

Following the period of infancy there is a quiescent phase in the mortality from tuberculosis, throughout which the deaths decrease year by year, although the number of infections, as shown by the tuberculin reaction, steadily increases. These infections are due probably to latent carriers, to individuals who from time to time shed tubercle bacilli. A daily examination of the sputum of a series of individuals, carried out carefully and for a period of months, might well be undertaken in order to test the validity of this hypothesis.

There is a second active phase in connection with the mortality from tuberculosis. This is commonly associated with the third quinquennium of life. If we study the deaths year by year, rather than by quinquenniums, we find that this period begins at 13 years of age, and that it is not coincident in females and in males. Among the former it sets in rather sharply at the age of 12, whereas among the boys the flare up does not manifest itself until the age of 16. This distinction was found to hold good for several large cities, and is governed probably by the onset of puberty. This uniform periodicity in the increase of deaths from tuberculosis, taking place in both girls and boys at a definite year of life, leads us to believe that the disease is due to an "autogenous reinfection" from some latent focus, rather than a fresh infection from a tuberculous individual.

Much good could be accomplished in combating tuberculosis both in infants and at puberty by providing country preventoriums in connection with the large cities. In view of the mortality incidence outlined above, it would seem advisable to change the present usage in regard to the older children and to restrict these institutions to girls from the ages of 10 to 16 and to boys from 14 to 18. In this way provision would be made for hygienic measures two years before the onset of the second period of high mortality.

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Health of Food Handlers in New York City.—New York City under its Sanitary Code has a regulation regarding the examination of food handlers, requiring them to obtain certificates of health from the health department. An analysis of the results of the examination of 1,980 of these persons, as set forth in an abstract in the *American Journal of Public Health*, shows that 1,590 of these were males and 390 females; 80.2 per cent. were in the age period between 20 and 44 years; 81 per cent. were restaurant and hotel workers and 16 per cent. were confectioners and bakers. Of the 81 per cent., 44.2 per cent. were waiters and waitresses, 19.6 cooks and 9.1 per cent. dishwashers and kitchen employees. The examination of these cases was very thorough. It was found that 32.1 per cent. of the males and 13.3 per cent. of the females were free from disease or any other condition worthy of mention. But among the diseases and defects found were 10 cases of active tuberculosis, 3 arrested cases and 12 suspected cases; 19 cases of active syphilis and 32 of suspected syphilis, and 6 cases of gonorrhoea. The prevalence of this latter disease among women was not determined. There were 370 cases of anemia, 112 cases showing eye diseases, 124 cases of heart disease, 64 of diseases of the arteries, 237 cases of varicose veins, 110 cases of colds and rhinitis, 25 cases of chronic bronchitis, 104 cases of pulmonary emphysema, 208 cases of pyorrhoea alveolaris, 288 cases of dental caries, 202 cases of pharyngitis, 62 cases of hernia and 133 cases of flatfoot. Bakers and cooks seem to suffer particularly from cardiovascular disease, anemia, flatfoot and respiratory disorders.

THE EFFECT OF HEAT ON THE SPORES OF BACILLUS BOTULINUS

ITS BEARING ON HOME CANNING METHODS: PART 1*

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There are four methods commonly used in the home for canning fruit and vegetables. They are:

1. The hot pack method, in which the fruit and vegetables are cooked in the open kettle till done and then sealed in clean jars while hot.
2. The cold pack method, in which the fruit or vegetables are first blanched, then packed in jars and the jars put into water and boiled for one continuous period of from one and a half to five hours.
3. Intermittent or fractional sterilization, the fruit or vegetables being packed cold in the jars and the jars placed in water and boiled from fifteen to sixty minutes on each of three successive days.
4. Pressure canning, in which the filled jars are sterilized with steam under pressure.

Of these methods the first two are probably more generally used because they require no special apparatus as for pressure canning, and the work is completed in one day, whereas the fractional method requires three days.

The latest books on bacteriology state that the spores of *B. botulinus* are easily killed, a temperature of 80 C. (176 F.) for sixty minutes being sufficient. If this statement were true of all strains of *B. botulinus*, any one of the four methods of canning mentioned would be amply sufficient to sterilize fruit or vegetables contaminated with *B. botulinus*, and development of toxin in jars would indicate gross carelessness on the part of the cook.

Seven cases of botulism were investigated by Dr. E. C. Dickson of Leland Stanford Junior University School of Medicine during the winter of 1917 and 1918, and in four of these cases I succeeded in isolating *Bacillus botulinus*, either from dead chickens, sick hogs or directly from the cans of fruit or vegetables. In two of these cases, vegetables were canned by the cold pack (one period) method. One of the cooks stated positively that she had kept the filled jars in a covered boiler with the water actively boiling for three hours. She did not, however, blanch the peas before placing them in the jars. In the third case of poisoning the beans were canned by the fractional method, and in the fourth case, in which the poisoning of eight persons followed the eating of canned apricots, the fruit was cooked in the open kettle and sealed, while hot, in the jars. As the cook was one of the first victims, there are no data as to just how long the fruit was cooked.

In view of these conflicting data, a series of experiments were begun to determine the effect of heat on the spores of *B. botulinus*. The work is being carried on under a special grant from the State Council of Defense of California, and has been performed in the Laboratory of Bacteriology and Immunity of Leland Stanford Junior University, where Dr. W. H. Manwaring has generously facilitated the work in every way.

There are in this laboratory ten strains of *B. botulinus*. Strains I and II came from the Museum of

* Report of the Botulism Research of the State Council of Defense of California, July, 1918.

Natural History, New York, and Columbia University, respectively, and I do not know where they were isolated. They produce very weak toxin and their colony formation is not uniform. Strains III, IV and V were isolated by Dr. Dickson from cases of botulism occurring in California and Oregon in 1916 and 1917. Strains III and IV produce a strong toxin and characteristic colonies. Strain V produces a weak toxin and its colonies are not uniform. Strain VI was isolated from cheese in Massachusetts. It produces strong toxin but its colony picture is not uniform. Strains VII, VIII, IX and X were isolated by myself during the past winter from the cases investigated by Dr. Dickson.¹ Strain VII came from Seattle, and the other three from California. They all produce strong toxin and form characteristic colonies. By means of toxin-antitoxin tests it has been shown that Strains III, IV, VII and IX belong to one homologous group, and that Strains VI, VIII and X belong to another. The relationship of Strains I, II and V has not been determined, as the toxin produced is very weak.

Spores develop most rapidly and abundantly in a culture medium made of sheep brains, cooked slightly with a little water and squeezed through cheese cloth. The medium is oil stratified in the tubes. In these cultures the spores are present in the fluid portion and also embedded in the brain tissue. In broth cultures, spores are formed very slowly, and for this reason only two experiments have as yet been performed with broth spores.

Each culture was examined in a smear, stained by Gram's method, and only those cultures were used that were found to contain free spores. Cultures showing only terminal spores were not used.

TECHNIC

Spore Mixtures.—For the broth spore experiments (Tables 1 and 2), about 1 c.c. of the sediment from the bottom of the broth culture was pipetted off with a sterile pipet into a sterile test tube and the volume made up to about 6 c.c. with sterile salt solution.

For the Arnold sterilizer experiment (Table 4), about 2 c.c. of the brain culture were pipetted off with a sterile pipet into a sterile test tube and diluted with about 10 c.c. of sterile salt solution. When the material was boiled in an open beaker (Table 3), about 10 c.c. of the brain culture were mixed in a sterile beaker with about 50 c.c. of sterile salt solution, and loss by evaporation was made up by the addition from time to time of hot sterile distilled water so that the boiling did not stop or the concentration of the fluid change materially. For the autoclave experiments (Table 5) about 0.5 c.c. of a brain culture was placed in a sterile test tube. No salt solution was added.

Apparatus.—Water Bath: A deep pot of water was used for the water bath with a cover having holes through which the tubes were pushed down into the water. The level of the water in the bath was always well above the level of the spore mixture in the tubes.

Arnold Sterilizer: The tubes containing the spore mixtures were placed in a rack in a regular Arnold sterilizer with a double door. The door was necessarily opened for about three minutes during each inoculation period. This, however, was done in a very warm room.

Autoclave: This was of the horizontal type.

Inoculation.—Inoculations were made from the heated material at definite intervals as specified in the respective tables. In all but the autoclave and part of the beaker experiments the inoculations were made by means of small sterile pipets. A separate pipet was used for each inoculation. The amount of material transferred was about 0.1 c.c.

1. Dr. E. C. Dickson has a report of these cases in process of publication.

This amount was increased somewhat in the inoculations made after the longer periods of heating. For the inoculations of the autoclaved spores, a platinum loop about one-fourth inch in diameter was used. The center of the loop was filled in with a coil of platinum wire to make a scoop so that a very rich inoculation was made. An ordinary platinum loop was used to make inoculations from the foam in the experiment of boiling the spores in an open beaker.

Media.—All inoculations were made into tubes of deep infusion or peptic digest agar, 2 per cent. glucose. The agar was boiled for twenty minutes and cooled to 47 C., at which temperature the tubes were inoculated. They were then at once thoroughly shaken and solidified in cold water. They were capped with a mixture of paraffin, beeswax and zinc oxid.

Incubation.—The cultures were incubated in an incubator which varied between 25 and 28 C.

Observation.—The cultures were observed each day for anaerobic growth and gas production. When growth appeared the cultures were examined under the dissecting microscope to determine whether the colonies were characteristic of the given strain of *B. botulinus*. This was done in order to rule out possible contamination with other organisms.

EXPERIMENTS AND RESULTS

Two experiments were performed in which spores from broth cultures were used. The spores were heated in a water bath. In the first experiment

TABLE 1.—RESULTS OF EXPOSING FREE SPORES FROM BROTH CULTURES OF *B. BOTULINUS* TO A TEMPERATURE OF 80 C. IN A WATER BATH; CULTURES INCUBATED FOR SIX WEEKS *

Length of Exposure at Time of Inoculation, Minutes	Temperature in Test Tube at Time of Inoculation, C.	Strains					
		I	III	V	VI	VII	IX
Control unheated	⊕ 1 d	+ 1 d ⊕ 2 d	⊕ 1 d	+ 1 d ⊕ 2 d	+ 1 d ⊕ 2 d	+ 1 d ⊕ 2 d
5	80.5	⊕ 1 d	⊕ 1 d	⊕ 1 d	⊕ 1 d ⊕ 2 d	+ 1 d ⊕ 2 d
10	80	⊕ 1 d	⊕ 1 d	⊕ 1 d	+ 1 d ⊕ 2 d	+ 1 d ⊕ 2 d
15	80.5	⊕ 1 d	⊕ 1 d	⊕ 1 d	+ 1 d ⊕ 2 d	+ 1 d ⊕ 2 d
20	79.5	+ 1 d ⊕ 2 d	⊕ 1 d	⊕ 1 d	⊕ 1 d	+ 1 d ⊕ 2 d
30	81	⊕ 1 d	⊕ 1 d	⊕ 1 d	+ 1 d ⊕ 2 d	⊕ 1 d
40	80.5	⊕ 1 d	⊕ 1 d	⊕ 1 d	⊕ 2 d	⊕ 1 d
60	80	⊕ 2 d	⊕ 2 d	⊕ 2 d	⊕ 4 d	⊕ 2 d
90	80	⊕ 2 d	⊕ 2 d	⊕ 2 d	⊕ 3 d	⊕ 2 d
120	80	⊕ 1 d	⊕ 1 d	⊕ 1 d	⊕ 3 d	⊕ 3 d

* In the tables, + indicates growth; ⊕ indicates growth and gas. Arabic numerals followed by "d" indicate the number of days of incubation before growth and gas appeared in the agar cultures.

(Table 1) the temperature of the water bath was approximately at 80 C., and the temperature inside the test tubes was maintained between 79.5 and 81 C. during the two hours. In the second (Table 2) the water in the bath was kept actively boiling and the temperature in the test tubes was maintained at 100 C. throughout.

Normal cultures of *B. botulinus* in agar grow and produce gas in from twenty-four to forty-eight hours, the more thickly seeded cultures showing up more quickly than thinly seeded ones. In Table 1 it will be observed that the broth spores of five strains survived a temperature of 80 C. for two hours and that 90 per cent. of the cultures developed in from twenty-four to forty-eight hours, while the others showed up

on the third or fourth day. It is evident, therefore, that the spores were practically uninjured by exposure to a temperature of 80 C. for two hours. Of the broth spores heated to 100 C. (Table 2), one strain survived after an exposure of fifteen minutes, another after twenty minutes, a third after thirty minutes and

TABLE 2.—RESULTS OF EXPOSING FREE SPORES FROM BROTH CULTURES OF *B. BOTULINUS* TO A TEMPERATURE OF 100 C. IN A WATER BATH; CULTURES INCUBATED FOR THREE MONTHS

Length of Exposure at Time of Inoculation, Minutes	Temperature in Test Tube at Time of Inoculation, C.	Strains			
		V	VI	VII	IX
Control unheated	...	+ 1 d ⊕ 3 d			
5	100	⊕ 1 d	⊕ 1 d	+ 6 d ⊕ 10 d	+ 3 d ⊕ 8 d
10	100	+ 1 d ⊕ 2 d	⊕ 4 d	+ 10 d ⊕ 15 d	⊕ 5 d
15	100	+ 5 d ⊕ 6 d	+ 10 d ⊕ 19 d	+ 5 d ⊕ 8 d
20	100	⊕ 8 d	⊕ 26 d	⊕ 6 d
30	100	+ 10 d ⊕ 19 d	+ 6 d ⊕ 8 d
40	100	+ 8 d ⊕ 12 d
60	100	+ 15 d ⊕ 17 d
90
120

a fourth after sixty minutes.. The control cultures and some of those inoculated after five and ten minutes' exposure developed in normal time. But the development of the other cultures was retarded. The incubation period was increased in direct proportion to the length of time the spores were exposed to the heat.

These two experiments parallel two which were made by Dr. E. C. Dickson² and myself last spring with spores from brain cultures. It was found at that time that spores from brain cultures of six strains survived a temperature of 80 C. for three hours and that growth appeared after a practically normal incubation period, and also that spores from brain cultures of five strains, exposed to a temperature of 100 C. for two hours, survived for the entire two hours, while the sixth strain survived for one and one-fourth hours. The negative cultures were not kept more than three weeks. The incubation period of the spores following exposure to 100 C. was increased in direct proportion to the length of time of exposure. Comparing these data with those in Table 2, it is evident that broth spores are somewhat less resistant than those grown in brain mediums, but that they are more resistant than has been hitherto reported.

In all experiments in which spores were exposed to temperatures of 100 C. or over, the subsequent development of the heated spores was more or less retarded (Tables 2, 3, 4 and 5). The longest period of incubation recorded in these charts is fifty-three days (Table 5, Strain X).

The cultures remain apparently sterile for days. No growth is visible even with the use of a dissecting microscope. Then suddenly, within a twenty-four to forty-eight hour period, normal growth and gas pro-

duction take place. There is a good deal of variation in the resistance of the spores of the different strains to heat and the amount of growth inhibition caused by exposure for any given time. For any one strain, however, the amount of inhibition varies directly with the length of exposure. An examination of the incubation periods noted for each of the cultures in the accompanying tables shows that there are very few exceptions to this rule. Several occur in Table 1; but, as I have already stated, all the cultures in this experiment developed in practically normal time, and the variations that occurred are due to difference in the thickness of seeding rather than to the effect of the heat. In Table 3, Strain VIII, there is a very decided break in the sequence between the spores heated for 150 minutes and those heated for 180 and 210 minutes. But, as will be explained more fully later, the early development of the 180 and 210 minute cultures was probably due to the accidental admixture of foam from the side of the beaker which had not been subjected to the same degree of heat as the liquid at the bottom.

The recognition of the fact that the incubation period of the spores of *B. botulinus* is very much lengthened following exposure to temperatures of 100 C. and over is very important. It means that in testing for the thermal death point all cultures of heated spores that do not show any growth must be incubated for months before they are finally reported

TABLE 3.—FREE SPORES FROM BRAIN CULTURES OF *B. BOTULINUS* BOILED IN A BEAKER OVER A FREE FLAME

Strain IV		Length of Boiling	Strain VI		Strain VIII	
Length of Boiling	Foam and Liquid		Liquid	Foam from Side of Beaker	Liquid	Foam on Surface of Liquid
Control unheated	⊕ 1 d	Control unheated	⊕ 2 d	No cultures	⊕ 2 d	No cultures
Just boiling after 4 min. over flame	⊕ 1 d	Just boiling after 5 min. over flame	⊕ 2 d	No cultures	+ 2 d ⊕ 4 d	No cultures
Boiled 2 minutes	⊕ 1 d	Boiled 5 minutes	⊕ 2 d	⊕ 2 d	+ 2 d ⊕ 4 d	No cultures
5 minutes	⊕ 1 d	10 minutes	⊕ 2 d	⊕ 2 d	+ 5 d ⊕ 6 d	No cultures
10 minutes	⊕ 1 d	15 minutes	⊕ 2 d	⊕ 2 d	+ 4 d ⊕ 6 d	No cultures
20 minutes	⊕ 2 d	20 minutes	⊕ 2 d	⊕ 2 d	+ 4 d ⊕ 6 d	No cultures
30 minutes	⊕ 2 d	30 minutes	⊕ 2 d	⊕ 2 d	⊕ 4 d	⊕ 4 d
60 minutes	+ 2 d ⊕ 3 d	60 minutes	+ 2 d ⊕ 3 d	⊕ 2 d	+ 4 d ⊕ 7 d	No cultures
90 minutes	⊕ 1 d	90 minutes	+ 2 d ⊕ 3 d	⊕ 2 d	⊕ 18 d	⊕ 6 d
		120 minutes	+ 2 d ⊕ 3 d	⊕ 2 d	⊕ 18 d	No cultures
		150 minutes	+ 2 d ⊕ 3 d	⊕ 2 d	⊕ 18 d	No cultures
		180 minutes	⊕ 3 d	⊕ 2 d	⊕ 5 d	+ 18 d ⊕ 20 d
		210 minutes	No cult.	No cultures	+ 8 d ⊕ 11 d	⊕ 4 d *

* Foam from side of beaker.

sterile. Therefore, whenever negative results are noted in the accompanying tables it must be understood that they represent incomplete data and do not indicate the thermal death point of the spores, for none of the cultures have been incubated for more than three months at the time of this writing.

2. Dr. Dickson has a report of these experiments in process of publication.

It is also very doubtful whether fractional sterilization on three successive days could sterilize spores whose development is so inhibited by exposure to heat. Even exposure for fifteen minutes to a temperature of 100 C. will inhibit certain spores so that they will not develop until the fourth to the tenth day. Two experiments have been performed to test fractional steriliza-

TABLE 4.—RESULTS OF HEATING FREE SPORES FROM BRAIN CULTURES OF *B. BOTULINUS* IN THE ARNOLD STERILIZER; CULTURES HAVE BEEN INCUBATED FOR THREE MONTHS

Length of Exposure	Strains								
	I	II	III	IV	V	VI	VII	VIII	IX
30 min.	⊕ 4 d	+ 3 d ⊕ 4 d	+ 3 d ⊕ 4 d	⊕ 4 d	⊕ 4 d	+ 4 d ⊕ 6 d	⊕ 3 d	⊕ 3 d
60 min.	⊕ 8 d	⊕ 8 d	+ 6 d ⊕ 8 d	⊕ 4 d	⊕ 4 d
90 min.	⊕ 6 d	⊕ 10 d	+ 6 d ⊕ 8 d	⊕ 4 d	⊕ 4 d
2 hrs.	+ 13 d ⊕ 18 d	⊕ 13 d	⊕ 8 d	+ 4 d ⊕ 6 d	+ 4 d ⊕ 6 d
2½ hrs.	⊕ 10 d	⊕ 6 d	+ 6 d ⊕ 8 d
3 hrs.	⊕ 16 d	+ 11 d ⊕ 12 d	⊕ 8 d
4 hrs.	+ 18 d ⊕ 20 d	+ 17 d ⊕ 18 d
5 hrs.
6 hrs.

tion. In both cases, spores from brain cultures were used and the spores were exposed to a temperature of 100 C. for one hour on each day. In the first experiment, two strains were used, and were heated on three successive days. Both strains survived after the third day's heating. The second time, six strains were tested, and they were sterilized on three successive days and again on the eighth day. Five strains survived the fourth period of heating on the eighth day. The other strain survived after the first period of heating, but so far the later cultures show no growth. The technic of these experiments was not, however, entirely satisfactory, and for that reason a detailed report of them will be reserved until there are more data.

It has been asserted that the blanching of the fruit and vegetables for five minutes in boiling water, as recommended in the cold pack (one period) method of canning, helps to sterilize the material. Also, many housewives can their fruit and vegetables by boiling them in an open kettle and packing them, hot, into clean jars. To test the reliability of these methods for *B. botulinus* spores from brain cultures of three strains were boiled in an open beaker (Table 3). Strain IV was boiled for ninety minutes, Strain VI for three hours, and Strain VIII for three and one-half hours. It was found that as soon as the brain mixture began to boil, a thick foamy scum formed on the surface of the mixture. In a few minutes this broke, leaving a clear portion in the center. For Strain IV, samples of both foam and liquid were taken each time. For Strains VI and VIII, duplicate cultures were made of the liquid and the foam. The foam inoculations of Strain VI were made from the foam adhering to the side of the beaker. For Strain VIII, the foam was taken from the surface of the fluid, except for the 210 minute inoculation, which was made from foam adhering to the side of the beaker.

Strain IV was uninjured by boiling for ninety minutes. Strain VI was uninjured by boiling for three hours, and Strain VIII survived for three and one-half hours, although the development of the spores was much retarded. The four samples of foam from Strain VIII show that the spores in the foam that adhered to the side of the beaker were not subjected to so much heat as those on the surface of the liquid, since the spores in the foam that was taken from the side of the beaker after 210 minutes developed much more quickly than those in the foam taken from the surface of the liquid after ninety and 180 minutes. The comparatively early development of the cultures made from the liquid after 180 minutes' and 210 minutes' boiling is probably due to the accidental admixture of some of the material from the side of the beaker at the time when hot water was added to make up loss by evaporation.

Blanching, therefore, cannot be relied on to kill or even materially injure spores of resistant strains of *B. botulinus*. Its value is as a cleansing agent. And to be effective it should be done before the fruit or vegetables are cut, for spores lodged on the skin may be crushed by the cutting process into the pulp, where they would be protected. The method of canning by boiling in an open kettle and then packing, hot, in the jars and sealing cannot be relied on to sterilize material contaminated with hardy strains of *B. botulinus* unless the boiling be continued for a good deal over four hours. A scum of foam always forms on the surface of boiling fruit or vegetables, and this may contain viable spores for a very long time.

Nine strains were tested in the Arnold sterilizer (Table 4) and ten in the autoclave (Table 5). The spores were obtained from the same set of brain cultures. A set of transplants in agar were made and incubated in order to test out the purity of the cultures.

TABLE 5.—RESULTS OF STERILIZING FREE SPORES FROM BRAIN CULTURES OF *B. BOTULINUS* IN THE AUTOCLAVE; CULTURES HAVE BEEN INCUBATED FOR THREE MONTHS

Pressure and Time	Time Required for Pressure to Rise, Min.	Time Required for Pressure to Fall, Min.	Strains									
			I	II	III	IV	V	VI	VII	VIII	IX	X
5 lbs. 5 min.	5	6	⊕1d	+1d ⊕5d	...	+1d ⊕2d	+1d ⊕2d	+1d ⊕4d	⊕4d	⊕3d	⊕3d	⊕3d
10 min.	4½	5	⊕16d	...	+12d ⊕14d	⊕12d	⊕12d
20 min.	5	4½
10 lbs. 5 min.	12	10	⊕16d
10 min.	12½	10	⊕50d
20 min.	10	13
15 lbs. 5 min.	22½	13
10 min.	18	16	⊕53d
20 min.	8½	12½

As these showed typical growth characteristics, no special controls were made for either the Arnold or the autoclave series.

The cultures of Tables 4 and 5 have been incubated for three months. More than half of the cultures are still negative, and none have developed since the fifty-third day. These tables, therefore, represent something approximating the thermal death point of the spores, though some further development may take place.

Spores of Strains VIII and IX survived in the Arnold sterilizer at a temperature of 100 C. for four hours. Therefore, one long period of heating, such as is recommended in the cold pack method of canning, is not a reliable method of killing spores of *B. botulinus*. The internal temperature of jars of fruit and vegetables does not reach 100 C. for about an hour after the surrounding water begins to boil. For these reasons even a period of five hours in boiling water would not sterilize the contents of jars, contaminated with spores of the more highly resistant strains of *B. botulinus*.

A month ago I received a report from a woman who canned a number of jars of string beans a year ago. Nearly the whole pack spoiled in the course of a few weeks. She stated that the beans were cut in pieces, washed carefully in water, packed in the jars, and the jars put in a boiler of cold water, the level of the water being up to the necks of the jars. The water was then brought to a boil and kept boiling for four and one-half hours. Whether any of the spoilage was due to *B. botulinus* is, of course, not known.

The data thus far obtained from the autoclaved spores show that a pressure of 15 pounds for a period of ten minutes will not kill all spores of *B. botulinus*.

There is apparently some variation in resistance to heat between spores of different cultures of the same strain. Strains I, III, IV and VI seem to be less resistant sometimes than at other times. The age of the cultures used, however, varied a good deal, because spores form more quickly in some cultures than in others. Strains VII, VIII, IX and X give more nearly uniform results. Strains VIII, IX and X, which are the most recently isolated strains, are the most highly resistant to heat.

CONCLUSIONS

1. Free spores of *B. botulinus*, grown in either broth or brain cultures, are highly resistant to heat.
2. Spores grown in brain cultures are more resistant than spores grown in broth cultures.
3. Exposure of the spores to a temperature of 100 C. or more inhibits the development of the spores of *B. botulinus* so that the incubation period is very much increased.
4. The method of canning by boiling the fruit or vegetables in an open kettle and sealing in clean jars cannot be relied on to sterilize material that is contaminated with spores of the more resistant strains of *B. botulinus*, since spores of *B. botulinus* will survive in boiling liquid for three and one-half hours; and in the foam that gathers on the side of the kettle they may live for a much longer period.
5. The sterilizing processes of the cold pack (one period) method are not sufficient to kill spores of *B. botulinus*. Blanching in boiling water for five minutes is effective as a cleansing agent, but it does not materially injure spores of resistant strains of *B. botulinus*. One period of heating in boiling water for five hours or less will not sterilize the contents of jars if they are contaminated with the more resistant spores of *B. botulinus*.
6. Fractional sterilization on three successive days is of doubtful value because, after exposure to a temperature of 100 C. for from fifteen to sixty minutes, the germination of the spores is retarded so that they do not develop before the third sterilization period.
7. Pressure canning is the only method of sterilization that at present can be considered safe. But a

pressure of 5, 10 or 15 pounds for ten minutes will not kill the more resistant spores of *B. botulinus*. Therefore a comparatively long sterilization period must be used.

8. With our present knowledge, the best way to guard against spoilage of canned goods due to *B. botulinus* is to prevent spores of this organism from entering the jars. The source of contamination is not at present known.³ It is quite certain that the organism is not present under the skin of perfectly sound fruit and vegetables that are not overripe. Therefore, fruit and vegetables of this sort should be used. Bruised and partially spoiled material, if it is fit to use at all, should be consumed at once and not stored for future use. The fruit and vegetables should be thoroughly cleaned before they are peeled or cut in order that no foreign matter may be crushed into the pulp where it would be protected. It is possible that the spores of *B. botulinus* get into the jars from some source in the kitchen. For this reason it is essential that the hands and all utensils be made as clean as possible and that flies be eliminated. The use of sound fruit and clean methods of handling are the most important factors in canning, so far as *B. botulinus* is concerned, because the sterilization processes in common use will not kill all the spores.

9. It is important that housewives be warned of the danger of *B. botulinus* spoilage so that they may examine every jar carefully when it is opened for table use. The contents of the jar must not be tasted to "see if it is spoiled." The smallest taste will be fatal if the toxin is strong. There are three signs of spoilage from *B. botulinus*, any one of which is sufficient to condemn the jar. These are:

- (a) Gas bubbles in the jars, the tops of the jars blown, and a squirt of liquid as the top is unscrewed.
- (b) An odor somewhat resembling rancid cheese.
- (c) A mushy or disintegrated appearance of the solid parts of the contents of the jars.

10. The toxin that *B. botulinus* produces after several weeks or months growth in a sealed jar may be entirely destroyed by boiling for five minutes, although the spores of *B. botulinus* are not killed by this treatment. It is the toxin and not the spores that produces illness, for the bacilli do not produce toxin when taken into the body. Therefore, any canned goods that are in the least suspicious should be emptied into a kettle and boiled for five minutes. They can then be eaten without danger.

11. Since *B. botulinus* produces toxin only in material that has been sealed in an air-tight container for a week or more, and since it produces no toxin in the human body, there is no danger of botulism from uncooked fruit or vegetables or from those freshly cooked.

3. I am collecting material from places where cases of botulism occurred during the past winter in an effort to secure data as to the source of the contamination.

The Lacteal Goddess.—If civilized peoples were to lapse into the worship of animals, the cow would certainly be their chosen goddess. What a fountain of blessing is the cow! She is the mother of beef, the source of butter, the original cause of cheese, to say nothing of shoehorns, hair combs and upper leathers. A gentle, amiable, ever-yielding creature, who has no joy in her family affairs that she does not share with man. We rob her of her children, that we may rob her thereafter of her milk; and we only care for her when the robbery may be perpetrated.—Charles Dickens.