

stimuli, objects in the laboratory at which she looked or nearby parts of the apparatus which she handled. No doubt, other factors would explain, in part, her failure to become conditioned sooner.

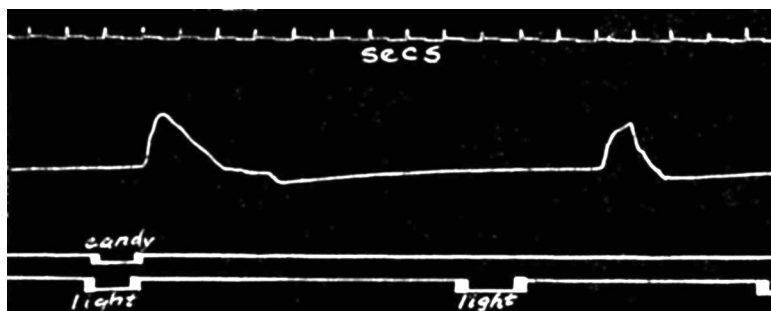


FIG. 1.

In the kymograph record (Fig. 1) the upper line is the time record in seconds, the line next below is the child's response. The third line from the top is the record of the signal lever for the candy stimulus, the fourth line is the record of the signal lever for the light stimulus to which the child is being conditioned. The response at the left of the figure (eighth or tenth joint stimulation) shows the light stimulus ("conditioned stimulus") accompanied ("reinforced") by the stimulus of the candy. The response at the right of the figure ("conditioned response") shows the child's reaction to the light stimulus ("conditioned stimulus") alone after 8 to 10 joint stimulations.

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Destruction of Botulinum Toxin by *Bacillus subtilis*.

C. N. STARK, J. M. SHERMAN AND PAULINE STARK.

From Cornell University, Ithaca, New York.

In previous papers^{1, 2} it has been shown that a variety of bacteria have the ability to destroy the toxin produced by *Clostridium botulinum*. This power, though definite, is relatively slight as compared with the ability of certain organisms to destroy the toxin

¹ Sherman, J. M., Stark, C. N., and Stark, Pauline, *PROC. SOC. EXP. BIOL. AND MED.*, 1927, xxiv, 546.

² Sherman, J. M., Stark, C. N., and Stark, Pauline, *J. Bact.*, 1928, xv, 35.

of the diphtheria organism.³ In our work on the destruction of the toxin of botulism there has not appeared to be any general relationship between the proteolytic activities of the organisms tested and their ability to destroy botulinum toxin. In fact, it has been found that such an active proteolytic organism as *Clostridium sporogenes* has little or no demonstrable capacity to destroy this toxin, under the conditions of our experiments.⁴ On the other hand, Dack,⁵ working with mice, showed a definite destruction of botulinum toxin by several strains of *Clostridium sporogenes* and also by a variety of other spore forming anaerobic organisms.

The purpose of this note is to report the results obtained with *Bacillus subtilis*, which shows a more marked destruction of botulinum toxin than does any one of the other organisms with which we have worked.

The toxin was made by growing *Clostridium botulinum*, type A, in a medium composed of: beef infusion, 4% peptone, 0.5% glucose, 0.7% di-basic potassium phosphate, and 0.3% mono-basic potassium phosphate. The culture was grown in this medium for 8 days at 37° C. It was then filtered and the sterility of the filtrate verified by cultural tests. Some of the filtrate was then inoculated with a laboratory culture of *Bacillus subtilis* and incubated for 14 days at 37° C. Other portions of the same filtrate uninoculated were similarly incubated as controls.

Destruction of Botulinum Toxin by *Bacillus subtilis*

	Approximate M. L. D. titer			
	10	25	300	350
Control			+	—
"			+	—
<i>B. subtilis</i>	+	—		
" "	+	—		

+ = Death of 250 gm. guinea pig in 4 days.

— = Survival of 250 gm. guinea pig.

As may be seen from the accompanying table, *Bacillus subtilis* reduced the toxin titer of the botulinum filtrate from about 300 M. L. D. to about 10 M. L. D. per cc. as determined by guinea pig inoculations.

³ Stark, C. N., Sherman, J. M., and Stark, Pauline, *J. Infect. Dis.*, in press.

⁴ Stark, C. N., 1927, Thesis, Cornell University.

⁵ Dack, Gail M., *J. Infect. Dis.*, 1926, xxxviii, 165.