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The Destruction of Botulinum Toxin by Intestinal Bacteria.

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In our studies of the destruction of toxins by bacteria a number of interesting, and perhaps important, observations have been made. Among these the destruction of the toxin of *Clostridium botulinum* by certain intestinal types of bacteria may be worthy of particular note.

Several investigators have shown that if large numbers of detoxified botulinum spores are fed to experimental animals death from botulism will ensue, indicating that, under some conditions, the organisms can grow and produce their toxin in the intestinal tract. Since it is well known that the organisms of botulism are frequently ingested with such foods as raw vegetables, fruits and milk, the question naturally arises as to why the disease is not commonly transmitted in this way. It may also be questioned whether some of the so-called autointoxications are in fact incipient botulism arising from the limited growth of these organisms in the intestinal tract, with the production of sublethal quantities of toxin.

It occurred to us that the probable explanation was to be found in the action of the intestinal bacteria upon the toxins which might be formed in the alimentary tract. In Table I are given data which

TABLE I.

Action of bacteria upon botulinum toxin.

M. L. D. Toxin Injected	1	2	3	4
Controls	+	+		
Bact. coli	—	—	+	+
Bact. communior	—	—	+	+
Bact. aerogenes	—	—	—	+
Proteus vulgaris	—	—	—	—

+ = Death of guinea pig with typical symptoms of botulism.
— = Survival of guinea pig.

indicate the destruction of botulinum toxin by *Bact. coli, Bact. communior, Bact. aerogenes* and *Proteus vulgaris.* In these experiments botulinum toxin (A type) was added to beef infusion broth so that the resulting medium contained approximately 100 m.l.d per cc. This toxin-broth mixture was inoculated with the different test organisms and allowed to incubate at 20° C. for two weeks. Unin-

oculated tubes of the toxin-broth mixture were of course incubated at the same temperature over the same period of time and subjected to the same filtering process in order to serve as controls. The unit amounts of toxin given in Table I are in terms of approximate m.l.d. values as determined on the control tubes after incubation.

The results obtained with these organisms do not reveal a very active destruction of the toxin (not nearly so great as results we have obtained with other organisms) but sufficient, we believe, to explain the fact that the ingestion of small numbers of spores of the organism of botulism leads to no apparent ill effects.

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The Labile Sulfur of Insulin.

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All observers agree that insulin material contains sulfur and Abel[1] has shown that sulfur is present in a very labile form, being split off as a sulfide by boiling with weak alkali. Abel believes that the sulfur is roughly proportional to the hypoglycemic potency. Brand and Sandberg[2] have shown that although the sulfur of cystine is relatively stable, the linkage of other amino acids with cystine so affects the sulfur that it is easily split out by boiling with weak alkali. Cystine can be determined quantitatively by the uric acid reagent of Folin and Denis, as shown by Folin and Looney.[3] The cystine is first reduced by sodium sulfite in saturated sodium carbonate to cysteine which then gives a deep blue color with the phosphotungstic acid reagent. Shonle and Waldo[4] found that insulin preparations respond to this test for cystine.

I have observed that insulin preparations do not give the reaction directly with the uric acid reagent but do so after reduction with sulfite. The insulin material upon boiling for thirty minutes with 0.1 *N* Na_2CO_3 gives a positive reaction without previous reduction, due to the formation of sulfide ion. Acid hydrolysates of the insulin material give the reaction only after reduction with sulfite. They do not, however, give the reaction directly with the uric acid reagent after boiling with 0.1 *N* Na_2CO_3, showing that the sulfur has somehow become more stable in the fragment split off by acid hydrolysis. It is necessary to reduce first with sodium sulfite to obtain the reaction as in the case of the unboiled acid hydrolysate.