as rendered by this method *without* the selective filter is in close correspondence with the curves recorded by the method of Einthoven. The murmurs are rendered better than by the microphone and the disturbances due to undamped motion of diaphragm and carbon particles being absent, the murmur records look less complicated.

A more detailed description with constants of the instruments and a discussion of results will be communicated after measurements with a galvanometer of higher natural period have been carried out.

89 (1671)

Concerning toxic byproducts of bacillus botulinus.

By J. BRONFENBRENNER, M. J. SCHLESINGER and P. F. ORR.

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

Attempting to shorten the incubation period of botulinus toxin by injecting gradually increasing amounts of toxin into mice, one of us (Orr) has observed early that when the amount of culture filtrate injected reach 0.5 c.c. animals frequently died within a few minutes after injection. Further study of the nature of this intoxication has brought out that when *B. botulinus* is grown on suitable medium there are produced, in addition to the specific toxin, other poisonous products. If such a culture is filtered through a Berkfeld candle and the filtrate treated with alcohol these secondary toxic products remain in solution. The alcoholic extract equivalent to 0.5 c.c. of the original filtrate is fatal when injected intraperitoneally into mice of 17–22 grams.

The first symptoms are noted immediately after injection and consist of restlessness and marked contraction of the abdominal muscles. Within a minute or two the animal shows increased response to external stimuli especially to sharp sound. Shortly the animal becomes prostrated. The increased excitability persists a few minutes longer, the respiration increases in depth and decreases in frequency, and may become as infrequent as 5 inspirations a minute. The animal goes into coma interrupted

by sharp convulsive seizures with contraction of the extensor muscles throughout the body. Death occurs in 5 to 15 minutes after the injection and during one of these convulsive seizures. If less than a lethal dose is injected the animal may exhibit all the symptoms described above but will recover completely in 2 to 4 hours.

The toxic substances responsible for these acute symptoms are quite distinct from the specific botulinus toxin and are not neutralized by the specific antitoxin. Besides we obtained similar products from the cultures of "atoxic" strains of *B. botulinus*, as well as from those of *B. sporogenes*, *B. tetani* and *B. proteus* when these organisms were grown on medium composed of minced meat broth.[1] Moreover, these toxic products are not of the nature of bacterial toxins since they are dialyzable, they act only in very large amounts (0.5 c.c. as compared with 0.002 c.c. of botulinus toxin) and since they exert their action immediately upon injection and not after a period of incubation characteristic of all bacterial toxins. In addition the toxic substances in question are thermostable and are not destroyed even in the autoclave when heated in a sealed tube. In the open container, however, and especially in presence of strong alkali, their toxicity diminishes with coincident volatilization of basic products. From the above it is evident that the toxic substances in question are chemical byproducts of the bacterial metabolism.

We feel justified in reporting these observation for several reasons: In the first place, the presence of toxic substances in cultures of different proteolytic bacteria have been at different times mistaken for specific toxin.[2] Secondly, in connection with the analysis of partly decomposed food products in which the presence of toxins is suspected, one usually injects into a test animal a comparatively large amount of the food extract. Since such extracts may contain poisonous salts, we wish to emphasize the necessity of controlling such animal inoculation by antitoxin neutralization experiments. When the latter is not available the test of thermostability of the toxic substances in neutral as well as in alkaline reaction may indicate the true nature of the substance.

[1] When grown on beef infusion broth or peptone water without minced meat, neither of these organisms produce the toxic substances in question.

[2] Barger, G., and Dale, H. H., *Brit. Med. Journ.*, 1915, 11, 808.