THE ACCELERATING EFFECT OF SUBLETHAL HEAT ON SPORE GERMINATION IN MESOPHILIC AEROBIC BACTERIA[1]

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In their early studies on disinfection, Koch, Gaffky and Loeffler (1881) reported that anthrax spores which had been heated to 90° and 95°C. required longer to produce visible plate colonies than unheated spores. Since that time the growth-delaying action of sublethal heat has been recorded by many investigators, both for spores and vegetative cells: Bredemann (1909), for *Bacillus amylobacter;* Weiss (1921), Esty and Meyer (1922), Esty and Williams (1924), Dickson (1928) and Sommer (1930), for *Clostridium botulinum;* Schultz (1940), for mesophilic aerobes; Allen (1923), for sporulating and non-sporulating mesophilic aerobes; Eijkman (1908) and Hershey (1939), for *Escherichia coli.* There is considerable evidence to indicate that the germination of thermophilic spores is not retarded by non-killing heat treatment; Bigelow and Esty (1920), Esty and Williams (1924), and Feier (1927). According to Sommer this also is true for *Bacillus subtilis.* Earlier observations by Williams (1929) lend support to this contention.

As a result of these reports the belief has become firmly established that sublethal heat has either a delaying or negligible action upon the germination of spores; the possibility that heat might provide a stimulus to germination has been generally overlooked. Eckelmann (1917), in a discussion of the causes of the heat-inhibition of spores, suggested that in some instances stimulation of germination might occur but offered no experimental proof. Allen noted that heat-shock reduced the subsequent generation time of one sporing culture, but it is our observation that a factor may have no influence upon the time required for spores to become heat-labile, and yet may change the rate of subsequent vegetative proliferation.

In this paper we will show that germination of the spores of the mesophilic aerobes may be consistently accelerated by exposure to heat through a wide range of temperatures. Attention has been given to some of the factors which exert an influence upon this reaction. Because of its potential usefulness in the processing of food and other materials this phenomenon possesses more than academic interest.

METHODS AND MATERIALS

The test organisms were the following: *Bacillus megatherium* (N. R. Smith #696), *Bacillus cereus* (#369, #720 N. R. Smith), *Bacillus subtilis* (A.T.C.C.

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#6051, #6634), *Bacillus cohaerens*, *Bacillus fusiformis* (Bureau Dairy Industry Collection), CC (National Canners Association #4149), #9499 (National Canners Association #9499). CC is closely allied to *Bacillus mesentericus*. The position of #9499 in the genus is not known. The plating medium was nutrient agar of the following composition: Difco peptone, 5 g.; Difco beef extract, 3 g.; sodium chloride, 5 g.; glucose, 3 g.; agar, 13 g.; water, 1,000 ml. pH 7.0. The broth was similar, except for the omission of agar. The evaporated milk was prepared from whole milk concentrated 2 to 1 and was not sterilized before use. Total bacterial counts of the evaporated milk prior to use varied between 300 and 700 per ml., of which less than 5 per ml. survived heat at 85°C. for 10 minutes.

The spores were produced on plain nutrient agar slopes; when sporulation was complete, the growth was washed off with distilled water, filtered through cotton, and centrifuged. The water was decanted and the washing process repeated twice. When clumping occurred the clumps were largely broken up by moderate shaking of the suspension with small glass beads. The concentrated stock suspensions thus prepared, practically 100 per cent spores, were plated to determine purity and count and were then held at 6°C. until used.

Germination was determined by the following procedure: A small quantity of the diluted stock suspension of spores was seeded into the test medium and the two thoroughly mixed. The uniformly dispersed suspension of spores was then divided into a series of equal portions, one of which, the control, received no preincubation heat, and was stored at 8°C., while the several remaining portions were preheated at selected temperatures for various periods. These, together with the unheated control, were then adjusted to and incubated at 37°C. for 3 to 5 hours. When the preheating medium was water or buffer solutions, small equal volumes of the heated spore suspensions were subseeded into glucose broth prior to incubation. Following incubation, all samples were heated at 85°C. for 10 minutes in order to kill the spores which had become heat-labile during the incubation interim. Subculturing of the final heated suspensions was carried out in glucose agar plates which were counted after 48 hours at 37°C. Assumption by the spores of the heat-lability characteristic of vegetative cells was accepted as evidence of germination. There was no appreciable change in the pH of the cultures during incubation.

The suspensions were heated in 8 ml. quantities in pyrex tubes in a stirred glycerol bath equipped with a thermo-regulator, which maintained the desired temperature ± 0.5°C.

EXPERIMENTAL

Germination of spores as affected by preincubation heat

It was observed early in this study that the effect of heat upon the germination of spores is materially influenced by the medium in which they are heated. Four different heating mediums were used.

In table 1 is shown the effect of heat upon the germination of spores, heated and incubated in glucose extract broth, when the suspensions were preheated

at 85°C. as indicated, then held at 37°C. for three hours, and finally heated at 85°C. for 10 minutes and plated. Under these conditions preincubation heat accelerated the germination of 7 of the 9 cultures; stimulation was greatest with *B. fusiformis*, *B. megatherium*, CC, and 9499. The data recorded in the lower part of the table were obtained when the spores received heat equivalent in amount to that of the upper part, except that all of the heat was applied at the end of the incubation period. The data, therefore, at 4–10 and 0–14, 8–10 and 0–18, etc., are directly comparable, in that each series received an equal amount of heat, differing only in the time at which it was applied. Differences in the counts of comparable series are a direct measure of the heat-induced acceleration or retardation of germination. With 7 cultures, preincubation heat mate-

TABLE 1

Effect of heat upon the germination of spores when heated and incubated in glucose-extract broth

PRE-INCUBATION HEAT	POST-INCUBATION HEAT	VIABLE SPORES AFTER INCUBATION FOR 3 HOURS WITH PRE- AND POST-INCUBATION HEAT AT 85°C. FOR THE PERIODS INDICATED								
		B. megatherium	*B. subtilis* (6051)	*B. subtilis* (6634)	*B. cohaerens*	*B. fusiformis*	*B. cereus* (369)	*B. cereus* (720)	CC	9499
		(70,000)	(82,000)	(95,000)	(96,000)	(91,000)	(155,000)	(190,000)	(98,000)	(130,000)
minutes	*minutes*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*
0	10	7,500	220	2,640	120	32,000	100	120	36,000	120,000
4	10	2,100	110	1,400	220	2,200			27,000	12,000
8	10	290	70	400	220	1,600	800	550	17,000	5,200
32	10	180	80	240	75	400	1,300	450	3,300	2,100
64	10	33	80	190	20	400	250	820	800	2,000
96	10	15	80	180	15	300	8	240	400	2,700
0	14	5,900	130	2,700	110	30,000			37,000	110,000
0	18	4,200	140	2,700	120	29,000	100	140	35,000	130,000
0	42	3,000	140	2,200	130	11,000	10	6	30,000	120,000
0	74	2,000	110	2,400	100	7,000	20	3	32,000	110,000
0	106	900	130	2,300	100	1,400	2	0	31,000	120,000

( ) Number of viable spores per ml. immediately after inoculation.

rially accelerated the change from the heat-stable to the heat-labile condition. With *B. cohaerens* stimulation was apparent only after the longer heating periods. The anomolous reaction obtained with the two strains of *B. cereus* is of interest in itself, and in connection with the behavior of this species in subsequent heating mediums. The data for 9499 require explanation. Our observations clearly demonstrate that a large proportion of the spores of this organism are normally dormant and will not germinate in the absence of preliminary heat treatment. Eight minutes of heating at 85°C. was found to increase the number of colonies which subsequently developed on plates, by about 35 per cent, as compared with the unheated control; thus, with this culture, when part of the heat is applied before incubation the resulting count reflects the effects both of accelerated

germination and heat activation of normally dormant spores, but with all the heat applied after incubation normal germination plus heat activation of dormant spores is indicated. In the latter instance the slight change in the number of heat-stable spores in relation to their initial concentration indicates an essential balance between normal germination and heat activation. The relatively constant values for CC, *B. cohaerens*, and *B. subtilis* (2 strains), when all of the heat was applied after incubation, suggests a correlation between delayed germination and heat-resistance. Although, as will be shown (figs. 1 and 2), the longer heating periods kill substantially more of the unincubated spores than the shorter heating periods, this is not reflected in any significant change in the number of

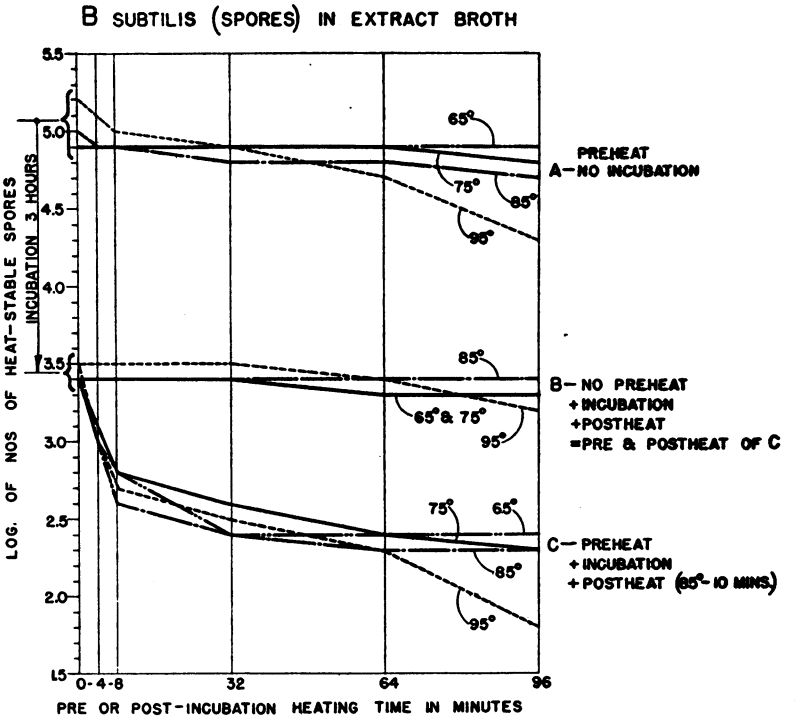

FIG. 1

spores that do not germinate (table 1, lower portion), indicating that spores of low heat resistance are not among those which exhibit slow germination.

In table 2 are shown the results obtained when the spores were heated in distilled water, and then transferred to glucose extract broth for incubation and postincubation heating. Under these conditions preliminary heat stimulated the germination of 5 of the 8 cultures. *B. cereus* (2 strains), retarded in their germination by heat in glucose broth, were somewhat stimulated by the same degree of heat applied to water suspensions. The reverse was true for *B. cohaerens* and the two strains of *B. subtilis*.

In another experiment the spores were seeded in evaporated milk, preheated

as shown and incubated for 5 hours, followed by the usual mild heating. As may be seen (table 3), preincubation heat under these conditions accelerated the germination of every culture. For eight minutes of preheating the minimum reduction in heat-stable spores over the unheated control was about 40 per cent, while the maximum was approximately 95 per cent.

Why relatively mild heat should speed the germination process is not known, but a possible clue is afforded by the work of Cook (1931) and Tarr (1933). The former found that the boiling of spores of *B. subtilis* prior to their seeding in tryptic medium reduced the lag in oxygen uptake over that of unheated spores; the latter showed that heat at 80°C. for 30 minutes greatly increased the dehydrogenase and oxidase activity of spores of *B. subtilis* suspended in a phosphate

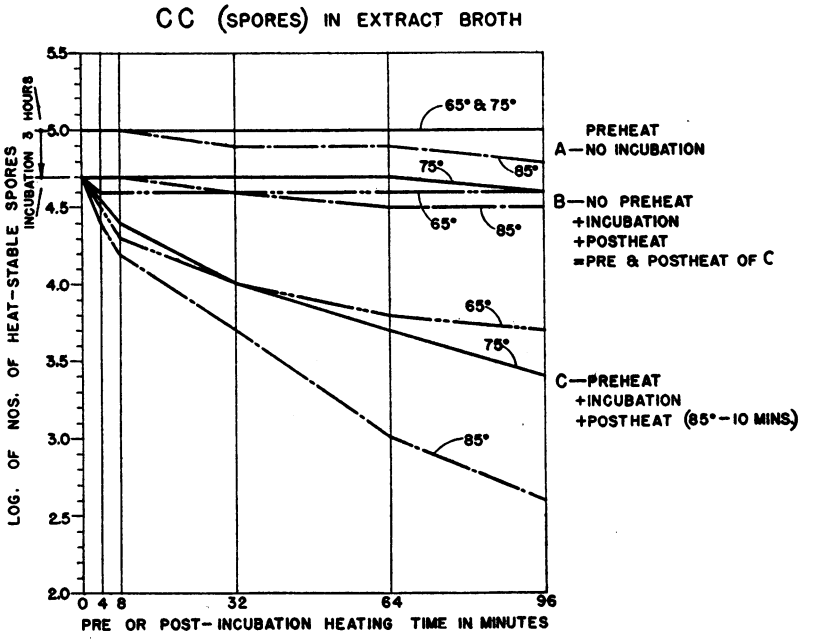

FIG. 2

buffer solution. Eighty degrees C. was more effective than 60°, 70°, or 90°. Tarr further showed that the increased oxygen uptake resulting from preheating the spores was maximal when the pH of the buffer suspension was 6.5, whereas it dropped off rapidly below pH 6.0. Prompted by this observation, we studied the effect of heating spores in phosphate buffers of differing pH followed by subculturing the spores in glucose broth at pH 7.0. The results are shown in table 4. The preincubation concentrations of spores, both with and without preheating are included, since the sporicidal action of heat at 85°C. varied with pH. It is evident from these data that the buffer medium is not a particularly favorable heating medium for demonstration of the heat effect. For *B. subtilis*, greatest acceleration of germination occurred at pH 6.3, with little demonstrable effect at pH 5.2, which accords with the effect of preheating of *B. subtilis* at 80°C. upon

the activity of respiratory catalysts reported by Tarr. With CC, preheating at pH 5.2 seemed to produce the greatest acceleration of germination. For *B. megatherium* the pH of the heating medium had no significant influence upon the preheating effect.

The importance of adequate nutritional stimuli for the subculture of heated spores has been previously emphasized by the publications of Süpfle and Dengler

TABLE 2

Effect of heat upon the germination of spores when heated in distilled water and incubated in glucose-extract broth

PRE-INCUBATION HEAT	VIABLE SPORES AFTER PREHEATING AT 85°C. AS INDICATED PLUS INCUBATION FOR 3 HOURS PLUS 85°C. FOR 10 MINUTES								
	B. megatherium	*B. subtilis* (6051)	*B. subtilis* (6634)	*B. cohaerens*	*B. fusiformis*	*B. cereus* (369)	*B. cereus* (720)	CC	9499
	(75,000)	(85,000)	(90,000)	(95,000)	(90,000)	(40,000)	(160,000)	(101,000)	(135,000)
minutes	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*
0	28,900	110	2,700	84	7,900	57	2.7	31,000	123,000
4	7,200	470	6,000	300	4,400	7.0	3.0	21,000	48,000
8	5,100	700	9,000	410	3,600	4.3	0.6	11,000	44,000
32	700	800	10,000	670	1,800	1.3	0.6	10,000	40,000
64	100	1,600	9,000	740	430	0	0.6	7,000	26,000
96	60	1,800	8,000	860	280	0.3	0	8,000	22,000

( ) = concentration of viable spores per ml. immediately after inoculation.

TABLE 3

Effect of heat upon the germination of spores when heated and incubated in evaporated milk

PRE-INCUBATION HEAT	VIABLE SPORES AFTER PREHEAT AT 85°C. FOR THE PERIODS INDICATED, PLUS INCUBATION FOR 5 HOURS, PLUS 85°C. FOR 10 MINUTES								
	B. megatherium	*B. subtilis* (6051)	*B. subtilis* (6634)	*B. cohaerens*	*B. fusiformis*	*B. cereus* (369)	*B. cereus* (720)	CC	9499
	(70,000)	(82,000)	(95,000)	(96,000)	(91,000)	(155,000)	(190,000)	(98,000)	(130,000)
minutes	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*
0	51,500	370	7,200	3,500	4,000	4,400	3,200	44,000	123,000
8	30,300	200	2,300	1,100	720	770	970	11,000	15,000
32	9,500	200	1,200	600	500	350	380	2,500	6,000
64	4,800	170	900	400	300	300	420	400	5,400

( ) = concentration of viable spores per ml. immediately after inoculation.

(1916), Morrison and Rettger (1930) and Curran and Evans (1937). The present results show that the heating medium, independently of the subculture medium, influences the subsequent behavior of heated spores.

A clearer picture of the action of sublethal heat upon the germination of spores is obtained when the data for a given culture (*B. subtilis* # 6634) are represented graphically as shown in figure 1. As may be seen, temperatures ranging from 65° to 85°C. with no incubation produced little change in the number of viable

spores. At 95°C. the direct killing was material when the heating was continued longer than 32 minutes. The 5.0 to 3.5 drop on the log scale at B represents the normal germination of the unheated spores during the incubation period of 3 hours. The much smaller number of heat-stable spores in series C shows that preheating materially accelerated the germination of the spores so treated. In general the preheating at 85°C. produced greater stimulation of germination than higher or lower temperatures. The lower counts obtained at 95°C., where the preheating time exceeded one hour, merely reflects the greater direct killing effected by this temperature. Figure 2 shows a similar series of curves for CC wherein the accelerating effect of preheat was more pronounced than for *B. subtilis*, and in which the differences produced by the changes in preheating temperatures were much wider. Data for 95°C. are omitted, since the highly lethal action of this temperature rendered the data of little significance.

TABLE 4

The germination of spores in glucose broth with 3 hours of incubation at 37°C. after previous heating in buffer solutions of differing pH followed by post incubation heating of 85°C. for 10 minutes*

	pH 5.2				pH 6.3				pH 7.4			
	No incubation		3 hours at 37°C.		No incubation		3 hours at 37°C.		No incubation		3 hours at 37°C.	
	No preheat	Preheat	No preheat	Preheat	No preheat	Preheat	No preheat	Preheat	No preheat	Preheat	No preheat	Preheat
B. subtilis #6634	94,000	67,000	1,360	1,050	92,000	86,000	1,160	220	100,000	87,000	840	220
B. megatherium.....	69,000	48,000	37,000	23,000	77,000	66,000	38,000	21,000	78,000	67,000	34,000	22,000
CC	106,000	84,000	30,000	10,000	97,000	86,000	22,000	12,000	78,000	65,000	21,000	18,000

* 85°C. for 8 minutes.

Persistence of the heat effect

We have shown that appropriate heat treatment accelerates the germination of bacterial spores if favorable cultural conditions are promptly provided. In the belief that light might be shed on the mechanism of the reaction, some observations were made upon the persistence of the preheat effect. This was determined by the delayed cultivation of spores in broth following their heating in distilled water. The data shown in table 5 indicate that heat-altered spores do not quickly revert to their former condition in the absence of favorable growth conditions. With the exception of *B. megatherium*, the heat-induced effects, whether of retardation or acceleration, showed little change throughout the observation period.

The advantage of preheating spores in reducing germination time

It was deemed of interest to ascertain the advantage of preheating spores as measured by the time required for heated and unheated spores to attain comparable germination levels. In an effort to accomplish this preheated (85°C. for 8 minutes) and unheated spores were suspended in glucose broth and incubated

at 37°C. for 3, 8, 17, and 24 hours, following which the heat-labile cells were killed by treatment at 85°C. for 10 minutes. The data are shown in table 6. As may be seen, the germination of unheated spores proceeded very slowly after

TABLE 5

Effect of delayed cultivation of heated spores upon their germination*

CULTURE	PRECULTIVATION TREATMENT	NO INCUBATION	TIME AFTER PREHEATING OF CULTIVATION† FOLLOWED BY HEATING OF 85°C. FOR 10 MINUTES		
			Immediately	1 day	7 days
		per ml.	*per ml.*	*per ml.*	*per ml.*
B. cohaerens	85°C.—10 min.	90,000	570	340	290
	No preheat	104,000	140	92	90
B. subtilis #6634	85°C.—10 min.	71,000	5,500	4,400	4,600
	No preheat	79,000	1,100	1,170	920
B. megatherium	85°C.—10 min.	88,000	8,100	14,000	15,000
	No preheat	95,000	28,000	27,400	29,700
CC	85°C.—10 min.	106,000	26,000	22,000	21,000
	No preheat	91,000	51,000	54,000	51,000
9499	85°C.—10 min.	150,000	26,000	22,000	25,000
	No preheat	113,000	102,000	107,000	116,000

* Heating medium, distilled water.

† 3 hours in glucose broth.

TABLE 6

The germination of preheated spores during 3 hours of incubation in glucose broth compared with that of unheated spores over a 24-hour incubation period

CULTURE	INITIAL CONCENTRATION OF SPORES	VIABLE SPORES AFTER INCUBATION FOR TIMES INDICATED, PLUS POST INCUBATION HEAT 85°C. FOR 10 MINUTES				
		Heated before incubation	Not heated before incubation			
		3 hours	3 hours	8 hours	17 hours	24 hours
	per ml.	*per ml.*	*per ml.*	*per ml.*	*per ml.*	*per ml.*
B. cohaerens	92,000	210	110	28	26	31
B. subtilis #6634	95,000	350	2,600	1,340	460	443
B. megatherium	78,000	260	8,000	4,400	3,000	2,500
CC	95,000	18,000	36,000	21,000	16,000	15,000
9499	127,000	5,800		106,000	102,000	98,000

the first three hours; little or no change in the number of heat-stable cells occurred during the last 8 hours. In general, three hours of incubation preceded by mild heating was superior to 24 hours of incubation without preheat as measured by the reduction in number of heat-stable spores. A longer preheating

of the *B. cohaerens* suspension would probably have brought the data on this culture into essential agreement with those of the other cultures (table 1). Although the descending order in the number of heat-stable spores speaks against resporulation, a separate experiment was performed to test this point. It was found that there was no resporulation during the 24-hour observation period with *B. subtilis*, *B. megatherium* and 9499. With *B. cohaerens* and CC resporulation occurred, but in numbers so small as to have no significant influence upon the results.

DISCUSSION

It is well known that many chemical poisons, including nearly all salts, will stimulate bacterial development if suitably diluted in a nutrient medium (Rahn, 1932; Topley and Wilson, 1937). Likewise there is considerable evidence that sublethal doses of ultraviolet radiation may stimulate bacterial growth (Coblentz and Fulton, 1924; Hollaender and Duggar, 1938). That there should be an analogous operation of this principle for temperatures beyond the natural range of growth of bacteria seems reasonable. For certain fungus spores this has been clearly demonstrated. Thus the ascospores of *Ascobolus* and *Neurospora* are normally dormant and will germinate only after they have been heated (Dodge, 1912; Shear and Dodge, 1927). Goddard (1935) later found that the heat necessary for the activation of these spores is correlated with a large increase in respiration (O_2 consumed). Evidence of the stimulative action of sublethal heat upon the respiratory enzymes of bacterial spores has been cited (Cook, 1931, and Tarr, 1937). It was shown by the latter that the real effect of heat was to reduce the time required for the attainment of a constant velocity of oxidation. It is, perhaps, significant that both the maximum stimulation of enzymic respiratory activity (Tarr) and the acceleration of germination occur at essentially the same temperature levels. Strain 9499 was noteworthy in that greatest stimulation followed preheating at 95°C. The greater heat-resistance of this species suggests that there may be a correlation between thermal resistance and the optimum preheating treatment essential for maximum acceleration of germination.

The observation that sublethal heat may speed rather than delay the germination of spores is contrary to general belief and the recorded observations of the investigators previously cited (page 513). The reasons for this discordance are not apparent; however, mention should be made that previous investigators based their conclusions on the relative periods required for heated and unheated spores to form visible colonies on plates—a process which involves both germination and vegetative multiplication.

The results obtained with the different heating mediums are of interest in connection with an earlier observation by Morrison and Rettger (1930a). These authors found that the spores of a spoilage organism when heated in water and subcultured in broth exhibited the familiar "skips" with a consequent ill-defined thermal death-time. When, however, the same suspension was heated in evaporated milk and subcultured on standard agar plates "skips" were absent and

the thermal death-time sharply defined and apparently constant. The similarity of this reaction with that observed for *B. subtilis* and *B. cohaerens* (tables 2 and 3) is significant and points to a better understanding of the irregularities frequently encountered in heating tests.

Although only a small proportion of spores is affected by preheating, these potentially dormant forms are nevertheless of primary economic significance, since delayed germination and high resistance are intimately associated phenomena; the factors which make for maximum resistance seem also to render the spores less responsive to nutritional stimuli.

SUMMARY

Sublethal preincubation heating of aerobic spores may be used to accelerate their germination.

Temperatures in the 65–95°C. range are effective accelerators of spore germination. In general, greatest acceleration was obtained with a preheating treatment of 85°C. for 8 to 10 minutes.

The effect of preheating upon spore germination is influenced by the nature of the medium in which the spores are heated and incubated. With some species preheating may accelerate or retard germination, depending on the nature of the preheated medium.

Preincubation heat accelerated spore germination in 7 out of 9 cultures heated and incubated in glucose broth.

Preincubation heat accelerated spore germination in 5 out of 8 cultures heated in distilled water, and incubated in glucose broth.

Preincubation heat accelerated spore germination in 9 cultures (no exceptions) heated and incubated in evaporated milk.

The effect of preincubation heat upon spore germination is influenced by the reaction of the preheated medium. The optimum pH for acceleration of germination apparently differs with the species.

When spores were heated, and held in distilled water, their altered capacity for germination was still manifest after 1 week.

In general three hours of incubation preceded by mild heating was equivalent or more than equivalent to 24 hours of incubation without preheating as measured by the reduction in numbers of heat-stable spores.

REFERENCES

ALLEN, P. 1923 The attenuation of bacteria due to temperature shock. J. Bact., **8**, 555–566.

BREDEMANN, G. 1909 *Bacillus amylobacter* in morphologischer physiologischer und systematischer Beziehung. Zentr. Bakt. Parasitenk., II., **23**, 385–568.

COBLENTZ, W. W., AND FULTON, H. R. 1924 A radiometric investigation of the germicidal action of ultraviolet radiation. U. S. Bur. Standards, Scientific Papers No. 495, **19**, 641–680.

COOK, R. P. 1931 Some factors influencing spore formation in *B. subtilis* and the metabolism of its spores. Zentr. Bakt. Parasitenk., I, Orig. **122**, 329–335.

CURRAN, H. R., AND EVANS, F. R. 1937 The influence of enrichments in the cultivation of bacterial spores previously exposed to lethal agencies. J. Bact., **34**, 179–189.

DICKSON, E. C. 1928 Dormancy or delayed germination of spores of *Clostridium botulinum* subjected to heat. Proc. Soc. Exptl. Biol. Med., **25**, 426–427.

DODGE, B. O. 1912 Methods of culture and the morphology of the archiicarp in certain species of the *Ascobolaceae*. Bull. Torrey Botan. Club, **39**, 139–197.

ECKELMANN, E. 1917 Ueber Bakterien, welche du fraktionierte sterilisation leben überdauern. Zentr. Bakt. Parasitenk., II. **48**, 140–178.

EIJKMAN, C. 1908 Die Überlebungskurve bei Abtötung von Bakterien, durch Hitze. Biochem. Z., **11**, 12–20.

ESTY, J. R., AND MEYER, K. F. 1922 The heat resistance of the spores of *B. botulinus* and allied anaerobes. XI. J. Infectious Diseases, **31**, 650–663.

ESTY, J. R., AND WILLIAMS, C. C. 1924 Heat resistance studies. I. A new method for the determination of heat resistance of bacterial spores. J. Infectious Disease, **34**, 516–528.

FEIRER, W. H. 1927 Studies on some obligate thermophilic bacteria from soil. Soil Sci., **27**, 47–56.

GODDARD, D. R. 1935 The reversible heat activation inducing germination and increased respiration in the ascospores of *Neurospora Tetrasperma*. J. Gen. Physiol., **19**, 45–60.

HERSHEY, A. D. 1939 Factors limiting bacterial growth. VII. Respiration and growth properties of *Escherichia coli* surviving sublethal temperatures. J. Bact., **38**, 563–578.

HOLLAENDER, A., AND DUGGAR, B. M. 1938 The effects of sublethal doses of monochromatic ultraviolet radiation on the growth properties of bacteria. J. Bact., **36**, 17–37.

KOCH, R., GAFFKY, G., AND LOEFFLER, F. 1881 Versuche über die Verwerthbarkeit heisser Wasserdämpfe zu Desinfectionszwecken. Mitt. kaiserliche Gesundheitsamte, *IX*, 322–340.

MORRISON, E. W., AND RETTGER, L. F. 1930a Bacterial spores. I. A study of heat resistance and dormancy. J. Bact., **20**, 299–311.

MORRISON, E. W., AND RETTGER, L. F. 1930b Bacterial spores. II. A study of spore germination in relation to environment. J. Bact., **20**, 313–342.

RAHN, O. 1932 Physiology of Bacteria. P. Blakiston's Son & Co., Inc., Philadelphia.

SCHULTZ, H. 1940 Untersuchungen über die Sporenötungszeit bei Bodenbakterien als diagnostisches Merkmal. Zentr. Bakt. Parasitenk., **102**, 433–485.

SHEAR, C. L., AND DODGE, B. O. 1927 Life histories and heterothallism of the red bread-mold fungi of the Monilia sitophila group. J. Agr. Research, **34**, 1019–1042.

SÜPFLE, K., AND DENGLER, A. 1916 Die Bedeutung optimaler Nährboden zur Nachkultur bei der Prüfung von Desinfections verfahren. Arch. Hyg., **85**, 189–197.

SOMMER, E. W. 1930 Heat resistance of the spores of *Clostridium botulinum*. J. Infectious Diseases, **46**, 1–30.

TARR, H. L. A. 1933 Some observations on the respiratory catalysts present in the spores and vegetative cells of certain areobic bacilli. Biochem. J., **27**, 136–145.

TOPLEY, W. W. C., AND WILSON, G. S. 1937 The principles of Bacteriology and Immunity. 2nd Ed. W. Wood & Co., Baltimore, p. 112.

WEISS, H. 1921 The heat resistance of spores with special reference to the spores of *B. botulinus*. J. Infectious Diseases, **28**, 70–92.

WILLIAMS, O. B. 1929 The heat resistance of bacterial spores. J. Infectious Diseases, **44**, 421–465.