FURTHER OBSERVATIONS ON THE EMPLOYMENT OF SPECIFIC AND NON-SPECIFIC ANTIGENS IN THE PERFORMANCE OF THE GONOCOCCIC COMPLEMENT-FIXATION TEST

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In a former paper1 read before the Philadelphia County Medical Society May 28, 1913, it was conclusively shown by Thomas and Ivy in an analysis of over 200 cases in which monavalent, trivalent, hexavalent and a commercial antigen of twelve strains of gonococci were employed, that the gonococcus complement-fixation test possesses great specificity so far as positive results are concerned. The results have proved that "the different strains of the gonococcus differ markedly one from another — so much so that the antibodies produced in the body by the toxin of one strain will in many instances not bind the complement in the presence of an antigen prepared from another strain. Therefore, if only one strain is used in the preparation of the antigen, a great many negative results would be obtained in positive cases; an antigen prepared from many strains fixes the complement whenever one of its component strains does so, and consequently the necessity of testing a serum against a number of antigens separately is avoided. It is not to be denied that there probably are other strains of gonococci differing widely from any present in the polyvalent antigen, so that at times a negative result will be obtained in a positive case."

Convinced from our previous study of the specificity of the gonococcus complement-fixation test in gonorrheal infections, namely, that although a negative reaction may be obtained in gonorrheal subjects and consequently is devoid of reliance, a positive reaction is most dependable and was not obtained in a large series of infectious and other diseases.

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In this article, in view of the finding of many Gram-positive and Gram-negative bacteria in the urine after massage of the prostate gland and seminal vesicles in the involvement of these organs in neisserian infection, a study has been made with respect to determining the specificity of the gonococcus antigen in the complement-fixation test by employing non-specific antigens made up from the various bacteria isolated from time to time.

Antigens were prepared from the following micro-organisms and utilized routinely in a series of serums from gonorrheal subjects:

Nine strains of gonococci.
Fifteen strains of meningococci.
Six strains of streptococci.
Six strains of the Micrococcus albus.
Six strains of the pneumococcus.
Six strains of Micrococcus aureus.
Three strains of the Micrococcus catarrhalis.
Six strains of Corynebacterium pseudodiphtheriticum.
Six strains of Bacillus coli.

TECHNIC OF THE PREPARATION OF THE ANTIGENS

As in the former work the best results were obtained with antigens prepared in the following manner:

Forty-eight-hour old cultures were washed off in sterile distilled water, shaken for one hour, and autolyzed for twenty-four hours in a thermostat at the temperature of 37 C. and heated in a water-bath at 60 C. for one-half hour. Before use this antigen is diluted 1:10 by the addition of 0.85 per cent. salt solution. The quantities of each antigen used is determined by preliminary standardization. The technic on which we have learned to place the greatest reliance is essentially the same as that employed by us in the performance of the Wassermann reaction — substituting the specific or non-specific antigen in each case for the syphilitic antigen, using always the carefully standardized single unit of complement and the routine standardization of antigen and amboceptor. This technic is fully described in the former paper on this subject.

Two hundred and sixteen serums in all were tested by the employment of various non-specific antigens. These added to the results of the previous work number 420 serums in which the complement-fixa-

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2. We are indebted to Dr. Parks of the Research Laboratory, New York Department of Health, for the fifteen strains of meningococci and the three strains of Micrococcus catarrhalis, and to Dr. A. P. Hitchens of H. K. Mulford Company for the nine strains of gonococci employed in this work.
tion test has been employed, using specific gonococcus and non-specific antigens.3

Of the 216 cases in which both the specific and non-specific antigens were employed, we have grouped the cases according to their clinical diagnoses.

1. Patients clinically cured, 9 cases.
2. Acute anterior urethritis, 10 cases.
3. Acute and subacute anteroposterior urethritis, 40 cases.
4. Chronic posterior urethritis, 84 cases.
5. Stricture, 7 cases.
6. Epididymitis, 30 cases.
7. Arthritis, 30 cases.
8. Gynecological affections, 3 cases.
9. Vulvovaginitis, 1 case.
10. Sexual impotence, 2 cases.

Results of Complement-Fixation Reactions with Specific Gonococcic and Non-Specific Antigens in Two Hundred and Sixteen Cases

<table>
<thead>
<tr>
<th>Antigens</th>
<th>No. Cases</th>
<th>No Positive</th>
<th>No. Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific: Gonoroccus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonvalent</td>
<td>216</td>
<td>67</td>
<td>149</td>
</tr>
<tr>
<td>Parke, Davis &amp; Co.</td>
<td>216</td>
<td>67</td>
<td>149</td>
</tr>
<tr>
<td>Non-Specific:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micrococcus catarrhalis</td>
<td>180</td>
<td>5</td>
<td>175</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>216</td>
<td>4</td>
<td>212</td>
</tr>
<tr>
<td>Micrococcus aureus</td>
<td>216</td>
<td>3</td>
<td>213</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>216</td>
<td>1</td>
<td>215</td>
</tr>
<tr>
<td>Corynebacterium pseudodiphtheriticum</td>
<td>160</td>
<td>1</td>
<td>139</td>
</tr>
<tr>
<td>Meningococcus</td>
<td>216</td>
<td>1</td>
<td>215</td>
</tr>
<tr>
<td>Micrococcus albus</td>
<td>216</td>
<td>...</td>
<td>216</td>
</tr>
<tr>
<td>Bacillus coli</td>
<td>160</td>
<td>...</td>
<td>160</td>
</tr>
</tbody>
</table>

Of this series of cases 135 serums gave negative results with the employment of specific and non-specific antigens.

Sixty-seven serums gave positive results with specific gonococcus antigens.

Fifteen serums gave positive results with non-specific antigens.

Of the complement-fixation tests, using non-specific antigens, the Micrococcus catarrhalis antigen gave positive results in 5 cases, the pneumococcus in 4, the Micrococcus aureus in 3, the streptococcus in 1, the Corynebacterium pseudodiphtheriticum in 1 and the meningococcus in 1 case.

3. Standardization of all the specific and non-specific antigens at the conclusion of this study as compared with their antigenic properties in the beginning demonstrated no deterioration.
Four of the foregoing non-specific antigens gave positive results when all other non-specific and specific antigens resulted negatively. They were:

1. Pneumococcus, 4 cases.
2. M. aureus, 3 cases.
3. M. catarrhalis, 1 case.
4. Corynebacterium pseudodiphtheriticum, 1 case.

Six of the foregoing fifteen non-specific fixation reactions occurred conjointly with the specific fixation reaction. They were:

The Micrococcus catarrhalis, 4 cases.
The streptococcus, 1 case.
The meningococcus, 1 case.

Our explanation for these occurrences in the complement-fixation reaction is that frequently a mixed infection complicates the gonorrheal urethritis, prostatitis, seminal vesiculitis, etc., also that not infrequently the gonococcus has ceased to be viable and that the active cause for the inflammation is a superimposed bacterium.

CONCLUSIONS

1. Although mixed infections are commonly found, antibodies of the non-specific organisms rarely bind complement in the presence of the non-specific antigens, and when such is the case, it can be attributed to the implantation of a superimposed mixed infection.

2. The specificity of the gonococcus complement-fixation test when positive in cases of neisserian infection seems to be clearly established; a negative reaction, on the contrary, means absolutely nothing from the clinical point of view.

3. Those organisms in cases of mixed infection capable of binding complement in our studies have been the Micrococcus catarrhalis, the pneumococcus, the Micrococcus aureus, the streptococcus, the Corynebacterium pseudodiphtheriticum and the meningococcus.

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