other solutions.

Summary. Activation of purified prothrombin in 25% sodium citrate solution can be inhibited by use of a small amount of serum or plasma or barium carbonate eluate. It is postulated that plasma and serum contain an inhibitor(s) that can retard autocatalytic activation of prothrombin.

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Effect of CA^{++} and MG^{++} on Anticomplementary Sera in Complement-Fixation Tests. (23340)

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The observation that mouse liver powder reduces anti-complementary (AC) activity of certain human sera(1) prompted a search for the active principle in the powder. Data have been obtained that indicate that Ca^{++} and Mg^{++}, both of which activate complement (2-8), may be involved.

Materials and methods. Since anticomplementary properties have been attributed to a relative increase in gamma globulin(9-15), 3 lots of commercial gamma globulin were used in the present study. Normal mouse liver powder was prepared and used as described previously(1). The chelating agent for removing Ca^{++} and Mg^{++} from liver powder was an isotonic aqueous solution of ethylene diamine tetra acetate (EDTA), pH 7.8(6). Calcium chloride and magnesium sulfate were used to prepare varying concentrations of Ca^{++} and Mg^{++}. All the reagents were diluted with 0.85% sodium chloride solution(16a). The technic for the complement-fixation test was unchanged(1). AC activity of gamma globulin was measured by its effects on one, two, and three 50% units of complement. According to our standards(1), 85% hemolysis with two 50% units of complement indicates AC activity. Titrations were also made to determine the effect of Ca^{++} and Mg^{++} on the 50% unit of complement(16b). Preliminary incubation for 24 hours at 3-6°C was followed by 30 minutes at 37°C for hemolysis.

Results. Fifty mg of mouse liver powder were shaken with 1-ml amounts each of distilled water, 0.85% sodium chloride solution, alcohol, ether, and acetone for one hour at room temperature to determine the solubility of the active principle. The mixtures were centrifuged, and the sediment and supernate of each dried in a vacuum desiccator over $CaCl_2$. Commercial gamma globulin solution was diluted 1:128 and 1 ml of this dilution, which was known to be AC, was shaken for one hour at room temperature with each of the dried materials, centrifuged, and tested for AC activity. The saline extract was the most effective in removing AC properties from the gamma globulin (Table I).

The active substance in the saline extract was dialyzable through a cellulose membrane.* Passage through an ion exchange

* Visking cellulose sausage casing, purchased from Visking Corp., Chicago, Ill.

TABLE I. Hemolysis Obtained with γ-Globulin Diluted 1:128 and Exposed to Extracts of Mouse Liver Powder.

Solvent	Hemolysis with one, two and three 50% units of complement: Dried supernate 1 u	2 u	3 u	Dried extracted liver powder 1 u	2 u	3 u
Distilled H_2O	15	25	60	15	20	45
.85% saline sol.	*60*	*95*	*100*	*10*	*20*	*55*
95% alcohol	0	10	40	25	35	75
Ether	0	0	15	0	0	10
Acetone	10	75	95	10	15	35
Controls:						
γ-globulin treated with mouse liver powder	25	65	75			
γ-globulin untreated	5	10	30			

Tests were incubated for 24 hr at 3-6°C.

resin (XE64, Na cycle, amberlite 200 mesh) resulted in loss of activity. Heating the dried saline extract to 100°C for 10 minutes slightly enhanced its ability to remove AC properties. The ash of untreated liver powder removed some AC properties.

Several 200-mg portions of mouse liver powder were treated for one hour at room temperature with varying saline dilutions of the EDTA solution, centrifuged, and the liver powder sediment washed 3 times with distilled water. The lowest concentration, 0.002 M solution of EDTA, removed little of the active substance, while the highest, 0.032 M, removed practically all of it.

Thus, the active substance in mouse liver powder appeared to be a divalent cation, possibly Ca^{++} or Mg^{++}. Gamma globulin was diluted with saline solution containing various concentrations of these ions and the AC activity was removed in a manner similar to the action of mouse liver powder. The ions were used in a ratio of 1 part Ca^{++} to 3 parts Mg^{++}. The effect of Ca^{++} alone was much less pronounced than that of Mg^{++}; the maximum effect was obtained when the two were combined.

Thirty-five human unfractionated sera were diluted 1:4 with saline solution containing varying concentrations of Ca^{++} and Mg^{++} and tested for AC activity. These ions markedly influenced activity of the sera with complement, both increasing and decreasing hemolytic activity. Optimum amounts for enhancing hemolytic activity varied with individual specimens. A few examples are shown in Table II.

The optimum amounts of Ca^{++} and Mg^{++} for enhancement of the hemolytic activity of complement alone were determined by including varying concentrations in the saline diluent for each reagent in the hemolytic system. These concentrations were incorporated in the saline diluent for all reagents and 28 human sera were tested for AC activity which was increased in 25 of the 28 sera.

Discussion. The experiments implicate Ca^{++} and Mg^{++} among the components of mouse liver powder most active in removing AC properties from serum. Varying the concentrations of these ions in the diluent for individual sera markedly affected their activity with complement, both increasing and decreasing hemolysis in absence or presence of antigen. From these observations, it is evi-

TABLE II. Results with Individual AC Sera Treated with Varying Concentrations of Ca^{++} and Mg^{++}.

Serum No.	50% units of complement	% hemolysis obtained with varying concentrations of Ca^{++} and Mg^{++}: *Ca: / *Mg:											
		.03 / .09	.023 / .07	.02 / .06	.017 / .05	.013 / .04	.01 / .03	.007 / .02	.00375 / .01125	.0027 / .008	.0017 / .005	.0033 / .001	None (control)
1	1 unit	20			45			30		20	20	15	10
2		40	40	45	50	50	50	50	15				0
3	2	0	10	20	20	45	40	65	70				50
4		5			5			0		0	5	25	40
5		90	90	90	95	90	95	85	55				40
6		85			90			80		70	70	65	45
7		95			80			55		35	30	5	5
8		70	45	50	30	30	30	35	25				5
9	3	50			55			45		15	10	0	0

* These figures relate to molarity. The tests were incubated for 24 hr at 3-6°C.

dent that the powder is superior to the ions alone in that it removes AC properties without a disproportionate drop in the antibody titer of the serum(1).

The ions cannot be used in the concentrations which enhance the hemolytic activity of complement alone because the inclusion of these amounts in all of the reagents involved in complement-fixation tests tends to increase AC activity in the majority of the sera. This same difficulty was encountered by Maltaner and Almeida(17). Some investigators determine the complement unit in the presence of Ca^{++} and Mg^{++} and then increase the amount of complement used in the diagnostic test sufficiently to overcome AC activity(18-21). The concentrations for eliminating AC activity would need to be adjusted for each individual specimen which would not be practical. Further studies may disclose that optimum ranges for different degrees of AC activity can be determined. The effect on specific antibody will need to be studied further.

It seems pertinent to pose the question at this time as to what produces AC activity. Do some sera, lacking Ca^{++} and Mg^{++}, remove these ions from the complement and thus inhibit its activity? The data suggest that artificial replacement of the ions either restores or in some way enhances hemolytic activity of complement to overcome AC activity. Experiments have shown that Ca^{++} and Mg^{++} do not have a lytic effect on either sensitized or unsensitized sheep cells in the absence of complement.

Summary. 1) The action of normal mouse liver powder in removing AC properties from sera appears to be associated with Ca^{++} and Mg^{++}. These ions eliminated AC activity from γ globulin in a manner similar to that of mouse liver powder. 2) Varying the amounts of Ca^{++} and Mg^{++} with individual human sera produced marked changes in their reactivity with complement. The introduction of Ca^{++} and Mg^{++} is less practical at present than the use of liver powder to eliminate AC properties.

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