitative precipitation test in the fashion previously described (8). The polysaccharide preparations used, Nos. 18 and 19, had been the subject of previous chemical and immunological analysis (8).

Protection tests were carried out in the manner already described, the results being expressed in provisional units (9). Values for absorbed sera were corrected for dilution, so as to be directly comparable to those for the original, undiluted sera.

### TABLE I

<table>
<thead>
<tr>
<th>Serum</th>
<th>Protective titer</th>
<th>Protective antibody removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units per ml.</td>
<td>per cent</td>
</tr>
<tr>
<td>A unabsorbed</td>
<td>50</td>
<td>88</td>
</tr>
<tr>
<td>A absorbed</td>
<td>7 5</td>
<td></td>
</tr>
<tr>
<td>B unabsorbed</td>
<td>420 420</td>
<td>87</td>
</tr>
<tr>
<td>B absorbed</td>
<td>55 55</td>
<td></td>
</tr>
<tr>
<td>C unabsorbed</td>
<td>740</td>
<td>99</td>
</tr>
<tr>
<td>C absorbed</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>D unabsorbed</td>
<td>100 100 104 125 110</td>
<td>95</td>
</tr>
<tr>
<td>D absorbed</td>
<td>5 7 4 5 6</td>
<td></td>
</tr>
</tbody>
</table>

The double entries for sera A and B represent the results of duplicate tests. The data for serum D were taken from three separate experiments.

### RESULTS

**Effect of Removal of All Antibody Precipitable by SI**

In the first group of experiments, the sera were treated with amounts of SI, calculated from the data of quantitative precipitin analyses, to give maximal precipitation of antibody and a small, but definite excess of the polysaccharide. The reactions were carried out at 0°C. for 48 hours and the hapten-antibody precipitates were removed by centrifugation at from 4 to 6°C.

The results of protection tests, summarized in Table I showed that the foregoing procedure removed from 87 to 99 per cent of the protective activity from the sera, and demonstrated, therefore, a close correlation between protective antibody and antibody to the polysaccharide preparation. At the time these experiments were done, it was not regarded as highly significant that the relatively small amount of from 5 to 13 per cent of the protective antibody was not precipitable from three of the sera. It was thought that this residue might represent species-specific antibody having protective power. On the other hand, the excellent results obtained with the freshly prepared
serum C suggested that a diminution of the precipitability of the antibodies had occurred in the other three sera, which ranged in age from approximately 2 to 20 years. Against this explanation, however, was the fact that 95 per cent of the protective antibody was precipitable by SI from serum D, the oldest one tested.

Kabat, Miller, Kaiser, and Foster (10) have reported recently that only from 30 to 60 per cent of the protective antibody was precipitable from an antimeningococcal horse serum by various preparations of SI, including one of the authors'. Absorption was even less complete in the case of two rabbit antisera and one chicken antiserum, and varied from 0 to 88 per cent in the case of four convalescent human sera. The residual protective antibody was shown to be type-specific by appropriate absorptions with intact meningococci. In view of these results, it appears most likely that the unabsorbed protective antibody in the present experiments corresponded to this new type-specific antibody, which constituted, however, but a small proportion of the total antibody in the four sera studied.

**Effect of Selective Removal of Antibody by SI**

A quantitative study of the precipitation reaction between SI and polyvalent antimeningococcal horse sera (8) indicated that SI consisted of (a) a true type-specific substance, "SSS," and (b) a component which was designated "X" because its nature was not then precisely defined, but which was most probably group-specific polysaccharide. The proportion of SSS to X in the available preparations of SI was estimated to be 4:1, whereas the polyvalent sera contained the antibodies in approximately the inverse proportion.

Fig. 1 depicts the course of the reaction between SI No. 18 and serum C, which was typical of six sera tested (sera B, C, D, and three sera studied previously (8)). According to the interpretation given (8), the first portion of the curve, described by the equation, \( N = 33.0 S - 307 S^2 \), represents the combined reactions (SSS + anti-SSS) + (X + anti-X). In the second portion of the curve, described by the equation, \( N = 0.30 + 17.0 S - 76.2 S^2 \), the precipitation of anti-SSS is complete and is represented by the constant (i.e., the anti-SSS content of the serum is 0.300 mg. of antibody N per ml.).

If the foregoing interpretation is correct and if protection of mice is afforded only by anti-SSS, then partial absorption of a serum at a point corresponding to A in Fig. 1 should be as effective as complete absorption in removing protective antibody, even though a considerable fraction of the anti-X remains in the serum. This expectation was realized in experiments with sera C and D, the results of which are summarized in Table II, and strongly support the conclusion that anti-X is devoid of protective action.

**Serum C.**—The results of the quantitative precipitin analysis of this serum showed that it contained 0.30 mg. of anti-SSS nitrogen and 1.09 mg. of anti-X
nitrogen per ml. A sample of the serum was partially absorbed in the manner discussed above, so that it still contained 0.44 mg. of anti-X nitrogen per ml., which was 40 per cent of the amount originally present.

Serum D.—The results of the quantitative precipitin analysis of this serum showed that it contained 0.27 mg. of anti-SSS nitrogen and 0.38 mg. of anti-X nitrogen per ml. Selective absorption in the manner described left 0.28 mg. of anti-X nitrogen per ml., or 74 per cent of the amount originally present.

![Typical precipitation reaction](image)

**Fig. 1.** Typical precipitation reaction between a Type I meningococcal polysaccharide preparation and polyvalent antimeningococcal horse serum. Polysaccharide preparation SI No. 18 and 1 ml. portions of serum C.

The foregoing results were correlated with the agglutination titers of the respective sera. In order to make the test as type-specific as possible, the test antigen consisted of a suspension of un殺lled organisms taken from a 4 hour culture on blood agar of a virulent strain of Type I meningococcus. The test mixtures were incubated for 2 hours at 37°C. and overnight in a refrigerator (11). The results, also given in Table II, showed good correlation between absorption of protective antibody and absorption of type-specific agglutinin.

All of these results define an anomaly: the Type I, specific, protective antibody of these antimeningococcal horse sera was but a minor fraction of the total, being secondary in amount to a group-specific, non-protective antibody which corresponded to a minor component of the preparations of polysaccharide separated by the authors from autolysates of Type I meningococci.
Kabat et al. (10) have obtained similar results in experiments of a somewhat different nature, and have shown in addition that the same relationships prevail in the immunological response of human beings to Type I meningococcal infection, treated with sulfonamides. These findings are in sharp contrast to the usual situation, exemplified by antipneumococcal sera (12), which is dominated by the formation of antibody to the capsular substance. This antibody is also the principal protective antibody.

### TABLE II

**Effect of Selective Absorption with Type I Meningococcal Polysaccharide Preparations on the Type I Protective Titer of Antimeningococcal Horse Sera**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Anti-SSS nitrogen</th>
<th>Anti-X nitrogen</th>
<th>Protective titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per ml.</td>
<td>mg. per ml.</td>
<td>units per ml.</td>
</tr>
<tr>
<td>C unabsorbed</td>
<td>0.30</td>
<td>1.09</td>
<td>740</td>
</tr>
<tr>
<td>C partially absorbed</td>
<td>0.00</td>
<td>0.44</td>
<td>6</td>
</tr>
<tr>
<td>C completely absorbed</td>
<td>0.00</td>
<td>0.00</td>
<td>6</td>
</tr>
<tr>
<td>D unabsorbed</td>
<td>0.27</td>
<td>0.38</td>
<td>104 125</td>
</tr>
<tr>
<td>D partially absorbed</td>
<td>0.00</td>
<td>0.28</td>
<td>5 6</td>
</tr>
<tr>
<td>D completely absorbed</td>
<td>0.00</td>
<td>0.00</td>
<td>4 5</td>
</tr>
</tbody>
</table>

**Agglutination Tests with the Above Sera**

<table>
<thead>
<tr>
<th>Dilution of serum</th>
<th>1:10</th>
<th>1:20</th>
<th>1:40</th>
<th>1:80</th>
<th>1:160</th>
<th>1:320</th>
<th>1:640</th>
<th>1:1280</th>
</tr>
</thead>
<tbody>
<tr>
<td>C unabsorbed</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>±</td>
<td>0</td>
</tr>
<tr>
<td>C partially absorbed</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C completely absorbed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D unabsorbed</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D partially absorbed</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D completely absorbed</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It seems possible that this peculiar behavior of Type I meningococcus is circumstantial, depending more upon the amounts of the respective antigens present at immunization than upon differences in their antigenicity. Type I meningococci readily undergo dissociation accompanied by loss of capsular antigen (11), they are easily decapsulated by chemical treatment (3), and in cultures more than 5 hours old, they lose virulence (1), a property presumably associated with the presence of the type-specific capsular antigen. There is, therefore, a considerable likelihood that a vaccine of Type I meningococci may be faulty in its content of type-specific capsular antigen and be dominated by one of the somatic antigens, the antibody to which would be expected to
be of low protective value. That this situation can be avoided is indicated by
the results of experiments reported previously. The present authors (7)
studied eight sera withdrawn from a horse over a period of 2 years, during
which the animal received repeated injections of young cultures of recently
isolated strains of Type I meningococcus. Under these conditions, the anti-
body response was almost exclusively type-specific.

It was pointed out previously (8) that there were considerable variations
in the antigen-combining capacity of the anti-SSS in different antimeningo-
coccal horse sera, a phenomenon similar to that noted earlier by Goodner and
Horsfall (13) with antipneumococcal horse sera. A like variability was ob-
served in the protective capacity of the antibody in the present experiments.
The protective titers per mg. of anti-SSS nitrogen were respectively: serum
B, 1000; serum C, 2470; serum D, 400. The data indicate, furthermore, that
the protective titer may not be proportional to the antigen-combining capacity
of the antibody. The amounts of S1 taken up per mg. of anti-SSS nitrogen,
at the point of complete precipitation of anti-SSS without excess SSS, were
estimated to be respectively: serum B, 0.071 mg.; serum C, 0.130 mg.; serum
D, 0.067 mg. On this basis, sera B and D should have had the same protective
titers per milligram of anti-SSS nitrogen, whereas the value for serum C should
have been 1.9 times as great. In view of the discrepancy between these pre-
dictions and the experimentally determined values, it becomes necessary to
conclude that the protective titer of antimeningococcal horse sera cannot be
estimated reliably by quantitative precipitin analysis.

On the Protective Effect of Agar-Specific Antibody

The sera of horses and rabbits, which have received injections of bacteria
cultivated upon media solidified by agar, frequently contain an antibody which
precipitates with agar, or with extracts and hydrolysates of agar (14). Pres-
umably agar, or a hapten derived from it, is adsorbed by the bacteria, thereby
acquiring antigenicity.

Agar antibody was present in each of six therapeutic antimeningococcal
horse sera tested for it, the amounts ranging from 0.05 to 0.47 mg. of antibody
nitrogen per ml. It was absent from two samples of the monovalent anti-
meningococcal sera prepared by the authors (7) and from a sample of commer-
cially prepared meningococcal antitoxin.

Because of the widespread occurrence of this antibody and because the
meningococci used in the protection test are customarily grown on media
solidified by agar, it seemed pertinent to ascertain if the antibody had any
effect upon the results of the protection test. To do this, the protective titers
of sera A, B, and D were determined before, and after, absorption with agar
(Difco) or an agar hapten. The latter was obtained in the course of an attempt
to prepare group-specific meningococcal polysaccharide from Type II organ-
isms cultivated on an agar-containing medium. It failed to react, however, with any of the available antimeningococcal sera, if these had been absorbed with agar, and consequently it was assumed to have only the specificity of agar. It was used as a test antigen for the absorption and quantitative estimation of agar-antibody in the present experiments because, unlike agar, it did not form precipitates in normal sera and, therefore, should not have removed antibody or other serum protein in an immunologically unspecific manner.

**TABLE III**

_Effect of Absorption with Agar or Agar-Hapten on the Type I Protective Titer of Antimeningococcal Horse Sera_

<table>
<thead>
<tr>
<th>Serum</th>
<th>Agar-antibody content</th>
<th>Absorbing antigen</th>
<th>Protective titer</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. N per ml.</td>
<td></td>
<td></td>
<td>units per ml.</td>
<td>units per ml.</td>
<td>units per ml.</td>
</tr>
<tr>
<td>A</td>
<td>0.00</td>
<td>None</td>
<td>50 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar hapten</td>
<td>50 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.47</td>
<td>None</td>
<td>420 420</td>
<td>324*</td>
<td>324*</td>
<td>324*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar hapten</td>
<td>370 420</td>
<td>324*</td>
<td>324*</td>
<td>324*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.09</td>
<td>None</td>
<td>100 100</td>
<td>104 125</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar hapten</td>
<td>45 50</td>
<td>87 104</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar</td>
<td></td>
<td>75 79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This experiment was carried out over a year after the first one with serum B, which apparently underwent some deterioration in the interim. Double entries in a single experiment are the results of duplicate tests.

Since serum A contained no agar-antibody demonstrable by the precipitin test, it was expected that the absorption would have no effect upon the protective titer, and such was the result (Table III). With one possible exception, there was likewise no effect in the case of serum B, which had the highest content of agar-antibody of the sera tested. With serum D, however, the absorptions caused an average decrease of 30 per cent in the protective titer. Because of the variability of the results, they were examined statistically and were found to be acceptable, since the difference between the means was 3.4 times its standard error.

Antimeningococcal horse sera may be encountered, therefore, from which a portion of the Type I protective antibody can be absorbed by agar. This might be interpreted to indicate a fortuitous relationship between the chemical structures of agar and Type I meningococcal polysaccharide similar to cross-reactions previously reported (15). Agglutination tests, carried out with
serum D simultaneously with those recorded in Table II, showed, however, no diminution in the Type I specific antibody titer following absorption by agar. This result supports an alternative explanation, namely, that in certain circumstances agar-antibody protects mice against infection by Type I meningococci, presumably acting upon the agar hapten adsorbed by the bacteria. Because serum D is unique in this respect at present, it does not seem permissible to draw a definitive conclusion, except that agar antibody is a potential cause of variability in mouse protection tests.

**SUMMARY**

Absorption tests indicated that the protective antibody (Type I) in four antimeningococcal horse sera corresponded closely to one of the components of the polysaccharide preparation previously isolated by the authors from Type I meningococci. This antibody was, however, a minor fraction in the three therapeutic sera tested, being secondary in amount to non-protective antibody corresponding to another component of the polysaccharide preparation, plus antibody corresponding to agar. In one case, removal of the agar-antibody diminished the protective titer by 30 per cent.

**BIBLIOGRAPHY**