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Toxin-Antitoxin Flocculation in Tetanus.

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While the flocculation-test with diphtheria-toxin and antitoxin has given fairly good results,[1, 2] the method has been found unreliable when applied to toxins and respective antitoxins of tetanus,[3, 4, 5] botulinus,[6, 7] staphylococcus,[8] and *Streptococcus scarlatinæ*.[9, 10]

Bronfenbrenner and Reichert[6] found that antitoxin obtained by immunizing animals against toxic filtrates of 24-day cultures of *B. botulinus* precipitated the filtrate while antitoxin prepared against toxic filtrates of 4-day cultures did not precipitate. They concluded that precipitation in the Ramon test may be influenced by the antibacterial antibody in the antitoxic serum. It seemed interesting to see whether these findings might also account for the discrepancies reported[3, 4, 5] in the *in vitro* titration of tetanus-toxin and antitoxin.

Rabbits were immunized with the formolized filtrates of *B. tetani* grown in 1% dextrose-ground beef-broth for 4 and 20 days, respectively, as well as with filtrates of 4-day and 20-day cultures of an atoxic variant of the same strain of *B. tetani*. As expected, the antisera prepared against the nontoxic filtrates were found to be entirely devoid of antitoxin as determined by the protection-test on mice, since as much as 0.5 cc. of undiluted serum failed to protect mice against even one m.l.d. of toxin. The sera containing antitoxin and those obtained against the nontoxic filtrates were used in the precipitation-test with the following antigens: (1) filtrates of 4-day cultures of the toxic and atoxic variants; (2) filtrates of 20-day cultures of the toxic and atoxic variants; and (3) 24-day filtrates of Type "A" botulinus-toxin (control). At the same time *in vivo* neutralization-tests were also carried out, and in the table

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3 Kalic, D. Z., *Compt. Rend. Soc. Biol.*, 1928, **98**, 649.

4 Inoue, T., *Bull. l'Inst. Past.*, 1932, **30**, 246.

5 Schmidt, S., *Ann. l'Inst. Past.*, 1928, **42**, 63.

6 Bronfenbrenner, J., and Reichert, P., *J. Exp. Med.*, 1926, **44**, 553.

7 Tani, S., *Japan. J. Exp. Méd.*, 1934, **12**, 33.

8 Sulkin, S. E. (to be published).

9 Eagles, G. H., *Brit. J. Exp. Path.*, 1927, **8**, 403.

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TABLE I.

	cc.	Antigen (2.0 cc. in each tube).* Filtrate of 4-day culture of toxic strain (10,000 mld per cc.)	Filtrate of 4-day culture of atoxic variant	Filtrate of 20-day culture of toxic strain (20,000 mld per cc.)	Filtrate of 20-day culture of atoxic variant	*Control* 24-day filtrate Type "A" botulinus toxin (2000 mld per cc.)
Antiserum against 20-day filtrate of toxic variant	.08	—	—	+	—	—
	.06	—	—	+	++	—
	.05	—	—	++ (N)	++	—
	.04	—	—	++	++	—
	.03	—	—	+++	+	—
	.025	— (N)	—	++	+	—
	.02	—	—	++	+	—
	.01	—	—	+	—	—
	.008	—	—	+	—	—
	.005	—	—	—	—	—
Antiserum against 20-day filtrate of atoxic variant	.09	—	—	—	+	—
	.07	—	—	+	++	—
	.05	—	—	++	++	—
	.03	—	—	++	++	—
	.01	—	—	+	+	—
	.009	—	—	+	+	—
	.007	—	—	+	+	—
	.005	—	—	—	—	—

(N) = neutral point in protection test.
+++ = copious precipitation.
++ = moderate precipitation.
+ = slight precipitation.
— = no precipitation.

Note: Antisera against 4-day toxic as well as that against 4-day atoxic filtrates failed to precipitate any of the antigens and therefore were omitted from this table.

the letter "N" indicates the neutral point as determined by this method. A wide zone of precipitation occurred when the antitoxic serum prepared against the 20-day formolized filtrate was mixed with either its homologous toxic filtrate or with the 20-day filtrate of the atoxic variant. The antiserum prepared against the 20-day filtrate of the atoxic variant also produced marked precipitation in the presence of both the homologous filtrate and of the 20-day filtrate of the toxic strain. On the other hand, in the case of the antitoxin obtained against the 4-day filtrate of the toxic variant no precipitation occurred in the presence of the various filtrates tested, although this serum neutralized the toxin in the *in vivo* tests. Similarly, no precipitation occurred when the antiserum prepared against the 4-day filtrate of the atoxic variant was combined with the respective antigens.

The results of these experiments indicate that (1) the filtrates of the old cultures of the toxic variant contain bacterial protein in addition to the toxin, thereby stimulating the production of both antibacterial antibodies and antitoxin; (2) that the filtrates of the young cultures of the toxic variant are relatively free from bacterial protein and hence their antisera contain antitoxin and no detectable antibacterial antibody; (3) that precipitation with tetanus-toxin and antitoxin does not result primarily from the union of toxin and antitoxin.

Similar experiments with staphylococcal toxin and antitoxin will be reported subsequently.

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Effect of Dinitrophenol on Agglutinin and Complement Titer.

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The prompt and striking pharmacologic actions, sustained increases in metabolism and body-temperature, produced in man and animals by dinitrophenol, suggested its employment to test the influence of these altered functions on immunologic reactions. Rabbits 1, 2, 3 and 4 were given daily or twice daily, subcutaneous injections of 10 mg. gradually increasing to 24 mg. of dinitrophenol (2.4) mg./k. body-weight over a period of 37 days. The solution was made up in 3% volume with ½ weight of $NaHCO_3$. On the