

and 0.1 c.c. of antigen. Increasing the amount of antigen beyond this relation tends to delay precipitation, while the employment of equal amounts of serum and antigen will inhibit the reaction in some cases.

133 (1880)

**The use of morphine in connection with serumtherapy of botulism.**

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Some time ago<sup>1</sup> we have observed the delay in the rate of absorption of botulinus toxin in the animals subjected to ether anesthesia. Combining ether anesthesia with the specific serum therapy, we have been able to save animals poisoned with botulinus toxin where antitoxin alone failed to do so.

In view of the fact that the application of ether anesthesia in cases of botulinus poisoning in men would be difficult on account of the pronounced respiratory distress present as a predominant symptom in such cases, we have attempted to find a satisfactory substitute for ether anesthesia.

Among various substances thus far tried with various degrees of success, morphine seems to give the best results. Thus, one-quarter of a cubic centimeter of botulinus toxin (125 M.L.D.) given by the mouth causes death of a guinea pig of 250 grams in from 10 to 12 hours. The administration of an excess of antitoxin intracardially does not save the animal if more than three hours have been allowed to elapse between the feeding of the toxin and subsequent injection of antitoxin.

If guinea pigs of 250 grams are similarly fed with 125 M.L.D. of botulinus toxin and if 0.02 gram of morphine is given to them subcutaneously (in 10 per cent. solution) soon after feeding of the toxin, such guinea pigs die in from 26 to 46 hours if not given any antitoxin. Thus 0.02 gram of morphine delays the death from botulinus poisoning and more than doubles the length of the life of the animal. If antitoxin is introduced intracardially into such

<sup>1</sup> PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 253.

animals (treated with morphine) the animals can be saved even as late as 24 hours after ingestion of toxin.

134 (1881)

**The state of aggregation of particles of botulinus toxin.**

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The fact that one cubic centimeter of botulinus toxin with only 10 per cent. of solids contains at least as many as  $10^{18}$  units of toxin when its hydrogen-ion concentration is adjusted to about  $P_H = 4$ ,<sup>1</sup> suggests that the molecules of this toxin must be very small and of a comparatively simple structure.

On the other hand we have shown<sup>2</sup> that saturation of the toxic filtrate of the culture of *B. botulinus* with  $(NH_4)_2SO_4$  precipitates the toxin, thus suggesting either that toxin is adsorbed by the coarser particles of the precipitate, or that, contrary to the above assumption, the matrix of the toxin is more complex and is capable of being salted out by the  $(NH_4)_2SO_4$ .

In order to determine which is the case a number of experiments were undertaken. While they did not yield as yet any definite answer to the question of the size or the nature of the molecule of botulinus toxin, they disclosed certain interesting, though not as yet correlated facts concerning the properties of this extremely active substance. We feel that these facts are sufficiently interesting to justify our reporting them at this time.

I. Crude toxin kills mice in the dose of  $3 \times 10^{-7}$  c.c. If its reaction is adjusted to about  $P_H = 4$  (at the temp. of  $37^\circ$  C.) its potency is increased so that the M.L.D. =  $3 \times 10^{-18}$  c.c.<sup>1</sup> If one adds to such an acidified toxin a solution of pepsin (at  $37^\circ$  C.), already 5 minutes after the addition of pepsin the potency of toxin is markedly reduced and after four hours of contact with

<sup>1</sup> Bronfenbrenner and Schlesinger, *PROC. SOC. EXP. BIOL. AND MED.*, 1921, xix, 1.

<sup>2</sup> Bronfenbrenner and Schlesinger, *PROC. SOC. EXP. BIOL. AND MED.*, 1921, xviii, 254.