10514

A Flocculative Reaction of *Perfringens* Toxin and Antitoxin.

A. J. WEIL AND C. H. PARSONS. (Introduced by M. Heidelberger.)

From the Lederle Laboratories, Inc., Pearl River, N. Y.

The assay of *Perfringens* antitoxin depends entirely on tests on the living animal. The only *in vitro* method used is the antihemolysin test, which, however, deals with a fraction of the antitoxic activity often considered of minor importance (Prigge,[1, 2] Le Fort, and Molina;[7] compare, however, the dissenting opinions of Dalling[8] and Weinberg.[4] We are studying this question of parallelism between general antitoxic power and antihemolytic qualities at the present time). Besides the hemolysin there is no toxic function of *Cl. welchii* (type A) known which would give a sure indication of a multiplicity of toxins.

The object of this communication is to report a flocculation of *Perfringens* toxin and antitoxin which seems to parallel the flocculation of other toxins with their antitoxins, such as that of diphtheric toxin-antitoxin.

The main feature of our method is the use of concentrated toxin.* The unconcentrated toxin shows no visible flocculation or even a turbidity if mixed in any usual proportion with the antitoxin. Filtrates of 18-hour broth cultures having a potency of about 50 MLD per cc (intravenous test on mice) were concentrated 16- to 35-fold by ultrafiltration with 8% Parlodion membranes. Slight turbidity of some of the concentrates was removed either by filtration or centrifugation.

The antitoxins used were either unrefined or refined sera of horses being hyperimmunized against *Perfringens* toxin. The refined sera were prepared by Parfentjev's method of partial peptic digestion.[5] The majority of the sera tested contained antitoxin against other

---

1 Prigge, R., *Z. Immunitätsf.*, 1936, **89**, 477.

2 Prigge, R., *Ibid.*, 1937, **91**, 456.

3 Dalling, T., and Ross, H. E., *J. Comp. Path. and Ther.*, 1938, **51**, 235.

4 Weinberg, M., Nativelle, R., and Prévot, A., *Les Microbes Anaérobes*, Paris, 1937, Masson Cié.

5 Parfentjev, I. A., 1936, U.S. Patent 2065, 196; further data, *J. Immunol.*, 1938, **35**, 399.

7 Le Fort, M. P., and Molina, G., *Rev. Inst. Bact. de Chile*, 1937, **6**, 49.

* The use of concentrated toxin to obtain a flocculative reaction was previously applied by Rane and Wyman in their work on streptococcal toxin.8

8 Rane and Wyman, *J. Immunol.*, 1937, **32**, 321.

toxins of the gas-gangrene group and some also tetanal antitoxin, as indicated in Table II.

After some preliminary tests the following procedure was adopted: Sera were diluted with saline solution and used in decreasing amounts, the intervals between being about 20% and the volume being made up to 1 cc. The degree of dilution depends of course on the antibody-

TABLE I.

Example of Toxin-antitoxin Flocculation.

1 cc of *Perfringens* toxin (concentrated 30-fold) was mixed with decreasing amounts of diluted serum as indicated below. Readings were made after 60 to 75 m. incubation at 45°C.

Test tube	Serum cc	Saline cc	Antitoxin 14, diluted 1/5	Antitoxin, 18, diluted 1/5	Antitoxin 28, diluted 1/20	Antitoxin 23, diluted 1/20
1	1.0	—	t	t	t	—
2	0.8	0.2	tt	tt	t	t
3	0.65	0.35	tt	tt	+	tt
4	0.5	0.5	+	(+)	++	++
5	0.4	0.6	t	++	+++	++
6	0.3	0.7	(t)	+++	+	t
7	0.25	0.75	—	++	t	(t)
8	0.2	0.8	—	t	(t)	—
9	0.15	0.85	—	—	—	—
10	0.12	0.88	—	—	—	—
11	0.1	0.9	—	—	—	—
12	—	1.0	—	—	—	—

Data on antitoxins used are given in Table II.
(t), t, tt: degree of turbidity.
(+), +, ++, +++: degree of flocculation.

TABLE II.

Comparison of Mouse Units and Flocculative Units of *Perfringens* Antitoxins.

Antitoxin Unrefined	Mouse-units	Flocculative-Units	Antitoxin Globulin Modified	Mouse-units	Flocculative-units
1. Univalent	50	55	19. Bivalent	700	700
2. "	50	55	20. "	1300	1125
3. "	200	240	21. Multivalent	700	840
4. Bivalent*	50	50	22. "	750	840
5. "	50	50	23. "	750	800
6. "	170	180	24. "	800	830
7. "	105	100	25. "	800	750
8. "	200	180	26. "	800	830
9. "	300	275	27. "	850	750
10. "	300	275	28. "	850	900
11. "	300	360	29. "	900	1000
12. "	600	600	30. "	900	900
13. "	270	250	31. "	950	1000
14. Multivalent†	180	175	32. "	1000	830
15. "	225	230	33. "	1000	950
16. "	275	260	34. "	1000	840
17. "	275	225	35. "	1100	1100
18. "	300	300			

*Bivalent: contains antitoxins against *Perfringens* and *Vibrion septique.*
†Multivalent: contains antitoxins against *Cl. welchii, Vibrion septique, Cl. novyi, Cl. sordelli, Cl. histolyticum.*

content of the serum. In our series of tests, sera below 100 mouse-units[6] were diluted ½, sera between 100 and 500, 1/3 or 1/5, and sera above 500 diluted 1/10 or 1/20. For unknown sera a preliminary test with 100% intervals should be used to find the proper range. Tests with 100% intervals are also helpful in finding the proper range for a new lot of toxin. The addition of 1 cc of concentrated toxin was found suitable in 3 out of 4 toxins. One toxin, concentrated only 16-fold, was used in 2 cc amounts. The mixtures were thoroughly shaken and incubated at 45°C in a waterbath. Turbidity appears rather quickly, particulation becoming visible after 1 to 4 hours. Flocculation-time varies more from serum to serum than between different lots of toxin. Readings were made as soon as flocculation appeared. Where 2 tubes showed the same degree of flocculation the value was calculated by interpolation. The flocculative unitage, X, was computed by the equation $X = S_2U/S_1$, where S_1 = amount (cc) of serum in "indicating" tube, S_2 = amount of standard serum tested in "indicating" tube, U = flocculative units of standard serum allotted arbitrarily as equal to its unitage in mouse-protective units according to the official standard of the National Institute of Health.[6] In 131 tests a very satisfactory correlation between results of the flocculation and mouse-test[6] was obtained. Table II gives the results with the toxin most frequently used. Numerous controls (normal sera, antitoxins against *Vibrion septique,* antibacterial sera, and antitoxic sera other than those of the bacteria causing gas gangrene) were added. None of them showed any trace of reaction.

The flocculate is transparent, gelatinous, similar to the antidiphtheric, but less copious. It sinks quickly, forming a loose agglomerate which is readily redispersed by shaking. This type of particulation is very characteristic. With 3 out of 4 lots of toxin there was only one zone of flocculation. The fourth also reacted in this way during the first month after being made. Later tests showed with some sera a coarse precipitation appearing in lower dilutions of the sera than those which gave the typical flocculation. The type of flocculation alone was sufficient to distinguish this from the correct zone; however, we think that toxin-concentrates showing this phenomenon should not be used in routine work. We are not able to give any explanation for this "coarse precipitate" at the present time.

The investigation of the practical usefulness of the test continues. Agreement on standardization should not be difficult.

The lack of purity of the concentrates with which we are working

[6] Bengtson, I. A., *Public Health Rep.*, 1934, **49**, 525.

at present makes it rather difficult to give any data on the theoretical side of the problem. The close agreement of protective and flocculative values indicates that the flocculation is a true toxin-antitoxin reaction. The agglutinative titer showed no correlation with the flocculative value.

The ratio of serum to toxin as shown by our tests indicates that the actual amount of toxin present in the unconcentrated filtrate of *Cl. welchii* is very small. This would mean that the low toxic qualities of such filtrates—low in comparison with other bacterial toxins—would not have to be ascribed to a relatively weak effect of the toxin but rather to a relatively small amount of toxin produced in broth cultures under conditions which we regard as optimal at the present state of our knowledge.

Summary. A flocculative reaction between the toxin of *Cl. welchii* (Type A) and its antitoxin is reported, which shows close correlation to the mouse-protective values of *Perfringens* antitoxins. Concentrated toxins are used as antigens.

10515 P

Calcium and Cephalin in Relation to the Clotting Power of Crystalline Trypsin.

JOHN H. FERGUSON AND B. NIMS ERICKSON.*

From the Departments of Pharmacology and of Pediatrics, University of Michigan, Ann Arbor.

It is true[1] that crystalline trypsin[2] can clot ordinary citrated plasma and can activate prothrombin without added calcium. As these experimental facts appear to conflict with the statement of Northrop and Kunitz[3] that a trace of ionized calcium is necessary, we have reinvestigated the point minutely, with an enzyme preparation kindly supplied by the Rockefeller workers.

It was found (Table I) that the trypsin is much more active in the presence of added calcium salt and that excess of citrate can inhibit its action. Whereas trypsin alone requires amounts of the order of 1-2 mg to coagulate 1 cc of citrated dog plasma and the clots quickly undergo fibrinolysis, much smaller quantities (0.01-

* Aided by a Sigma Xi research grant.

1 Eagle, H., *J. Gen. Physiol.*, 1937, **20**, 543.

2 Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1932, **16**, 267.

3 Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1935, **18**, 456.