have lost the power to break through the trapdoor which leads to the acetaldehyde stage in which they are capable of exercising antiketogenetic effects. Apparently the only way the carbohydrates affect ketogenesis is when they have come down to the acetaldehyde stage.

The modus operandi of the action of acetaldehyde on the ketone bodies has already been discussed by Ringer and Frankel. They suggested the possibility of acetaldehyde combining with β-hydroxybutyric acid or acetoacetic acid giving rise to a substance which is not ketogenetic.

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Botulism.[1] A method for determining the thermal death time of the spores of Bacillus botulinus.

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In the course of a series of experiments dealing with the determination of the thermal death point of spores of *Bacillus botulinus* in which the method of procedure recommended by Bigelow and Estey[2] was followed with minor modifications, it was found that in the daily transplanting of several hundred specimens to the tubes in which the heated material was to be incubated, it was inevitable that a certain small percentage of the tubes became contaminated. The number of proved contaminations was not large, less than 1 per cent. in a test of approximately 2,000 tubes, but because of the fact that one could not be absolutely certain that any particular tube was free from contamination, it was impossible to draw accurate conclusions in any instance where an unusual survival time was indicated. It was therefore imperative that a method be devised in which the necessary number of tubes per day could be handled with rapidity, and, at

[1] These experiments are a part of an investigation of Botulism which is being made in California by the U. S. Public Health Service, Leland Stanford Junior University and the University of California under a grant from the National Canners' Association, the Canners' League of California and the California Olive Association.

[2] Bigelow and Estey, *Jour. Infect. Dis.*, 1920, xxvii, 602.

the same time, with absolute protection against any possibility of contamination after their contents had been subjected to the heating process.

After a number of trials we have adopted the following method of procedure in our experiments, and, although it cannot be adapted to the investigation of all the problems which present themselves in thermal death time experiments, it has proved to be satisfactory for the problems under immediate investigation.

Soft glass tubes, 10 x 150 mm., are used in the experiments. Three cubic centimeters of 1 per cent. glucose peptic digest liver broth, adjusted so that the final P_H is between 7.3 and 7.5, are placed in each tube and covered with a thin layer of oil to prevent evaporation. The medium is sterilized at fifteen pounds pressure for thirty minutes.

Immediately before they are to be inoculated the tubes of broth are exposed to live steam for twenty minutes to expel the air. A known number of spores is added to each tube in ½ c.c. of the medium in which they have grown, the number of spores in the suspension being determined by actual count in a counting chamber. The tubes are then sealed in an oxygen flame and are ready for heating.

Although it has been found by actual counts that there is no appreciable change in the number of spores within five hours after they have been placed in the tubes, not more than ninety minutes are allowed to elapse after the tubes are inoculated before they are submitted to the heat.

The spores are heated by immersing the sealed tubes in racks into oil which is maintained constant at the required temperature and vigorously agitated. At the end of the required time of heating the tubes are removed from the oil, placed in deep pans of cold water to cool and immediately labelled. They are then incubated at 37.5° C.

The appearance of growth in the sealed tubes is characteristic and easily detected. Incubation is continued for at least ten days after growth is recognized in each instance to allow time for the formation of toxin, after which the tubes are opened under sterile precautions, deep agar, broth and meat mediums are inoculated for the purpose of observing the cultural characteristics

and guinea pigs are immediately injected for the demonstration of toxin and determination of its type. No test is considered positive unless the broth culture within the sealed tube contains a virulent *botulinus* toxin at the time the tube is broken.

This technic, because it eliminates all possibility of contamination of the contents of the tubes after they have been subjected to the required amount of heat, ensures that any bacteria which are alive within the tube must be the growth from bacteria or spores which have survived the heating process. It also demonstrates beyond any possibility of doubt not only when the *botulinus* spores have survived the given exposure to heat but whether they have retained their ability to form toxin.