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The precipitation of diphtheria antitoxin by means of precipitins.

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This work was undertaken partly to check and to review the previous observations by one of us a number of years ago on the precipitation of diphtheria antitoxin from solutions by means of antibodies prepared by injecting diphtheria antitoxic globulin and globulin of normal horse serum into rabbits,[1] partly to determine whether such a method could be used in the further purification of diphtheria antitoxin.

In these earlier experiments it was found that the antibody formed by the injection of globulin of normal serum into rabbits threw out of solution diphtheria antitoxin just as well as the antibody formed by the injection of diphtheria antitoxic globulin.

We undertook these last experiments with the purest diphtheria antitoxin fractionated from the globulin, and serum globulin fractionated in the same way and under the same conditions. The sera were kept at 56° C. for fifteen hours to convert, as far as possible, pseudo-globulin into eu-globulin. The diphtheria antitoxin under these conditions remains unchanged with the pseudo-globulin.[2] The solution was then half saturated with ammonium sulphate and the precipitated globulin filtered off. The precipitate was washed with saturated sodium chloride to remove the pseudo-globulin. The sodium chloride solution was precipitated with acetic acid and again filtered and squeezed between filter paper to remove excess of salts, especially ammonium sulphate, and finally dialyzed to free it as completely as possible from salts. Putrefaction was prevented by the presence of chloroform. The dialyzed globulin in the case of the diphtheria antitoxin was highly concentrated and is the antitoxin of commerce today.

Both sera, antitoxic and normal, were put through exactly the same process in preparing the globulin for injection.

[1] J. P. Atkinson, *Med. News*, 1904, lxxxiv, 375.

[2] Banzhaf, *Proc. of the Soc. for Exp. Biol. and Med.*, 1908, vi, 24.

Injections and development of antisera.—Nine rabbits were immunized against diphtheria antitoxic globulin and eight against normal serum globulin as follows:

Antitoxic globulin, preparation No. 163.

Injections begun March 7, 1909. Three rabbits injected at intervals of three days.
No. 1—4 injections of 2.5 c.c. produced an immune serum of 1/20,000 strength.
No. 2—6 injections of 2.5 c.c. produced an immune serum of 1/20,000+ strength.
No. 3—9 injections of 2.5 c.c. produced an immune serum of 1/30,000+ strength.

Injections begun May 1, 1909; 2 rabbits subcutaneously.

5 injections, each of 2.5 c.c. every 2 days.

Injections begun December 2, 1909; 2 rabbits.

6 injections, each 2.5 c.c.

Normal serum globulin.

Injections begun March 24, 1909; 3 rabbits injected every 3 days.

No. 1—5 injections of 2.5 c.c.
No. 2—5 injections of 2.5 c.c.
No. 3—9 injections of 2.5 c.c.

Injections begun May 1, 1909; 1 rabbit subcutaneously.

5 injections of 2.5 c.c. every 2 days.

Injections begun December 2, 1909; 2 rabbits.

5 injections each of 2.5 c.c.

The rabbits were bled ten days after the final injection. It was not considered necessary to test the strength of the immune sera after the first tests, since it was all needed for the experimental work.

Two explanations are applicable to these reactions.

1. Diphtheria antitoxin is a globulin which is not changed by heat to a form insoluble in saturated solution of sodium chloride and is consequently precipitated by a globulin antibody of the same nature. We have not yet tried the effect of the addition of an antibody for pure eu-globulin on diphtheria antitoxin.

2. Diphtheria antitoxin is carried down mechanically in the precipitation of the globulin by the antibody as a mordant carries a dye out of solution and holds it.

If the first explanation is true then the combination of the diphtheria antitoxin with the antibody is a comparatively loose one, because normal saline solution and glycerine partially separate them, and the precipitate will neutralize diphtheria toxin.

If the second explanation is true, the precipitation of the globulins by means of a precipitin acts as a very complete and powerful mordant, using the term "mordant" in the broad sense of a cleaning agent.

The table shows the results of the addition of immune antitoxic and normal globulin to diphtheria antitoxin.[1]

Preparation of diphtheria antitoxin.	Units per c.c.	Dilution with saline sol.	Immune antitoxic globulin.	Hours at room temperature.	Strength of clear centrifugalized fluid in units.[2]	Per cent. of antitoxin in units in precipitate.[2]	Precipitate extracted with distilled water in units.	Precipitate extracted with glycerine in units.[2]	Precipitate emulsified and tested in units.[2]
163	700	1 c.c. of 1/10+8 c.c.	+1 c.c.	24 hrs.	700				
163	700	" 1/100+5 c.c.	+1 c.c.	24 "	675	4	none.		
163	700	" 1/200+5 c.c.	+1 c.c.	24 "	650	7	"		
163	700	" 1/400+5 c.c.	+1 c.c.	24 "	575	18	"		
163	700	" 1/400	+6 c.c.	24 "	175	75	"		500
			Immune normal glob.						
163	700	" 1/400	+6 c.c.	24 "	175	75	"		500
			Immune antitoxic glob.						
172 B	3000	" 1/3000+	1 c.c.	48 "	300	90	"	725	1500
172 B	3000	" 1/3000+	3 c.c.	48 "	150	95	"	750	1500
			Immune normal glob.						
172 B	3000	" 1/3000+	1 c.c.	48 "	300	90	"	700	2000
172 B	3000	" 1/3000+	3 c.c.	48 "	150	95	"	300	1500
155	850	" 1/200+	No. 3 16 c.c.	48 "	40	95	"	225	500
155	850	" 1/200+	16 c.c.	48 "	40	95	"	225	500
			Gray rabbit.						
155	850	" 1/200+	16 c.c.	48 "	170	80	"	200	400
155	850	" 1/200+	16 c.c.	48 "	170	80	"	200	400

[1] Later experiments showed that the precipitate extracted with sodium chloride yielded some antitoxin. It is evident that a relatively large amount of immune serum must be added to the antitoxin to throw out the antitoxin in anything like quantitative amounts.

[2] Unit strength is calculated on the basis of the undiluted original serum.