Reversal of the Bactericidal Reaction of Serum by Magnesium Ion

LOUIS H. MUSCHEL and JEAN E. JACKSON

Department of Microbiology, University of Minnesota, Minneapolis, Minnesota

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ABSTRACT

MUSCHEL, LOUIS H. (University of Minnesota, Minneapolis), and JEAN E. JACKSON. Reversal of the bactericidal reaction of serum by magnesium ion. J. Bacteriol. 91:1399–1402. 1966.—Magnesium ion was found to reverse the bactericidal action of the antibody-complement system. Concentrations of 0.03 to 0.11 mM MgSO4 or MgCl2 were effective, provided that the reaction of the antibody-complement system did not proceed longer than 50 min. Salts of other monovalent and divalent cations, the polyamine, spermine, and several anticomplementary substances were inactive in reversing the serum bactericidal reaction. Thus, the bactericidal reaction of serum may be bacteriostatic under certain conditions, and this finding is compatible with the persistence of infection in certain diseases despite high levels of antibody.

In the older literature, antibodies that lysed bacteria in conjunction with complement were called bacteriolysins, and antibodies that killed them without visible lysis were called bactericidins. Despite this distinction, it was difficult in practice to distinguish between them, and generally it was only the bactericidal effect which was determined. Methods in current use for estimating the number of bacteria surviving serum action do not distinguish actual lysis of the cell from any reaction which merely inhibits multiplication (7). When gram-negative bacteria have been subjected to the action of serum antibody and complement, or their growth has been inhibited in certain other ways, these bacteria may be lysed by the enzyme lysozyme, or, in the presence of stabilizing media, converted to spheroplasts (8). For the estimation of immune bacteriolysis, a method based on the photometric determination of released nucleic acid has been developed (1). Obviously, lysis results in death, but the possibility of a bacteriostatic effect as opposed to a bactericidal effect without obvious cellular disintegration can never be rigorously excluded, since bactericidal agents may turn out to be bacteriostatic when an appropriate reversing agent is found.

Preliminary experiments in our laboratory indicated that, when Salmonella typhosa O901 was subjected to the action of antiserum and complement and the surviving organisms were assayed by plate counts, an increased number of colonies did not result, even when the plates were examined as long as 7 days after the reaction. Under the limited conditions of this experiment, the effect of the antibody-complement system seemed to be bactericidal. Different experimental approaches were subsequently attempted to determine whether the bactericidal reaction of serum may be bacteriostatic. Heparin, an anticomplementary agent, magnesium ion, which stabilizes spheroplasts of gram-negative bacteria (3), and spermine, which reduces the osmotic fragility of lysozyme-ethylenediaminetetraacetic acid (EDTA) spheroplasts (4), were tested to determine their capability in reversing the action of the antibody-complement system.

MATERIALS AND METHODS

Photometric growth assay. The quantitative photometric growth assay method (10) for the determination of the surviving organisms of the bactericidal reaction was used as originally described, except that the reaction of antiserum and complement with the test organism, S. typhosa O901, was generally limited to 25 instead of 60 min. Also, 0.05 ml of untreated normal guinea pig serum was used as a complement source instead of larger amounts of specifically absorbed serum. In most experiments, two different amounts of antiserum were used to provide a range in the percentage of killed organisms. The diluent for those experiments in which the effect of magnesium ion was being studied was 0.146 M NaCl instead of the usual diluent of 0.146 M NaCl and 0.003 M MgCl2*6H2O.

Experimental procedures. Appropriate amounts of
antiserum, normal guinea pig serum as a complement source, and diluent were mixed with the test culture in triplicate along with controls of antiserum, complement, and diluent, each alone with the culture. After a suitable period (usually 25 min) at 37 °C, the number of surviving organisms in one of the sets was assayed by subculture in broth. At that time, the substance being tested for its possible effect in reversing the bactericidal effect of serum substances was added to the second set, and those mixtures were allowed to incubate for an additional 5 min prior to subculture and assay for the number of surviving organisms. In addition, the triplicate set without the test material was assayed after a reaction period of 30 min, so that for each experiment there were two control sets, with the reaction period in one being 5 min longer than in the other.

It was observed that the divalent cations calcium and strontium formed a precipitate with the Brain Heart Infusion Broth (Difco) usually used for the cultivation of the test organism. Therefore, in testing the effect of salts of these ions, nutrient broth was used, and the surviving organisms were assayed by plate count on meat extract-agar.

RESULTS

Preliminary tests indicated that the addition of magnesium salts was capable of reversing the bactericidal reaction of serum. Accordingly, MgSO₄, giving a final concentration of 0.11 m, was added to the mixtures of antiserum, complement, and culture after 25 min. The surviving organisms were assayed after 5 min (Table 1).

The results indicated that MgSO₄ is capable of partially reversing the bactericidal action of the antibody-complement system. The greater survival in the control set with a 25-min reaction period compared to the control set with a reaction period of 30 min indicated that the bactericidal reaction was not completed at 25 min, and that the reversal of the bactericidal reaction may have been effected while the reaction was still in progress in the absence of the added salt. The possibility that the MgSO₄ itself may have influenced the survival and growth of S. typhosa was excluded by additional control tubes. Lower concentrations of Mg ion (0.03 m MgSO₄) were almost as effective as 0.11 m, but a concentration as low as 0.01 m gave a barely detectable effect. Similar results were obtained with MgCl₂, but not with Na₂SO₄, indicating that the reversal of the bactericidal reaction may be attributed to Mg ion.

It was of interest to determine the relationship between the length of time of the bactericidal reaction and its reversibility by Mg ion. The results noted in Table 2 indicate that there was no detectable bactericidal effect after 6 min and, therefore, no possibility of reversal at the time. The reaction was reversible after it had been in progress for as long as 50 min. No evidence of reversibility, however, was observed after 100 min.

Magnesium sulfate was also capable of partially reversing the bactericidal activity of normal guinea pig and human serum against both S. typhosa O901 and Escherichia coli Lilly. The latter is a rough organism that is extremely sensitive to serum (9). Thus, the action of fresh normal serum, contributing both "normal antibody" and complement, against gram-negative bacteria was tested in Table 2.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Complement</th>
<th>Per cent survival of Salmonella typhosa O901</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test* with 0.11 m MgSO₄ Controls (no Mg ion)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Per cent survival at 25 min† 30 min‡</td>
</tr>
<tr>
<td>ml</td>
<td>ml</td>
<td>25 min† 30 min‡</td>
</tr>
<tr>
<td>2.6 × 10⁻⁴</td>
<td>0.05</td>
<td>56   19   7</td>
</tr>
<tr>
<td>1.3 × 10⁻⁴</td>
<td>0.05</td>
<td>96   57  17</td>
</tr>
<tr>
<td>None</td>
<td>0.05</td>
<td>100  100 100</td>
</tr>
<tr>
<td>2.6 × 10⁻⁴</td>
<td>None</td>
<td>100 100 100</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>100 100 100</td>
</tr>
</tbody>
</table>

* Reaction period of 25 min, addition of MgSO₄, and assay of surviving bacteria after 5 min.† Reaction time.

**TABLE 2. Relationship between the time of the bactericidal reaction and its reversibility by magnesium ion**

<table>
<thead>
<tr>
<th>Reaction time</th>
<th>Test with 0.11 m MgSO₄</th>
<th>Control (no Mg ion)</th>
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<tbody>
<tr>
<td>min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100† 100</td>
<td>100 100</td>
</tr>
<tr>
<td>12</td>
<td>100 86</td>
<td>100 100</td>
</tr>
<tr>
<td>25</td>
<td>52 37</td>
<td>78 68</td>
</tr>
<tr>
<td>50</td>
<td>11 0</td>
<td>38 27</td>
</tr>
<tr>
<td>100</td>
<td>0 15</td>
<td>12 15</td>
</tr>
</tbody>
</table>

* The volume of complement (0.05 ml) was the same in all tests.
† Represents per cent survival with 1.6 × 10⁻⁴ and 0.8 × 10⁻⁴ ml of antiserum, respectively.
also may be bacteriostatic under these conditions.

Many other substances tested over wide concentration ranges were not able to reverse the bactericidal reaction of serum. These included anticomplementary substances such as heparin, dicumarol, sodium citrate, sodium oxalate, and EDTA. Other materials tested, but also without effect, were dextrose, sucrose, ovalbumin, gelatin, heated guinea pig serum, polyethylene glycol, sodium sulfate, potassium chloride, ammonium chloride, strontium chloride, and calcium chloride. Finally, concentrations of spermine phosphate and spermine tetrahydrochloride (10^{-3} to 10^{-4} M) were without effect in reversal of the bactericidal reaction.

**Discussion**

These results indicate that the action of the antibody-complement system upon gram-negative bacteria may not inevitably result in a bactericidal effect, but that under certain experimental conditions the injury sustained by these organisms may be repaired by magnesium ion. Many other substances, including anticomplementary agents, polyamines, proteins, and other monovalent and divalent cations, were without demonstrable effect under those conditions in which magnesium ion gave a readily demonstrable reversal of the bactericidal reaction. It is interesting to note that the action of complement, which requires a small concentration of magnesium ion (6), may be partially reversed in the bactericidal reaction by the addition of larger amounts of that ion.

Gram-negative organisms such as *S. typhosa* may be eliminated from the body, not only by phagocytes, but by the immune bactericidal reaction. Specific immunity to typhoid fever probably depends on the presence of antibodies, but whether these act mainly by opsonization or by sensitization of the organism to the action of complement is still doubtful. It is conceivable that the action of the antibody-complement system does not invariably result in a bactericidal effect, and that body fluids may provide protective substances that behave like magnesium ion in vitro. The paradox of continued infection in diseases such as brucellosis or typhoid fever, despite the presence of high levels of bactericidal antibody, has been attributed generally to the intracellular growth of the microbes (2). An additional factor in the survival of these organisms may be related to the fact that the antibody-complement system may result in bacteriostasis. Of course, some very effective antibiotics are bacteriostatic in their action, so that these considerations need not contradict the possible contribution of the antibody-complement system to host defense mechanisms. On the other hand, it may help to explain certain stubborn infections with microbial persistence.

By analogy with the immune lysis of red cells, it may be postulated that the action of complement upon sensitized gram-negative bacteria is directed primarily against the cell membrane. The locus of the antigen-antibody complex which activates complement is probably on the cell wall, but the complement target may be the cell membrane (7). It is well established that complement-mediated red-cell lysis may result from the combination of antibody with surface antigens, which need not be integral components of the red cells (5). This fact suggests that the antigen-antibody reaction serves merely to concentrate complement at a susceptible structure. Antigens of the cell wall or capsules of gram-negative bacteria may act, therefore, like an adsorbed antigen on the cell membrane. In both hemolysis and bactericidal action, the damage that results in red-cell lysis or bacterial cell death may result from membrane damage caused by an enzyme of complement, or by activation of enzymes in the cells themselves. Lysis of the bacterial cell results, then, from further enzymatic attack upon the cell wall by other agents, such as lysozyme (8). The action of magnesium in reversing the action of serum bactericidal reaction lends support to this concept. Magnesium ion also protects *Aerobacter aerogenes* and other gram-negative organisms against the lethal effect of chilling (11), and is effective in stabilization of spheroplasts of gram-negative bacteria (3, 8). These effects of the metal may be related to its effect in preventing the release of lipid residues of the plasma membranes (12), thereby preventing a loss of permeability control by these membranes. Magnesium has proved to be uniquely effective in cold shock, the stabilization of spheroplasts, and in reversal of the bactericidal reaction, probably as a result of its action in membrane stabilization.

**Acknowledgment**

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**Literature Cited**


3. Lederberg, J. 1956. Bacterial protoplasts induced


