Agar-Gel Precipitin-Inhibition Technique for C-Reactive Protein Determinations

III. Quantitation of C-Reactive Protein in Serum Specimens

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Received for publication 21 September 1964

ABSTRACT

SHAY, DONALD E. (University of Maryland School of Dentistry, Baltimore), and JOHN G. RAY, JR. Agar-gel precipitin-inhibition technique for C-reactive protein determinations. III. Quantitation of C-reactive protein in serum specimens. Appl. Microbiol. 13:305-307. 1965.—A quantitative C-reactive protein serological procedure has been developed. By use of this method, which is performed in agar-gel plates, from 2 to 654 μg of C-reactive protein per ml of titrated human serum can be detected. The method is based on the inhibition of a specific C-reactive protein antigen-antibody precipitate formed in agar-gel by the minimal reactive dilutions of each reagent in 48 hr. It is simple, sensitive, and readily reproducible.

Wood and McCarty (1951), Librettii, Kaplan, and Goldin (1955), Rapport and Graf (1956), and Fukuda, Heiskell, and Carpenter (1959) employed various techniques in an effort to quantitate the C-reactive protein (CRP) in human acute-phase serum samples.

The agar-gel precipitin-inhibition (AGPI) technique described by Ray and Shay (1965) evidenced a high degree of sensitivity and reproducibility, and it was decided to employ this method to quantitate the CRP in acute-phase positive serum specimens.

MATERIALS AND METHODS

The procedures used were described previously (Ray and Shay, 1965). The block titration of the reagents in agar-gel determined the sensitivity of the test procedure.

RESULTS

The protein content was determined by the micro-Kjeldahl technique, with the use of a lyophilized crude CRP preparation (BBL). The material was amorphous in character and was not like the crystalline rhomboid plates described by McCarty (1947).

Because the product was assumed to be impure, the lipid and carbohydrate contents were ascertained by extraction with alcohol-ether. The material remained at the interphase, and no phosphorus was detected. The Molisch and Anthrone tests were negative for reducing sugars. The material was soluble in water and was antigenic to rabbits, and produced an antiserum corresponding to that obtained commercially. It did not cross-react with normal human serum components. Ninhydrin and biuret tests were positive, indicating the presence of amino acids in a peptide linkage.

Micro-Kjeldahl determinations on lyophilized CRP. Three portions each of two lots of lyophilized CRP product were analyzed for total nitrogen and nonprotein nitrogen by the micro-Kjeldahl method. Lot 1 assayed 1.9% total nitrogen and 0.145% nonprotein nitrogen. Thus, 1.0 mg of the lyophilized product contained 110 μg of protein, expressed as CRP.

The second lot contained 2.56% total nitrogen and 0.13% nonprotein nitrogen. Therefore, 1.0 mg was equivalent to 152 μg of protein. The two lots equated to an average of 131 μg of CRP per mg of lyophilized product, for quantitative purposes.

Reaction of lyophilized CRP preparations in agar-gel. Milligram portions of the lyophilized preparation were dissolved in 1 ml of physiological saline and titrated by the recommended AGPI method. The titers of these 0.5-, 1.0-, 1.5-, 2.0-, and 3.0-mg portions were equated to the amount of CRP in milligrams per 0.2 ml of physiological saline-reconstituted CRP (Table 1).
TABLE 1. Computation of the content of C-reactive protein in titratable human serum based on micro-Kjeldahl and agar-gel precipitin-inhibition (AGPI) analyses

<table>
<thead>
<tr>
<th>Lyophilized prepn</th>
<th>AGPI titer*</th>
<th>Protein (micro-Kjeldahl)</th>
<th>Protein in 0.2 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>a/b</td>
<td>mg/ml</td>
<td>μg</td>
</tr>
<tr>
<td>0.5</td>
<td>1:8</td>
<td>65</td>
<td>13</td>
</tr>
<tr>
<td>1.0</td>
<td>1:16</td>
<td>131</td>
<td>26</td>
</tr>
<tr>
<td>1.5</td>
<td>1:32</td>
<td>196</td>
<td>39</td>
</tr>
<tr>
<td>2.0</td>
<td>1:64</td>
<td>262</td>
<td>52</td>
</tr>
<tr>
<td>3.0</td>
<td>1:128</td>
<td>392</td>
<td>78</td>
</tr>
</tbody>
</table>

*Therefore, a 1:4 titer should contain 0.5 μg of CRP per 0.2 ml, a 1:2 titer should contain 3.3 μg of CRP per 0.2 ml, and a 1:1 titer should contain 1.6 μg of CRP per 0.2 ml.

The titrations of three more successive weighings indicated that a sample of 1.0 mg/ml, when used in 0.2-ml quantities, contained 26 μg of the CRP. The degree of sensitivity of the test in the detection of 1.6 to 3.3 μg of CRP per 0.2 ml of human serum can be noted.

These titrations should compare with the amount present in the serum specimen if the specimen has the same titer as the lyophilized CRP preparation, and all of the protein in the lyophilized preparation is considered as CRP reacting with its homologous antiserum.

DISCUSSION

The determination of the CRP in human sera, relative to the acute stages of many diseases, has proved to be a valuable diagnostic aid, as well as the prognosticator of the disease process and an indicator of correct therapeutic treatment.

Micro-Kjeldahl analysis revealed that a 1.0-mg sample of lyophilized amorphous material contains 26 μg of CRP per 0.2 ml of physiological saline-reconstituted CRP. This is shown in Table 1, which also shows the amount of CRP contained in 1 ml of serum to be 131 μg, providing that the serum shows a titer of 1:16 by the AGPI test. Likewise, a serum titer of 1:32, when obtained by the AGPI method, would indicate the presence of 196 μg/ml, or 39 μg per 0.2 ml, of CRP in the serum specimen.

The sensitivity of the AGPI method is calculated to detect between 1.6 and 3.3 μg of C-reactive protein per 0.2 ml of serum, depending upon the accuracy of the serological dilutions. The quantitative serological tests advocated by Wood and McCarty (1951) and Rapport and Graf (1956) detected from 2 to 6 μg of CRP in 1.0 ml of serum; thus, they appear to be more sensitive than the recommended AGPI method. However, Fishel, Vedros, and Rothlauf (1960) showed that, when acute-phase serum is treated with C-polysaccharide, complete removal of the CRP does not occur. Positive CRP serum, when subsequently absorbed with the C-polysaccharide, still produced a positive reaction in the range of a trace amount to 4 mm by the capillary tube method. This may be explained in part by the occurrence of a C-polysaccharide antibody in the serum.

The reported range of detectable CRP in the previous quantitative tests is from 0 to 360 μg/ml of serum. The theoretical range of the AGPI test is from 0 to 654 μg of CRP per ml of serum, based on a titer of 1:512.

In summary, a quantitative method is described for the serological determination of CRP in human serum, varying in quantities from 0 to 654 μg/ml. This test procedure is simply performed, has a high degree of sensitivity, and appeared to be completely reproducible when employed by several technicians who are unfamiliar with the method.

The main deterrent of this recommended test procedure is the equipment required for its performance in the clinical laboratory. Otherwise, once the test sensitivity has been determined by block titration of the reacting agents, any soluble diffusible antigen-antibody system may be adapted to this agar-gel procedure, eliminating the use of an indicator system.

ACKNOWLEDGMENTS

Acknowledgment is made to Marvin H. Rosenfeld for supplying the C-reactive protein and to John M. Ladino and Joseph M. Lynch for biochemical analyses.

LITERATURE CITED


