THE PARALLEL EFFECTS OF MAGNESIUM ON THE COMPLEMENTARY AND COAGULATIVE ACTIVITIES OF BLOOD SERUM

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The close relationship between the role of calcium in the clotting and complement activities of serum has already been shown. More recent studies have demonstrated the effect of magnesium in the hemolytic activity of complement and in the clotting reaction. Since it is well known that magnesium salts do not replace calcium in the clotting process, this work suggested that the part of magnesium be further explored. It has been found that the effect of magnesium ions in both phenomena is parallel, either alone or in the presence of such antagonists as human serum and disodium phosphate. The practical importance of ionized magnesium salts in complement-fixation tests has also been emphasized by other investigators. Experiments were therefore made to determine whether addition of these salts affects the quantitatively standardized complement-fixation procedure developed in this laboratory.

Materials and Methods

Complement was obtained from frozen pools of guinea pig serum used in routine complement-fixation tests.

Inactivated human serum was a pool of sera inactivated at 56 C. for one-half hour prior to use.

Disodium phosphate was prepared by diluting 1/14th molar solutions with physiologic salt solution.

Magnesium chloride and calcium chloride were similarly prepared from molar solutions of these salts.

Cephalin was phosphatidyl serine prepared by the method of Folch, further purified by reprecipitation from hot methyl alcohol, and dissolved in petroleum ether. It was suspended in distilled water from the dried state as 0.1 per cent solution, was dialyzed overnight, made isotonic, and, diluted 1:10 with physiologic salt solution.

Dioxalated plasma, used for titration of prothrombin activity, was prepared from cell-free 0.1 per cent oxalated plasma obtained by the carotid bleeding of guinea pigs, using paraffined canulee and tubes chilled in ice; nine volumes of blood were collected in one volume of 1 per cent sodium oxalate dissolved in 0.5 per cent sodium chloride. After removal of blood cells at low speed, the plasma was transferred to paraffined tubes and the platelets removed as completely as possible by high speed centrifugation for one to two hours in the refrigerated centrifuge. The horizontal position in the centrifuge should be used and the time required depends on the speed available. A plasma that clots in ten minutes or longer in glass tubes and after optimum recalcification may be used but much more stable plasma is obtainable by these procedures. The dioxalated plasma was prepared by diluting oxalated plasma with four volumes of 0.2 per cent sodium oxalate in physiologic salt solution.

The hemolytic system was prepared from washed 5 per cent sheep cells and antisheep cell amboceptor.

Clotting Technic

The quantity of complement selected for use in clotting tests was one unit as employed in the quantitatively standardized complement-fixation test for syphilis; i.e., the amount required for 50 per cent hemolysis of a standard dose of maximally sensitized sheep cells in fifteen minutes in a water bath at 37 C. Amounts of calcium

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chloride and cephalin were used that, when incubated for six minutes in preliminary titration with one unit of complement in a total volume made up to 0.6 ml. with physiologic salt solution, clotted 0.1 ml. of added dioxalated plasma in ten minutes. In the experiments recorded below, this corresponded to 0.1 ml. of M/400 calcium chloride and 0.1 ml. of 0.01 per cent phosphatidyl serine.

In the tests of the inhibiting effect of inactivated human serum and disodium phosphate and of the enhancing action of magnesium chloride, varying amounts of these reagents were pipetted into the test tubes followed by complement, calcium chloride, cephalin, and physiologic salt solution to a volume of 0.6 ml. The mixture was incubated in the water bath at 37°C for six minutes and 0.1 ml. of dioxalated plasma was then added. The clotting time was recorded in minutes.

Technic of Complement Titration

Two methods of complement titration were employed, one based on the time and the other on the amount required for 50 per cent hemolysis at constant time. Similar results were obtained with both methods but only the former is described since it provides a more convenient comparison with the results of the clotting tests.

Determinations of time of hemolysis were made in a total volume of 2.0 ml. in the calibrated tubes of a Coleman Junior spectrophotometer at wave length 580 μ. The T per cent transmission reading corresponding to 50 per cent hemolysis was determined by measurement of color standards prepared from known proportions of hemolyzed and unhemolyzed cells and an amount of inactivated complement similar to that used in the tests. The readings were made with the cells in suspension. In the preliminary titration, complement was used in amounts of 0.4, 0.2, 0.15, and 0.1 ml. of a 1:25 dilution. Volumes were equalized with physiologic salt solution to 1.6 ml. before the addition of 0.4 ml. of sensitized sheep cells. Incubation was at 37°C in the water bath and the time required for 50 per cent hemolysis was recorded. An amount of complement which required twelve minutes for 50 per cent hemolysis was used in the following experiments. The different amounts of inactivated serum, disodium phosphate, and magnesium chloride were added to this unit quantity of complement, the volumes made up to 1.6 ml. with physiologic salt solution, and 0.4 ml. of sensitized cells added. Readings were made at frequent intervals during incubation and the time required for 50 per cent hemolysis recorded.8

Technic of Quantitative Titration of Syphilitic Sera by Complement Fixation

Method 1 was employed but in one set of tests physiologic salt solution containing 12 micrograms of magnesium per ml. for diluting antigen, amboceptor, and complement, and for equalizing volumes in different tubes of titrations were used.

Results

The effect of magnesium chloride on the inhibition of prothrombin activation by inactivated human serum and by disodium phosphate. Figure 1, curve 1 shows the effect of magnesium chloride on the prothrombin activation of complement. The clotting
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Time was reduced from ten to two minutes by the addition of 0.1 ml. of a M/800 solution of magnesium chloride. Curve 2 shows the effect of magnesium chloride in the presence of an inhibiting dose of inactivated human serum which without magnesium chloride gave a clotting time of fourteen minutes. The inhibitory effect was neutralized by magnesium chloride, maximum activation resulting with 0.18 ml. in three minutes. Curves 3 and 4 show the effect of magnesium chloride on inhibition by disodium phosphate. One-tenth of a milliliter of a M/200 solution of the phosphate showed a clotting time of fourteen minutes. This was reduced progressively with increasing quantities of magnesium chloride to two minutes. With double the amount of disodium phosphate, prothrombin activation of one hemolytic unit of complement was completely inhibited but twice the amount of added magnesium chloride completely neutralized the effect of this dose. These results suggested an equivalent relationship in the effect of these two salts.

![Diagram](image_url)

**Fig. 1.**—Curves 1 and 2: Effect of inactivated human serum on the enhancing action of magnesium chloride in the prothrombin activation of complement.

Curves 3 and 4: The effect of disodium phosphate (Na₂HPO₄) on the enhancing action of magnesium chloride in the prothrombin activation of complement.

**Effect of magnesium chloride on the inhibition of the hemolytic activity of complement by inactivated human serum and disodium phosphate** (figures 2 and 3). The time required for 50 per cent hemolysis in the absence of serum and magnesium chloride was 12.2 minutes. This was increased in the presence of increasing amounts of serum to 18.8 minutes with 0.2 ml. When this dose of inactivated human serum was tested with magnesium chloride in varying amounts, the inhibiting effect of the serum was neutralized by approximately 0.35 ml. of a M/100 solution, and 0.6 ml. resulted in a further enhancement as indicated by the reduction in time for 50 per cent hemolysis to 11 minutes. Similarly, in tests with the same quantity of complement and varying quantities of M/20 sodium phosphate, slight activation was observed with 0.1 ml. and inhibition with larger amounts. Five-tenths of a milliliter required 22.5 minutes for 50 per cent hemolysis as shown in the first part of figure 3. As shown in the second part of figure 3, when varying quantities of magnesium chloride were used with this dose of disodium phosphate, 0.25 ml. of an M/500 solution of magnesium chloride completely neutralized the inhibitory effect.
Effect of the phosphate and further enhancement resulted with increasing quantities up to 0.6 ml.

Effect of magnesium chloride on the titer of syphilitic sera as determined by the quantitatively standardized complement-fixation procedure (table 1). Under the conditions employed in the test a linear relationship is observed between the amount of complement required for 50 per cent hemolysis and the amount of serum tested; the total change in complement activity or total fixation of complement is determined by linear extrapolation. The unit value as determined by titration of complement alone does not represent the unit value under the conditions of the
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test, i.e., in the presence of serum and antigen. The cardiolipin antigen used in these tests has, in itself, little or no effect upon complement. The serum of the test, however, has a variable effect. Therefore the titer is taken as the ratio of the total change to the change resulting from incubation with serum alone. In table 1 it may be seen that the total reaction of serum plus antigen observed with 6 different sera in tests with and without magnesium, varied markedly but the titers when expressed as the ratio

\[
\text{serum + antigen} \quad \text{serum alone}
\]

were essentially the same.

| Table 1.—The Effect of Mg++ Treated Complement in the Quantitative Complement-Fixation Test for Syphilis |
|---|---|---|---|---|---|---|
| Complement unit | Reaction | | | | | |
| Serum no. | Saline | MgCl2 Saline | Serum + antigen | Serum alone | Titer: | Serum + antigen | Serum alone |
| | Saline | MgCl2 saline | Saline | MgCl2 saline | | Saline | MgCl2 saline |
| 174261 | 0.0016 | 0.00096 | 75 | 111 | 1.19 | 1.78 | 60 | 62 |
| 174262 | 0.0015 | 0.0007 | 74 | 99 | 1.31 | 1.54 | 56 | 64 |
| 174263 | 0.0016 | 0.00096 | 72 | 116 | 1.12 | 1.78 | 64 | 70 |
| 184442 | 0.0015 | 0.00088 | 333 | 434 | 1.39 | 2.00 | 234 | 117 |
| 192579 | 0.0015 | 0.00088 | 477 | 751 | 1.78 | 3.00 | 263 | 250 |
| 197004 | 0.0015 | 0.0010 | 331 | 416 | 1.60 | 2.00 | 207 | 208 |

DISCUSSION

The results confirm those of previous investigators in showing the enhancement of the hemolytic effect of complement by magnesium. They demonstrate also a parallel effect of magnesium on the coagulative activity of serum. In both cases the influence of magnesium is inhibited by serum and by disodium phosphate. It has been suggested that the greater effect of magnesium ions over calcium or other cations on the hemolytic activity of complement, implies its greater importance in this phenomenon. Indeed the explanation has been offered that calcium may act by displacement of magnesium from a complex. It should be borne in mind that the opposite may also be true, namely, that the enhancing effect of magnesium ions is due to a sparing of calcium ions from the serum phosphates. The fact that the addition of ionized calcium salts to complement does not increase significantly its hemolytic activity appears to render this explanation unlikely, but it is indeterminate whether or not the addition of amounts of Ca++ equivalent to those that might be liberated as a result of the sparing action of Mg++ would increase the calcium ion concentration of serum. On the other hand, the idea of a sparing effect on calcium seems logical in relation to the clotting phenomenon, in which ionized magnesium salts appear to be inactive in the absence of calcium. The parallel behavior of magnesium in enhancing the clotting and complement activities of serum, and parallel behavior of disodium phosphate and of serum in antagonizing this effect suggest a common cause, whatever it may prove to be.

The use of magnesium chloride may introduce error into complement-fixation tests when the effect on the reaction of syphilitic serum and antigen is not considered in relation to the effect on the reaction of serum alone.
Magnesium chloride enhances the coagulation and complement activities of blood serum in parallel degree. These enhancing effects are inhibited by inactivated serum or by disodium phosphate.

Distortion in the results of complement-fixation tests occurs with the addition of ionized magnesium salts to the system. This is due to the antagonistic effect of the inactivated test serum. In the quantitatively standardized test, however, where the titer is expressed as the ratio of the reaction of serum and antigen to that of serum alone, the findings are the same with or without added magnesium salts.

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