INFLUENCE OF VACUUM UPON GROWTH OF SOME AEROBIC SPORE-BEARING BACTERIA[1]

L. D. BUSHNELL

Kansas Agricultural Experiment Station

Received for publication September 11, 1921

In the course of a study of the bacteria present in canned foods, we found that certain aerobic spore-bearing bacteria were present in jars which showed no evidence of spoilage, if they were properly sealed. These results were reported from this laboratory in 1918 (Bushnell 1918), but no attempt was made to determine the types present.

Vaillard, in 1900 and 1902 examined bacteriologically, many cans of meat, and found living organisms in seventy or eighty percent of them. He believed that the bacteria survived in a dormant state in the cans from five to seven years. Among the spore-bearing types he isolated *B. subtilis* and *B. mesentericus*, (three varieties, *vulgatus*, *ruber*, and *fuscus*).

Deichsetter, in 1901, reported on the examination of preserved food provided for the Bavarian Army during a period of five years and failed to find microörganisms in canned foods, save in cans in which the food was sent in under suspicion. He considered that Vaillard's findings were probably due to faulty technic.

Pfuhl, in 1904, examined canned meats from five firms and found bacteria in 29 out of 106 cans. He considered that the findings of both Vaillard and Deichsetter were correct and that the difference in results was due to a difference in the care with which the foods were sterilized.

Very little work had been reported upon this point until 1919 when Weinzirl published the results of his findings on commercial

[1] Contribution Number 37 from the Bacteriological Laboratory of the Kansas Agricultural Experiment Station.

canned foods. He states that in commercial canned foods giving no evidence of spoilage, microörganisms were found in 179 out of a total of 782 cans, or in 23 per cent of the cans. The spore-bearers were practically the only organisms present, due to their superior resistance to the sterilizing process. Viable spores were found in 19.2 per cent of the non-leaking cans. Of the types of bacteria isolated, *B. mesentericus* predominated, with *B. subtilis* next. This author concludes that the living spores in commercial canned foods are unable to grow, due to the absence of oxygen, and that the vacuum is essential to the preservation of canned foods under the present method of processing.

Cheyney, in 1919, reported *B. mesentericus* in apparently perfect cans, which were given a standard processing.

Hunter and Thom, in 1919, made an examination of 530 cans of canned salmon and found 237 unsterilized. 234 of these cans contained the same organism of the *B. mesentericus* group, either in pure culture or in connection with other species. Only 13 showed active spoilage.

From the above it is evident that the aerobic spore-bearing types predominating in unspoiled canned foods belong to the *B. mesentericus* and *B. subtilis* groups of bacteria.

We may consider three reasons why these organisms predominate in foods under such conditions:

1. Spores of certain types predominate on the product as it goes into the container.

2. Spores of certain types are more resistant to heat than spores of other types.

3. The spores of certain types are not all destroyed during the processing period and those remaining are able to grow under conditions as they exist in the container.

From the results obtained in this laboratory, it is evident that *B. mesentericus* predominates, with *B. subtilis* second in number among the aerobic spore-bearing types. We have no idea of the number of each type upon the raw product as it went into the jars, so that it is not possible to consider this point except in so far as we may apply the work of Bruett (1919) upon the death of bacteria. She concluded that the death rate followed

the laws of monomolecular reactions. According to this law, if all spores were of equal resistance to heat, those present in the largest number would be the last to disappear. However, spores of different species of bacteria are not of equal resistance to heat, and while those of a particular species may follow this law, it cannot be applied in a comparison of the thermal death rate of different species.

We have found that the several cultures of *B. mesentericus* with which we have worked, are less resistant than the strains of *B. subtilis*. Regardless of this decreased resistance *B. mesentericus* predominated among the organisms isolated. Our cultures had grown for some time upon culture media and the resistance may have changed by this treatment. Weinzirl considers that *B. mesentericus* predominates in canned foods because of its superior heat-resisting qualities.

Lawrence and Ford (1916) state that the spores of *B. subtilis* survive steaming one and one-fourth hours in the Arnold sterilizer and autoclaving up to and including 19 pounds pressure but are usually destroyed at 20 pounds. The *B. mesentericus* spores survived one hour in the Arnold sterilizer and autoclaving at 19 pounds pressure, being killed by 20 pounds pressure. These statements would indicate that their cultures of *B. subtilis* were somewhat more resistant than those of *B. mesentericus*.

From some previous work upon these two types we had considered that *B. mesentericus* could grow in the absence of oxygen more readily than *B. subtilis*. Cheyney in 1919, also calls attention to this fact in his recent article on the bacteriology of canned foods.

We have occasionally isolated *B. mesentericus* from the deeper layers of agar in our search for anaerobes, but we have never isolated *B. subtilis* under such conditions, although it has been found on the surface several times. It must be admitted that we did not make quantitative determinations of the types present in each jar. The predominance merely means that in the routine isolation of colonies more of the *B. mesentericus* type were isolated, although we are convinced that this organism did predominate in the jars.

In an attempt to determine why *B. mesentericus* predominated, we undertook some experiments, using these two types. The types used were isolated from jars of asparagus and were rapid spore formers, although they had been grown in the laboratory for more than a year.

The thermal death point of the spores of the *B. subtilis* culture used in these experiments was from ninety to one hundred and twenty minutes in steam at 98°C.; for *B. mesentericus* eighty to one hundred minutes. The time at which all are killed depends to some extent upon the numbers present.

For the experimental work, the organisms were grown upon plain extract agar from four to six days. The growth was scraped from the medium and suspended in a small amount of sterile normal saline. This was shaken in a heavy walled bottle with glass beads and filtered through sterile cotton to remove clumps. The suspension was heated at 80°C. for twenty minutes to kill the vegetative cells.

Experiment 1. In this experiment we wished to determine the influence of different amounts of air upon the growth of the organisms. The number of spores indicated in the table were added to tubes of extract broth, 0.50 per cent N/1 acid to phenolphthalein pH 5.9. In this case the column of air above the medium was about 5 cc. The tubes were exhausted to various points and sealed. The results are shown in table 1.

From this table we may conclude that there was slight growth of both organisms during fifty-one days incubation at room temperature in the less exhausted tubes.

Experiment II was conducted in order to determine the influence of varying amounts of salt and air upon the growth of *B. subtilis* and *B. mesentericus*. In this case, known amounts of air were left above the liquid.

An attempt was made to remove all possible traces of air from the medium. To do this, the tubes were partially filled with a known amount of broth to which varying amounts of salt had been added. The tubes were then drawn out to a slender neck, as close to the liquid as possible, and heated in a seamer for fifteen minutes. The tubes were next cooled in cold water, and

1 cc. of a suspension of spores added. The spores were suspended in salt solutions to correspond to that in the tube, so that the salt concentration was not changed. All the tubes were filled to a mark on the constriction, with broth containing corresponding amounts of salt. A certain per cent of this was then removed and the tubes exhausted and sealed at the mark. The tubes were then incubated at 37°C. for twenty-seven days and plates made.

TABLE 1

DAYS INCUBATION	B. SUBTILIS					B. MESENTERICUS				
	Number spores added	Tubes exhausted to following mm. Hg. on manometer				Number spores added	Tubes exhausted to following mm. Hg. on manometer			
		175	350	525	685		175	350	525	685
5	3110	2740	2220	3000	2280	1300	1830	1730	1370	1440
5	31	25	18	43	58	13	32	31	36	30
12	3110	1170	7000	1900	1000	1300	2100	2000	1090	640
12	31	69	60	16	71	13	31	18	19	36
19	3110	2300	3500	2500	8000	1300	3200	1830	1020	120
19	31	50	41	50	30	13	60	38	13	20
41	3110	3200	4000	5000	6000	1300	4000	1990	1260	260
41	31	49	29	11	12	13	90	72	27	8
51	3110	9000	7000	3200	5000	1300	4000	2460	1120	370
51	31	90	120	12	11	13	150	71	18	11

Since each tube had a slightly different amount of broth added, it was necessary to calculate how many organisms were present in each centimeter of liquid in the beginning. These numbers are in one column and the number at the end of the incubation period in a parallel column. The volume of air varied from 0.3 cc., in the tubes containing 1 per cent air to 1.5 cc. in the tubes containing 25 per cent air, each tube being of somewhat different volume from the others. The results are shown in tables 2 and 3.

These tables show the same as table 1, that there is some growth in these tubes. There is an interesting point relative to the action of increased amounts of salt. In every case, when the average is taken for all tubes in the same concentration of salt, there is an increase over that of a lower concentration. This point is somewhat more in evidence in connection with

TABLE 2

Influence of varying amounts of salt and air upon the growth of B. subtilis in broth

EXHAUSTED TO MM. Hg.	AIR	1 PER CENT NaCl		2 PER CENT NaCl		4 PER CENT NaCl	
		Bacteria added per cubic centimeter	Bacteria determined per cubic centimeter	Bacteria added per cubic centimeter	Bacteria determined per cubic centimeter	Bacteria added per cubic centimeter	Bacteria determined per cubic centimeter
	per cent						
0	5	167,000	100,000	149,000	190,000	175,000	320,000
	10	146,000	150,000	157,000	200,000	165,000	250,000
	25	152,000	117,000	151,000	240,000	151,000	210,000
50	5	146,000	210,000	151,000	102,000	157,000	320,000
	10	152,000	160,000	157,000	110,000	165,000	240,000
	25	141,000	180,000	149,000	110,000	162,000	270,000
100	5	149,000	111,000	151,000	160,000	162,000	180,000
	10	162,000	220,000	143,000	240,000	143,000	270,000
	25	162,000	220,000	146,000	260,000	149,000	
Average		153,000	163,000	150,000	179,000	158,000	258,000
175	1	113,000	129,000	98,000	28,000	104,000	164,000
	5	135,000	76,000	119,000	27,000	124,000	384,000
	10	135,000	112,000			117,000	104,000
	25	113,000	102,000	129,000	158,000	113,000	424,000
350	1	113,000	89,000	129,000	200,000	124,000	448,000
	5	123,000	78,000			117,000	342,000
	10	132,000	98,000	124,000	200,000	118,000	476,000
	25	123,000	55,000	104,000	348,000	107,000	688,000
525	1	113,000	37,000	114,000	152,000	108,000	444,000
	5	113,000	84,000	114,000	256,000	113,000	436,000
	10	118,000	126,000	129,000	240,000	104,000	428,000
	25	123,000	76,000	114,000	220,000	118,000	416,000
685	1	123,000	97,000	124,000	26,000	119,000	516,000
	5			119,000	44,000	113,000	458,000
	10	141,000	48,000	119,000	100,000	113,000	454,000
	25	135,000	145,000	132,000	160,000	114,000	472,000
Average		123,000	90,100	118,000	153,000	114,000	416,000

TABLE 3

Influence of varying amounts of salt and air upon growth of B. mesentericus in broth

EXHAUSTED TO MM. Hg.	AIR	1 PER CENT NaCl		2 PER CENT NaCl		4 PER CENT NaCl	
		Bacteria added per cubic centimeter	Bacteria determined per cubic centimeter	Bacteria added per cubic centimeter	Bacteria determined per cubic centimeter	Bacteria added per cubic centimeter	Bacteria determined per cubic centimeter
	per cent						
0	5	340,000	320,000	328,000	280,000	323,000	720,000
	10	307,000	220,000	314,000	310,000	323,000	750,000
	25	345,000	450,000	328,000	260,000	340,000	860,000
50	5	328,000	340,000	328,000	450,000	341,000	940,000
	10	340,000	260,000	328,000	440,000	341,000	870,000
	25	323,000	290,000	345,000	480,000	328,000	Broken
100	5	323,000	Broken	314,000	440,000	328,000	560,000
	10	328,000	210,000	292,000	290,000	314,000	630,000
	25	340,000	190,000	345,000	360,000	353,000	810,000
Average		331,000	285,000	324,000	367,700	332,300	767,500
175	1			15,300	35,000	17,400	23,000
	5	15,300	29,000	15,500	36,000	15,300	25,000
	10	14,900	24,000	17,700	36,000	15,300	45,000
	25	17,400	22,000	14,300	60,000	14,500	24,000
350	1	15,200	18,000			17,700	22,200
	5	16,000	11,000	15,900	26,000	17,400	24,000
	10	17,700	32,000	18,500	15,000	15,900	48,000
	25	18,100	31,000	15,700	34,000	17,700	29,000
525	1	16,600	26,000	15,300	27,000		
	5	17,600	26,000	14,900	32,000	17,400	24,000
	10	18,600	18,000	16,000	27,000	15,700	34,000
	25	16,000	27,000	16,200	22,000	16,600	29,000
685	1			17,400	29,000	16,000	25,000
	5	17,700	15,000	16,000	27,000	15,700	30,000
	10	16,000	13,000	18,600	30,000	17,400	24,000
	25	16,100	12,000	15,700	20,000	17,400	21,000
Average		16,600	21,500	16,900	30,400	16,700	31,300

B. mesentericus than with *B. subtilis*. The very small amount of air remaining in these tubes apparently has no influence upon the amount of growth. There were relatively no more organisms present in tubes merely sealed and with 25 per cent volume of air, that there were in those tubes which were exhausted and with but 1 per cent of the volume of air. We believe that the organisms in the tube exhausted to 685 mm. were under as completely anaerobic conditions as it is possible to obtain.

According to Bitting and Bitting (1916) an ordinary tin can shows a vacuum of about four inches of mercury when exhausted at a temperature of 130°F. and tested at 85°F.

We may conclude from the results obtained, that the degree of vacuum plays no part in the destruction of the spores of these two organisms. When one per cent of salt is present, there may be a slight decrease in the twenty-seven days of incubation. This is somewhat more marked in higher vacuum than in the lower, but the differences are not marked. In the presence of larger amounts of salt, there appears to be an actual increase in the number of viable bacteria. This is apparently not due to accidental conditions, since we have made numerous parallel determinations and find that the averages of those determined at the end of the incubation period are two or three times as high as the number added. Why there should be a decrease in the presence of 1 per cent, and an increase in the presence of 4 per cent salt we are unable to say. Of course there is considerable variation in the determinations, but the averages indicate a real increase.

Experiment III. This experiment was set up parallel with that of experiment II, except that varying amounts of acid were added to the broth. The organisms were treated in the same way as those in the last experiment, except that they were suspended in acid broth after heating to kill the vegetative forms. The tubes were incubated at 37°C. for twenty-three days for *B. subtilis* and twenty-two days for *B. mesentericus*. The results are shown in tables 4 and 5.

Apparently *B. subtilis* spores are more sensitive to acid than those of *B. mesentericus*. The degrees of vacuum had no influence

TABLE 4

Influence of varying amounts of acetic acid and air upon the growth of B. subtilis in broth

EXHAUSTED TO MM. Hg.	AIR	PER CENTS OF N/1 ACID AND pH							
		0.5 per cent pH 5.6		1 per cent pH 5.00		2 per cent pH 4.30		4 per cent pH 4.00	
		Added	Determined	Added	Determined	Added	Determined	Added	Determined
	per cent								
0	5	715,000	429,000	800,000	173,000	890,000	241,000	800,000	184,000
	10	783,000	450,000	858,000	219,000	872,000	205,000	890,000	170,000
	25	716,000	457,000	737,000	265,000	715,000	256,000	828,000	145,000
50	5	813,000	392,000	907,000	223,000	800,000	234,000	858,000	176,000
	10	872,000	465,000	737,000	232,000	828,000	247,000	858,000	166,000
	25	783,000	472,000	813,000	175,000	761,000		800,000	186,000
100	5	858,000	408,000	872,000	209,000	858,000	198,000	813,000	52,000
	10	813,000	430,000	858,000	250,000	847,000	236,000	761,000	100,000
	25	783,000	472,000	813,000	273,000	828,000	234,000	800,000	130,000
Average		802,000	443,000	836,000	224,000	822,000	231,000	823,000	146,000
175	5	409,000	256,000	410,000	214,000	410,000	157,000	391,000	
	10	383,000	250,000	451,000	270,000	409,000	171,000	391,000	70,000
	25	409,000	303,000	383,000	190,000	451,000	192,000	400,000	170,000
350	5	400,000	212,000	409,000	250,000	409,000		409,000	140,000
	10	374,000	270,000	429,000	230,000	440,000	148,800	409,000	103,000
	25	451,000	303,000	440,000	137,000	409,000	141,600	400,000	210,000
525	5	440,000	266,400	366,000	128,000	409,000	162,400	451,000	100,000
	10	383,000	259,400	429,000	178,000	463,000	146,400	391,000	180,000
	25	410,000	273,600	409,000	210,000	366,000	165,600	409,000	110,000
685	5	463,000	276,000	440,000	230,000	410,000	176,800	400,000	140,000
	10	400,000	258,000	391,000	220,000	488,000	162,400	429,000	190,000
	25	391,000	216,000	463,000	225,000	429,000	158,400	400,000	131,000
Average		409,400	262,300	418,000	206,900	424,400	162,100	407,700	140,000

upon the decrease in numbers, but the higher amounts of acid were more active than the smaller amounts. As with *B. subtilis* in the presence of salt, there is a marked decrease even in the tubes with smaller amounts, but unlike the higher amounts of

TABLE 5

Influence of varying amounts of acid and air upon the growth of B. mesentericus in broth

EXHAUSTED MM. Hg.	AIR	PER CENTS OF N/1 ACID AND pH							
		0.5 per cent pH 5.60		1 per cent pH 5.00		2 per cent pH 4.30		4 per cent pH 4.00	
		Added	Determined	Added	Determined.	Added	Determined	Added	Determined
	per cent								
0	5	116,000	90,000	114,000	120,000	114,000	240,000	109,000	120,000
	10	104,000	80,000	114,000	150,000	102,000	220,000	102,000	130,000
	25	102,000	89,000	102,000	140,000	118,000	280,000	101,000	230,000
50	5	108,000	54,000	108,000	140,000	102,000	240,000	106,000	140,000
	10	111,000	56,000	96,000	68,000	118,000		91,000	150,000
	25	104,000	75,000	104,000	160,000	108,000		111,000	150,000
100	5	111,000	45,000	114,000	110,000	91,000	120,000	96,000	113,000
	10	101,000	52,000	109,000	110,000	109,000	190,000	102,000	113,000
	25	109,000	48,000	104,000	200,000	102,000		108,000	140,000
Average		107,000	66,000	107,000	199,000	107,000	215,000	103,000	151,000
175	5	40,000	19,200	42,900	16,800	47,600	18,000	38,300	49,600
	10	41,000	16,000	33,200	22,800	45,100	20,000	37,400	33,600
	25	47,600	19,200	36,000	23,200	39,000	36,000	42,900	47,200
350	5	42,900	14,800	36,600	25,200	42,900	49,000	46,300	36,800
	10	44,000	15,200	45,100	26,800	40,900	35,000	46,300	48,000
	25	40,000	12,000	41,000	19,200	46,300	60,000	45,100	65,600
525	5	40,000	14,400	40,900	14,000	36,100	28,000	41,000	43,300
	10	41,000	16,500	44,000	16,800	48,800	30,000	38,300	59,200
	25	39,000	16,000	39,100	14,000	47,600	27,000	37,400	40,000
685	5	44,000	16,000	41,000	18,400	45,100	17,000	39,100	48,000
	10	48,800	18,800	40,000	21,600	45,100		46,300	43,000
	25	45,000	12,800	40,000	13,600	45,100	34,000	44,000	51,200
Average		42,800	15,980	40,300	19,360	44,100	31,700	41,800	47,140

salt there is a marked decrease in the higher concentrations of acid. Even sealing seems to cause a marked decrease in the number of viable spores of both types. Perhaps if the incubation period had been lengthened there would have been a still greater

decrease in case of *B. subtilis*. Parallel tubes which were not sealed showed heavy growth of these organisms in all concentrations of salt, but only a trace of growth in the higher amounts of acid. In the sealed tubes there was a very faint visible trace of surface growth in the tubes containing larger amounts of air. This was easily broken up and did not re-form on standing. The plates made from these tubes checked much better than would generally be expected, when it is considered that organisms of this type produce such heavy surface growths in open tubes. However, these organisms do not form such adherent growths in sealed tubes, and by vigorous shaking a fairly uniform suspension may be obtained.

In the case of *B. mesentericus* there was much less marked action of acid. Either there is not so much decrease in the higher amounts of acid or there is a slight increase of this organism after the initial decrease. At the end of the incubation period there were about the same numbers as at the beginning of the experiment.

In *Experiment IV* an attempt was made to determine the influence of the amount of air and salt upon the thermal death point of *B. subtilis* and *B. mesentericus*. The results are shown in tables 6 and 7. The tubes and spore suspensions were prepared as above.

It is evident from these tables that *B. mesentericus* spores are somewhat more easly killed by heat than those of *B. subtilis*. We have found this to be true in numerous other tests upon the thermal death point of these organisms.

The amount of salt or the amount of air has practically no influence upon the thermal death point, particularly in the case of *B. subtilis*, the larger amounts of salt seeming, however, to protect the organisms to some extent.

Experiment V shows the influence of acetic acid and varying amounts of air upon the thermal death point of *B. subtilis* and *B. mesentericus*.

In this experiment the spores were prepared as above described. The liquid in which the spores were suspended during the heating was extract broth plus 0.5 percent; 1 per cent; 2 per cent; 4 per

cent of N/1 glacial acetic acid, giving the pH as shown in the tables.

Two centimeters of this acid broth were added to the tubes which were then heated in the steamer for fifteen minutes and cooled as rapidly as possible to remove air from the liquid. To

TABLE 6

Influence of varying amounts of salt and air upon thermal death point of B. subtilis spores. Original numbers of spores added 32,000,000 per cubic centimeter

SALT	MM. Hg.	TIME OF HEATING AT 98°C.			
		15 minutes	30 minutes	60 minutes	120 minutes
per cent					
1	Open	460,000	140,000	6,100	15
	175	110,400	40,000	7,600	31
	350	520,000	170,000	6,500	49
	525	640,000	200,000	8,300	15
	685	400,000	121,000	9,300	32
Average		426,000	134,000	7,560	28
2	Open	670,000	180,000	8,700	38
	175	500,000	250,000	9,800	21
	350	400,000	120,000	6,400	42
	525	580,000	210,000	14,600	71
	685	700,000	160,000	10,400	24
Average		570,000	184,000	9,980	39
4	Open	860,000	480,000	18,000	36
	175	840,000	200,000	14,500	56
	350	840,000	190,000	33,600	66
	525	473,000	200,000	26,000	82
	685	860,000		44,000	72
Average		774,600	302,500	27,200	62
General average		590,200	206,800	14,910	44

these tubes was added 1 cc. of a heavy suspension of spores suspended in acid broth, similar to that in the tubes. The tubes were then filled to the mark with similar broth and the volume noted. Amounts equal to 5 per cent, 10 per cent, 25 per cent of the total volume were removed from the tubes. They were

then exhausted to the points desired and sealed at the mark. The tubes were all placed in a steam sterilizer and heated for one hour. Tables 8 and 9 show the results obtained. Since each tube was of slightly different volume the liquid remaining after removing the above amounts would contain slightly different

TABLE 7

Influence of varying amounts of salt and air upon the thermal death point of B. mesentericus spores. Original number of spores added 3,780,000

SALT	MM. Hg.	TIME OF HEATING AT 98°C.			
		15 minutes	30 minutes	60 minutes	120 minutes
per cent					
1	Open	8,000	700	17	2
	175	2,400	200	8	0
	350	1,000	130	34	0
	525	3,600	500	17	0
	685	1,600	200	8	0
Average		3,320	346	17	1
2	Open	2,300	1,100	98	0
	175	1,100	800	30	0
	350	1,400	400	27	0
	525	1,000	520	39	0
	685	1,000	700	74	0
Average		1,360	704	53	0
4	Open	1,700	390	69	0
	175	1,000	640	56	0
	350	2,300	700	88	3
	525	1,400	320	20	0
	685	1,500	200	70	0
Average		1,580	450	61	1
General average		2,087	500	44	1

numbers per cc. The reduction in numbers in this experiment is so striking that the numbers are not included in the tables in each case. The average number for each cc. of the liquid remaining in the tubes was about 37,400,000 per centimeter for *B. subtilis* and 1,760,000 for *B. mesentericus*.

The above tables show a very marked influence of acid upon the thermal death point of both organisms. The fact that canned fruits and vegetables containing, or treated with acid, kept so much better than fruits and vegetables containing no acid, was

TABLE 8

Influence of varying amounts of acetic acid and air upon the thermal death point of of B. subtilis spores

MM. Hg.	AIR	PER CENTS OF N/1 ACETIC ACID			
		0.5 per cent pH 5.5	1 per cent pH 5.10	2 per cent pH 4.5	4 per cent pH 4.10
	per cent				
175	5	590	9	3	0
	10	490	0	0	0
	25	700	0	2	0
Average		593	3	1	0
350	5	482	0	0	0
	10	142	0	2	0
	25	336	0	6	0
Average		320	0	2	0
525	5	43	0	0	0
	10	113	0	0	0
	25	46	0	0	0
Average		67	0	0	0
685	5	44	0	2	1
	10	600	3	4	2
	25	702	1	0	0
Average		448	1	2	1
Open		372	0	3	1
General average		360	1	2	1

formerly thought to be due to the fact that the acid inhibited growth. From the results which we have obtained, we are inclined to believe that the keeping is due, not so much to the influence of the acid in inhibiting growth as to the fact that most or all of the organisms present are killed by the heating process.

The few remaining are probably unable to grow to any extent in the highly acid medium.

Here, also, the amount of air present in the containers has no influence whatever upon the thermal death point of the bacteria present.

TABLE 9

Influence of varying amounts of acetic acid and air upon the thermal death point of B. mesentericus spores

MM. Hg.	AIR	PER CENTS OF N/1 ACETIC ACID			
		0.5 per cent pH 5.0	1 per cent pH 5.10	2 per cent pH 5.50	4 per cent pH 4.10
	per cent				
175	5	4	1	0	0
	10	10	1	0	0
	25	8	0	0	0
Average		7	1	0	0
350	5	11	0	0	0
	10	2	1	0	0
	25	16	1	0	0
Average		9	1	0	0
525	5	13	2	0	0
	10	10	1	0	0
	25	10	3	0	0
Average		11	2	0	0
685	5	2	2	0	0
	10	5	0	0	0
	25	16	2	0	0
Average		7	1	0	0
Open		12	2	0	0
General average		9	1	0	0

Experiment VI shows the influence upon the thermal death point of *B. subtilis* and *B. mesentericus* of several of the more common organic acids found in fruits. The spores were prepared as described in experiment V and placed in the acid solutions

after heating at 80°C. for twenty minutes to kill the vegetative forms. The results are shown in table 10.

TABLE 10

Influence of organic acids upon the thermal death point of B. subtilis and B. mesentericus spores

	TIME OF HEATING	PER CENT OF N/1 ACID ADDED TO BROTH			
		0.5 per cent	1 per cent	2 per cent	4 per cent
B. subtilis added 7,305,000 per cubic centimeter					
	minutes				
Lactic	15	74,400	17,300	3,200	273
	30	3,400	2,000	50	0
	60	80	10	0	0
B. mesentericus added 2,500,000 per cubic centimeter					
Lactic	15	18,000	100	90	10
	30	8,200	0	0	0
	60	13	0	0	0
B. subtilis added 17,500,000 per cubic centimeter					
Tartaric	15	520,000	410,000	52,000	2,000
	30	73,000	54,000	3	3
	60	600	576	2	0
B. mesentericus added 2,145,000 per cubic centimeter					
Tartaric	15	14,000	1,630	4	10
	30	1,200	220	0	0
	60	39	3	0	0
B. subtilis added 27,000,000 per cubic centimeter					
Citric	15	2,000,000	110,000	200	500
	30	240,000	600		200
	60	1,200	4	4	0
B. mesentericus added 17,000,000 per cubic centimeter					
Citric	15	24,000	60	50	12
	30	2,150	48	0	0
	60	0	0	0	0

The results show that there is very little difference in the action of these organic acids. The acetic is perhaps a little more effec-

tive, but not enough to be of practical importance. This table also shows the influence of the amount of acid, and also the fact that *B. mesentericus* is somewhat more easily destroyed by heat than *B. subtilis*.

SUMMARY

From the results obtained, we are inclined to believe that *B. mesentericus* predominates in canned foods because it is capable of growing to some extent in absence of air, rather than because its spores are more heat resistant than some other types of aerobic bacteria.

The amount of vacuum under which spores of these organisms are placed during the heating does not influence the thermal death point.

The small amount of acid present had but slight retarding influence upon the growth of these organisms in air, but did have a marked influence upon the thermal death point. It may be that the beneficial influence of acid upon the keeping of canned foods is due more to the lowering of the thermal death point than to the inhibition of growth of the organisms.

The amount of air remaining above the liquid has little influence upon the growth of these bacteria, since sealing the tubes prevents all but minimum growth. This inhibiting influence is more marked in case of *B. subtilis* than in case of *B. mesentericus*.

REFERENCES

BITTING, A. W., AND BITTING, K. G. 1916 Canning and how to use canned foods. Bull. 14, National Canners Assoc., Washington, D. C., 191 pp.

BRUETT, E. M. 1919 Utility of blanching in food canning. Effect of cold shock upon the bacterial death rates. Journ. of Ind. & Eng. Chem. **11**, 37–39.

BUSHNELL, L. D. 1918 The influence of cold shock in the sterilization of canned foods. Jour. of Ind. & Eng. Chem. **6**, 431–436.

CHEYNEY, E. W. 1919 A study of the microörganisms found in merchantable canned foods. Jour. of Med. Res., **40**, 177–197.

DEICHSETTER, J. 1901 Ueber den Keimgehalt den Fleischkonserven. Zeitschrift für Untersuch, d. Nahr und Genussmit., **4**, 1115.

HUNTER, A. C., AND THOM, C. H. 1919 An aerobic spore-forming bacillus in canned salmon. Jour. Ind. & Eng. Chem., **11**, 655–657.

LAWRENCE, J. S., AND FORD, W. W. 1916 Studies on aerobic spore-bearing non-pathogenic bacteria. Jour. Bact. **21**, 273–320.

PFUHL, E. 1904 Beiträge zur Bakteriologischen Untersuchungen den Fleischkonserven, Zeitschrift für Hygiene. **48**, 121–134.

VAILLARD, L. 1900 Les Conserves, Rept. to 10th Congress of Hygiene and Dem., Paris.

VAILLARD, L. 1902 Les conserves de la viande: Les accidents qu'elles provoquent leurs causes; les moyens de les prevenir. Rev. d'Hygiene, **24**, T. 17–35 and 109–121.

WENZIRL, J. 1919 The bacteriology of canned foods. Jour. of Med. Res. **39**, (New Series **34**) 349–413.