eviscerated rat lacks a pancreas and its hormone, insulin, it is capable of utilizing some glucose, as is readily shown when the organs responsible for the endogenous formation of glucose, *i.e.*, the liver and kidneys, are absent. The rate at which such animal removes glucose from the blood is further accelerated by muscular work. We propose to study the effect of muscle work upon the glucose load which the eviscerated rat can tolerate in the presence and absence of insulin.

*Summary.* In eviscerated and in eviscerated-nephrectomized rats stimulation of the gastrocnemius muscle accelerates the fall of blood glucose and shortens the time of survival.

4 Reinecke, R. M., *Am. J. Physiol.*, 1942, **136**, 167.

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## Enhancing Effect of $Mg^{++}$ on Clotting Activity of $Ca^{++}$

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Recent studies[1] on the activating effect of metallic salts on the hemolytic function of complement led to the conclusion that free bivalent cations were essential to the process and that the importance of $Mg^{++}$ was more decisive than $Ca^{++}$ or other cations studied.

In previous publications we have reported experiments indicating a close correlation between cephalin and $Ca^{++}$ and their role in the coagulative and complementary activities of blood plasma or serum.[2-4] Moreover, in the clotting process the action of ionized calcium salts has been shown to be highly specific in that salts of even closely related elements, strontium and barium, had relatively little effect.

It seemed of importance therefore to determine whether magnesium, although inactive alone, might enhance the coagulative activity of calcium for plasma or serum.

The technic used in these experiments was similar to that described previously[3] except for the use of guinea pig plasma instead of rabbit plasma. Two preparations were employed: a 0.1% cell-free oxalated plasma for determining the direct coagulating effect of $Ca^{++}$ or other ions and a more strongly oxalated and diluted plasma, the dioxalated plasma of Bordet and Delange[5] which is more resistant to clotting by direct recalcification and provides a satisfactory reagent for the detection of "thrombin" activity.

*Oxalated plasma* was obtained by bleeding from the carotid artery over paraffined surfaces into 1% sodium oxalate containing 0.5% NaCl, 1 part to 9 parts of blood, and removal of cells and platelets by centrifugation at 2°-6°C.

*Dioxalated plasma* was prepared by mixing 1 volume of cell-free oxalated plasma with 4 volumes of 0.2% sodium oxalate containing 0.85%NaCl.

The solutions of $MgCl_2$ and $CaCl_2$ were prepared by diluting molar solutions with 0.85% saline.

The cephalin was a 0.01% solution of phosphatidyl serine in 0.85% saline.

In the experiments of Fig. 1, 0.1 ml amounts of the molar concentrations of $CaCl_2$ or $MgCl_2$ indicated were used, the volume was made up to 0.6 ml with saline and 0.1 ml of

1 Mayer, M. M., Osler, A. G., Bier, O. G., and Heidelberger, M., *J. Exp. Med.*, 1946, **84**, 535.

2 Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *J. Immunol.*, 1936, **30**, 417.

3 Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *J. Immunol.*, 1937, **33**, 297.

4 Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *Am. J. Physiol.*, 1937, **119**, 80.

5 Bordet, J., and Delange, L., *Ann. de l'Institut Pasteur*, 1912, **26**, 657.

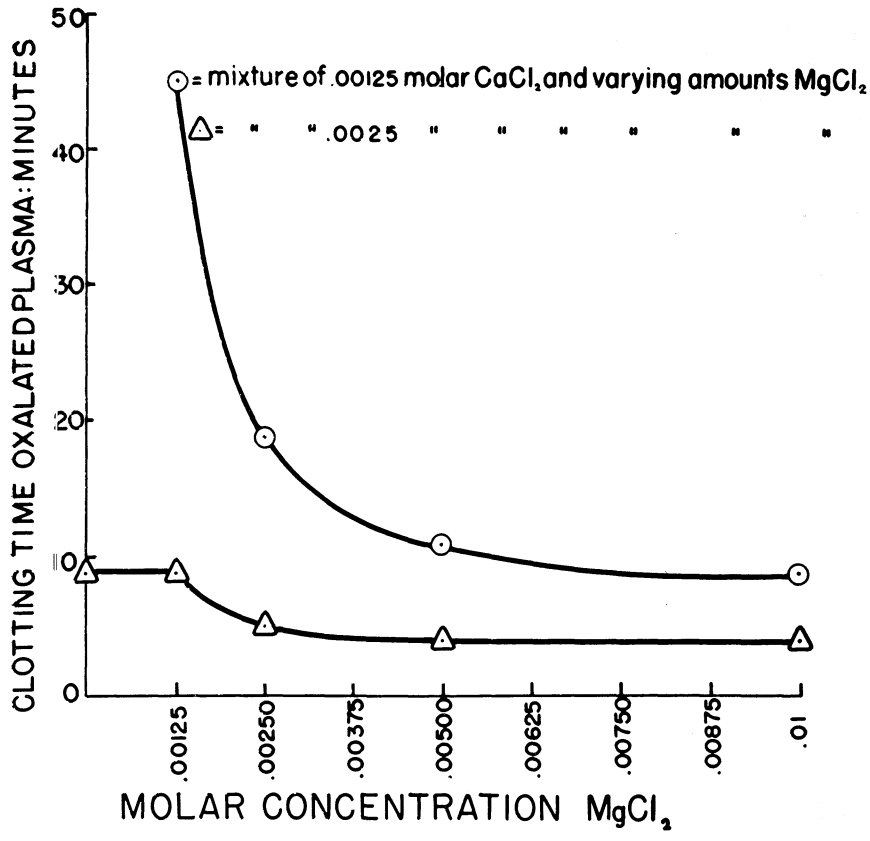


FIG. 1.
The enhancing effect of $MgCl_2$ on the coagulation of recalcified oxalated guinea pig plasma. No clotting was observed with any concentration of $MgCl_2$ without $CaCl_2$ during two hours' observation. 0.00125 molar $CaCl_2$ alone failed to clot within 2 hours.

oxalated plasma was added. Thus the effective molar concentration was one-seventh of that indicated in the figure. The tubes were placed in a 37°C water bath and clotting times determined.

In the experiments of Fig. 2 the quantities of guinea pig complement indicated were pipetted from 1:10 and 1:100 dilutions in saline, 0.1 ml of 0.00125 molar $CaCl_2$ and 0.1, 0.2, 0.3, or 0.4 ml of 0.00125 molar $MgCl_2$ were then added, and finally 0.1 ml of a 0.01% solution of phosphatidyl serine. The volume was brought to 0.6 ml with saline. The effective molar concentration of $CaCl_2$ or $MgCl_2$ was thus one-sixth of that indicated in the figure. Mixtures were incubated 6 minutes in a 37°C water bath, 0.1 ml of the oxalated and diluted plasma was then added, and the clotting time determined. In the absence of complement the indicated amounts of other reagents, either alone or in combination, had no clotting action on dioxalated plasma nor did the complement alone have such activity.

The enhancing effect of $MgCl_2$ on the coagulation of recalcified cell-free oxalated guinea pig plasma is illustrated in Fig. 1.

The effect of $MgCl_2$ on the clotting activity of guinea pig serum (complement) resulting from preliminary incubation of the serum with cephalin and $CaCl_2$, the so-called prothrombin activation, is illustrated in Fig. 2. These results were obtained with minimum quantities of $CaCl_2$. Similar results were obtained when larger doses of $CaCl_2$ were used but the effect of $MgCl_2$ did not appear so great as the optimum dosage of $CaCl_2$ was approached.

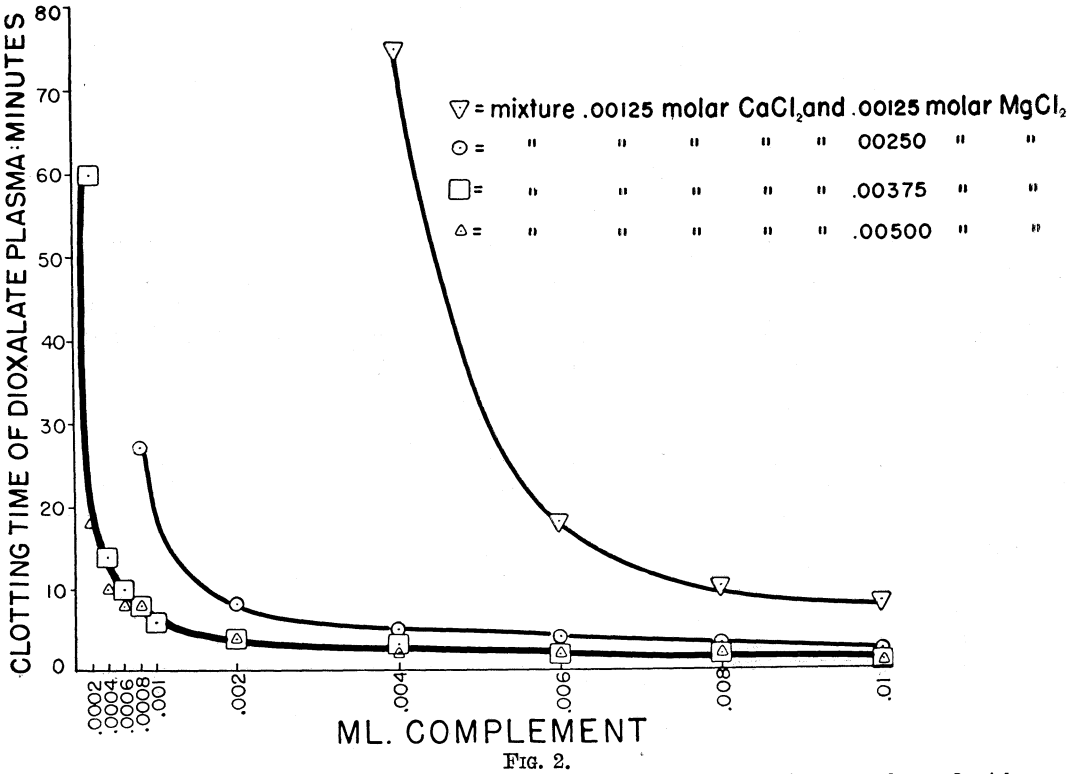


FIG. 2.

Enhancement of clotting activity of serum (complement). No clotting was observed with 0.00500 molar $MgCl_2$ without $CaCl_2$ during 2 hours' observation. 0.00125 molar $CaCl_2$ alone failed to clot within 2 hours.

*Conclusions.* The results of the experiments demonstrated that whereas ionized magnesium salts ($MgCl_2$) alone do not possess the coagulative activity of calcium salts, they do markedly enhance the effect of calcium. The enhancement is more apparent when the Ca ion concentration is relatively low and the concentration of magnesium salts exceeds that of calcium salts. Thus the action of $Mg^{++}$ is secondary or accessory, the more essential and decisive role in clotting being played by $Ca^{++}$.

The parallelism between the enhancing effect of $Mg^{++}$ in the coagulative and the complementary activities of the blood provides additional support for the close relationship of these two phenomena.