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.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

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 $P_H = 4$,[1] suggests that the molecules of this toxin must be very small and of a comparatively simple structure.

On the other hand we have shown[2] 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 3×10^{-7} c.c. If its reaction is adjusted to about $P_H = 4$ (at the temp. of 37° C.) its potency is increased so that the M.L.D. $= 3 \times 10^{-18}$ c.c.[1] If one adds to such an acidified toxin a solution of pepsin (at 37° C.), already 5 minutes after the addition of pepsin the potency of toxin is markedly reduced and after four hours of contact with

[1] Bronfenbrenner and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 1.

[2] Bronfenbrenner and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 254.

pepsin the M.L.D. of toxin is reduced to that of the original filtrate (M.L.D. = 3×10^{-7} c.c.), but no further reduction of toxicity could be observed during further incubation for 48 hours.

That the reduction of toxicity is not due to peptic digestion follows from the fact that toxicity was only partly destroyed and in fact was reduced exactly to the level at which it existed in the crude toxin. Besides, identical reduction of potency was observed when *heated* (80° C.) pepsin was added to the acid toxin. Neither could the reduction of toxicity be ascribed to spontaneous deterioration of toxin, since the control mixture consisting of toxin and acid buffer alone maintained its high toxicity to the end of the experiment (48 hours). On the other hand the failure of pepsin to completely digest the toxin is not due to the possible presence in the toxin of some inhibiting substance, since the activity of pepsin against edestin in the controls proceeded equally well in the presence as in the absence ot the culture filtrate of *B. botulinus.*

Thus the reduction of potency of the acidified toxin in the presence of pepsin is not due to its digestion, but most likely to some physical phenomenon such as ready adsorption (?), which possibly returns the particles of toxin from the state of high degree of dissociation to the more stable state where they exist in a condition of undissociated aggregates.

II. As we have previously stated [1] the crude toxic filtrate of the culture of *B. botulinus* killing mice by the intraperitoneal injection in the amount of 3×10^{-7} c.c. can be rendered so potent by acidification that it will kill mice in the dose of 3×10^{-18} c.c. However, if this crude filtrate is salted out with $(NH_4)_2SO_4$, the precipitate which contains all of the original toxin in a concentrated state *can no longer* be rendered more potent by acidification.

If the tentative assumption that the toxicity of botulinus toxin may depend on the degree of dispersion of its particles is correct, and if in the original toxin the coarser aggregates may be dispersed by acidification, it seems that precipitation with ammonium sulphate renders the particles uncapable of dispersion.

III. If the changes in the structure of botulinus toxin, brought about by the adjustment of its hydrogen-ion concentration are of the nature of an increase of dispersion of its particles, one might

expect that the smaller particles of such a dissociated toxin might dialyze easier than the coarser aggregates of the crude toxin. The experimental evidence indicates the reverse to be the fact. While the crude toxin dialyzes with a comparative ease, the acidified toxin remains quantitatively inside the parchment thimble.

May not this phenomenon be explained on the basis of the theories offered by some physical chemists[3] namely, that while the coarser aggregates of protein carry no charge, the increase in the dispersion resulting upon dilution and especially upon acidification confers the electrical charge on such dissociated particles. In virtue of this charge these smaller particles are adsorbed to the membrane whereas the coarser particles of undissociated substance were able to go through the pores of the membrane.

135 (1882)

The diagnosis of kala-azar by blood culture.

By CHARLES W. YOUNG and HELEN M. VAN SANT (by invitation)

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

The usual method for diagnosis of kala-azar by means of cultures has been from spleen juice obtained by puncture and aspiration with a syringe and needle. Prolonged bleeding-time is fairly constant in advanced kala-azar. The risk of bleeding from the needle wound in the spleen together with the possibility of tearing that organ by a sudden movement of the patient during puncture, makes a safer method for cultural diagnosis desirable.

Meyer and Werner[1] reported in 1914, five successful cultures from a single specimen of blood. Row[2] and Korke[3] each report one. Cornwall and LaFrenais[4] succeeded in seven cases; however,

[3] Robertson, T., Brailsford. The Physiological Chemistry of the Proteins, 1918, p. 153.

[1] Meyer, M., and Werner, *Deutsch. Med. Wchnschr.*, 1914, xl, 67.

[2] Row, R., *Indian Jour. Med. Res.*, 1914, July (quoted in (4)).

[3] Korke, V., idem.

[4] Cornwall, J. W., and LaFrenais, H. M., *Indian Jour. Med. Res.*, 1915–16, iii, 698.