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The flocculation of botulinus toxin antitoxin mixtures.

By J. BRONFENBRENNER and P. REICHERT.

[From the Laboratories of the Rockefeller Institute for Medical Research, New York City.]

Neutral or nearly neutral mixtures of diphtheria toxin and antitoxin give rise to a flocculent precipitate, appearing earliest in those tubes approaching most nearly the point of toxin neutralization. This phenomenon was adapted by Ramon¹ to the *in vitro* titration of antitoxin, a method the value of which has since been confirmed by a number of investigators.

In the course of an attempt to develop an early specific reaction for the diagnosis of botulism it was hoped that the precipitin test might be useful to detect small quantities of toxin in the circulating blood of laboratory animals suffering from this disease. For the production of the precipitating serum, rabbits were immunized with the formalinized filtrates of four day old botulinus cultures. Before the addition of formalin these filtrates contained approximately 100,000 guinea pig MLD per cc.

The sera obtained were titrated in mice, and 0.1 cc. was found to protect against 6,000 fatal doses. These sera, however, were not flocculated by their homologous toxin. Toxins of various age and potency, and at various pH all gave negative results. A sample of botulinus antitoxin received from the New York City Department of Health gave only a weak and irregular reaction; 0.1 cc. of this serum protected a mouse against 10,000 fatal doses. Under parallel conditions the diphtheria toxin antitoxin mixtures gave typical quantitative precipitations.

We had previously observed that precipitation takes place when the antitoxin is combined with the extracts of foods infected with *B. botulinus*,² as well as with bacterial autolysates, although these reactions were found to be not strictly type speci-

¹ Ramon, G., *Compt. rend. de la Soc. de Biol.*, 1922, lxxxvi, 711; *Ann. de l'Institut Pasteur*, 1923, xxxvii, 1001.

² Bronfenbrenner, J., and Schlesinger, M. J., *Proc. Soc. Exp. Biol. and Med.*, 1919, xvii, 24, 25.

fic.³ In the light of these observations the negative results obtained above led us to suspect that the appearance of the precipitate might be due, not to the actual antitoxin content, but to the presence in the serum of an antibacterial antibody. A new set of rabbits was accordingly immunized with the filtrates of 16-24 day old cultures of *B. botulinus* similarly treated with formalin. These older filtrates were actually somewhat less toxic than the four day filtrates, yet the antitoxic sera obtained were flocculated sharply by the original antigen and by other old filtrates. The four day filtrates used above flocculated these sera definitely but more weakly and in a narrower zone.

This is illustrated by the following composite protocol:

Amount of Antigen used in cc.	0.05 cc. of Antiserum to a 4-day toxin		0.05 cc. of Antiserum to a 16-day toxin	
	Flocculated by a 4-day toxin	Flocculated by a 16-24-d. toxin	Flocculated by a 4-day toxin	Flocculated by a 16-24-d. toxin
1.000	—	—	—	—
.750	—	—	—	—
.500	—	—	—	—
.300	—	—	—	—
.200	—	—	—	—
.150	—	—	—	weak
.100	—	—	—	+
.075	—	—	—	+
.050	—	—	—	+
.030	—	—	weak	++
.020	—	—	+	+
.015	—	—	weak	weak
.010	—	—	weak	weak

It is evident that a young toxin, though it be physiologically more potent than an older one and productive in animals of a highly antitoxic serum, may not produce precipitins, whereas a filtrate from a culture old enough to contain presumably a relatively high concentration of bacterial protein in addition to toxin produces a flocculating as well as antitoxic serum when used for immunization of animals.

The use of flocculation for the *in vitro* titration of botulinus antitoxin is now shown, therefore, to be limited by the fact that the flocculating power is not strictly parallel to toxicity but depends upon the presence of bacterial proteins in the antigen. That

³ Bronfenbrenner, J., Schlesinger, M. J., and Calazans, J. C., *Proc. Soc. Exp. Biol. and Med.*, 1921, xix, 21.

a botulinus toxin rendered nontoxic—by formalinization, for instance—still retains its flocculating power constitutes a further restriction. In the case of the titration of diphtheria antitoxins this latter restriction has already been definitely pointed out.^{4, 5}

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Permanence of results obtained by *L. acidophilus* therapy.

By NICHOLAS KOPELOFF.

[From the Department of Bacteriology, Psychiatric Institute,
Ward's Island, New York City.]

Having demonstrated that constipation and diarrhea are alleviated during the ingestion of *L. acidophilus* milk, it is of interest to establish whether such benefits are permanent or transitory. In other words, is it necessary to continue the ingestion of *L. acidophilus* milk in order to maintain the benefits of treatment?

Of 34 cases where daily observations have been carefully recorded after discontinuing treatment, 27 cases, or 80 per cent continued to have more normal defecations; 6 cases, or 18 per cent, had approximately the same number, and 1 case, or 3 per cent, had fewer normal defecations than before treatment. One case which prior to treatment averaged one normal defecation per week, had daily normal defecations *without exception* for 924 days after treatment was discontinued. Three other cases showed benefits over a year after treatment was discontinued, and 10 cases showed benefits from 3 to 6 months afterwards. There were 14 cases where the treatment was of relatively short duration, *i. e.*, about 2 months or less, and the benefits persisted for considerably longer than that time.

While the results of *L. acidophilus* therapy obviously vary from individual to individual, there is here good evidence for believing that in most instances the beneficial effects of *L. acidophilus* persist after treatment has been discontinued.¹

⁴ Glenny, A. T., and Okell, C. C., *J. Path. and Bact.*, 1924, xxvii, No. 2, 187.

⁵ Bayne-Jones, S., *J. Immunol.*, 1924, ix, 481.

¹ Kopeloff, N., *Lactobacillus acidophilus*, 1925, Williams and Wilkins Co. (In press).