

# Chemical States of Bacterial Spores: Heat Resistance and Its Kinetics at Intermediate Water Activity

GORDON ALDERTON AND NEVA SNELL

*Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710*

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Bacterial spore heat resistance at intermediate water activity, like aqueous and strictly dry heat resistance, is a property manipulatable by chemical pretreatments of the dormant mature spore. Heat resistances differ widely, and survival is prominently nonlogarithmic for both chemical forms of the spore. Log survival varies approximately as the cube of time for the resistant state of *Bacillus stearothermophilus* spores and as the square of time for the sensitive state. A method for measuring heat resistance at intermediate humidity was designed to provide direct and unequivocal control of water vapor concentration with quick equilibration, maintenance of known spore state, and dispersion of spores singly for valid survivor counting. Temperature characteristics such as  $z$ ,  $E_a$ , and  $Q_{10}$  cannot be determined in the usual sense (as a spore property) for spores encapsulated with a constant weight of water. Effect on spore survival of temperature induced changes of water activity in such systems is discussed.

Traditionally, bacterial spores have been considered to have two kinds of heat resistance, wet and dry. In general, wet has referred to test environments in which liquid-phase water was present, dry to measurements made in the absence of liquid water. Murrell and Scott (10) showed that in the absence of liquid water the water vapor activity ( $a_w$ ) or relative humidity of the test environment had a very large effect on spore heat resistance. They showed that heat resistance was maximal at environmental  $a_w$  values in the 0.2 to 0.4 range. The heat resistance effects they obtained by variation of the relative humidity of the test environment were large, amounting to many-fold. Thus, those categories of spore heat resistance based on the water status of the test environment could no longer be confined to two, wet and dry, but must be increased to at least three general classes: wet, meaning in the presence of liquid water at  $a_w$  values near 1; dry, meaning the total absence of water activity in the test environment; and third, resistance in the absence of liquid water but at an intermediate water vapor activity. In the last category, environmental water activity must be specified precisely because of the large variation of resistance with relative humidity. As a practical probability, most heat challenges of bacterial spores in the absence of liquid water

would fall into the third category since quite rigorous precautions are required to insure the total absence of water activity (3). This inadequacy of the traditional term "dry heat" as a sufficient specification of a nonaqueous environment for spore heat resistance has also been pointed out by Angelotti et al. (5), Pflug and Schmidt (11), and others, but the term seems to persist for a description of environments with water activities from zero to near one so long as obvious liquid water is absent. Since Murrell and Scott's work (10), several recent studies of Angelotti (5), Fox and Pflug (7), Mullican and Hoffman (9), Hoffman, Gambill, and Buchanan (8), and Bruch and Smith (6) on so-called dry-heat resistance have included comments on the pertinence of these water activity effects to the results. Unfortunately, in none of these studies could the level of water activity at the lethal temperature be specified quantitatively.

For ordinary aqueous heat resistance, we have shown that mature bacterial spores can exist in sensitive and resistant states (1, 3, 4). These different resistance states are prepared and interconverted by in vitro chemical pretreatments of the spores. The changes of aqueous heat resistance between the states can amount to about a thousand-fold. These changes or differences in

heat resistance reside within the spores rather than being a response to environmental conditions during the heat resistance test since the changed resistance properties persist when the reagents used to effect the change of state are removed and the spore is transferred to a new environment for measurement of its heat resistance capacity. Thus, to the extent of three orders of magnitude, aqueous spore heat resistance for *Bacillus stearothermophilus* is an inducible property, and meaningful measurements of heat resistance potential are not possible without knowing the chemical state of a spore sample. Chemical events accompanying these changes of heat resistance state have been described (1, 4). Later, we showed (3) that these same chemical states of the spore also have different resistances to strictly dry heat and we presented a method for dry-heat resistance measurements designed to avoid artifacts due to interference by unknown spore chemical state, water activity effects, and uncertain mechanical recovery of the dry-heat-challenged spores.

Here we show that the sensitive and resistant states of spores also exist for heat resistance at intermediate environmental water activity. Characteristics of the nonlogarithmic survivor curves and their temperature dependence are given for each of the chemical states at an optimal environmental water activity. A method is presented for testing heat resistance at intermediate water activity. The method is designed to avoid the interfering factors mentioned above and also to furnish known and easily controlled environmental water activity at the test temperature. Some consequences of the existence of a maximum in the survivor versus  $a_w$  relation are given for the temperature dependence of survival rates of spores encapsulated with a definite weight of water.

#### MATERIALS AND METHODS

**Preparation of spore crop and the spore chemical states.** Spores of *B. stearothermophilus* NCA 1518 were grown and cleaned as previously described (3). The preparation of the sensitive state, hydrogen form, spores, and the three preparations of the resistant state spores were the same as before (3).

**Method for measurement of heat resistance at controlled water activity.** The general approach to water activity control was direct rather than through the use of humidity-controlling solutions. It was arranged to have a known weight of water in an otherwise evacuated sealed glass tube of known volume at a known temperature. The amounts of water required to give the desired  $a_w$  at the lethal test temperature were taken from handbook tables of the properties of saturated steam. The ratio of space volume to spore weight should be chosen such that these amounts of

water are large by comparison with any expected emission or uptake of water by the spores themselves.

Borosilicate glass thermal death time (TDT) tubes (9 by 150 mm) were precontracted to facilitate later flame sealing. The volume of the tubes up to the middle of the constriction (about 4 ml) was then determined by filling with water. The tubes were segregated into lots whose members had a volume variation range of less than 0.1 ml. The tubes were then filled and covered with distilled deionized water and autoclaved for 1 hr to leach out soluble alkali near the surface of the glass (2). The leaching process was repeated and the tubes were allowed to dry.

Very small piles (200  $\mu\text{g}$ ) of the "dry" (freeze dried) spores were weighed into the tubes. The amount (steam tables) of water required to give the desired water activity at the test temperature was then injected by a Hamilton microliter syringe as a drop on the inner wall of the tube. The area of the tube around the water drop was pressed against a piece of dry ice until the drop froze firmly. The open top of the tube was then quickly connected to a piece of gum-rubber tubing connected to, but sealed off by a spring clamp from, an oil pump vacuum. The TDT tube with the drop still frozen was then inserted up to the constriction into powdered dry ice in a Dewar flask. After about 1.5 min, the spring clamp to the preexisting oil pump vacuum was removed and vacuum was pulled for about 20 sec. The TDT tube still attached to the oil pump vacuum was then quickly sealed in a gas-oxygen flame and the seal was annealed in a smoky flame. The sealed TDT tube was then canted against the sharp edge of a slab of dry ice until needed for the lethal heating.

The water drop can be moved quickly from place to place within the sealed tube by chilling a small area against dry ice. This serves as a vacuum test and was applied to each tube both before and after the lethal heating. Also, it was confirmed that no significant loss of water occurred during the above described vacuum sealing procedure. This was done by weighing, before and after drying, severed portions of previously prepared TDT tubes into which the water had been collected into one end by such spot chilling with dry ice.

For the lethal heating step, the tubes were enclosed singly in flat wire cages ( $\sim 1.5$  by 10 by 12 cm) fabricated from 0.64-cm mesh wire (hardware wire cloth). Prior to insertion of the tubes into the cages, the chilled spot on the tube was thawed with fingers. The tubes were heated for measured times in an oil bath controlled to better than 0.01 C. After the elapsed heating time, the wire cages were quickly plunged into cool water for a few seconds. The cages were then set upright to a depth of about 2.54 cm in warm ( $\sim 40$  C) water for a few seconds to drive the condensed water away from the spores. The tubes were then removed from the cages, quickly wiped free of oil, and again slanted against the sharp edge of a slab of dry ice to collect all the condensed water into one spot away from the spores. If plating for survivor count was to be delayed to another day, the tubes at this point were stored in crushed dry ice.

The tubes were then snapped open after scoring

thoroughly to facilitate a clean, even break. The spores were washed out with four 1-ml rinses of Tryptone broth into a Teflon homogenizer cup (A. H. Thomas Co., Catalog #4288, size A) and homogenized thoroughly enough to disperse them singly, as judged by direct microscopic count on oil-cleared membrane filters (12). The homogenized spore suspension was then further diluted as required with Tryptone broth for plating (Tryptone, 1%-glucose 0.5%-soluble starch, 0.1%). Incubation was for 2 days at 53 C. Direct microscopic counts were made on the Tryptone broth dilutions by the method of Snell (12) for each tube. The number of spores in the original little pile of spores was based on this direct count. This avoided needing to know and control the moisture content of the original spore sample and the problems of accurate weighing in the microgram range as well as that of correcting for possible loss in opening the evacuated tubes.

After rinsing the spores out of the opened tubes, the volume of the two halves of each of the tubes was measured by filling (level meniscus) with water. With some practice in flame sealing and the preselection for volume uniformity mentioned above, the standard deviation of the measured volumes of the opened tubes within a lot was about 0.4% of the mean. However, the volume of both halves of each tube was always checked because of the obvious possibility of serious volume variation from the flame sealing operation. In actual practice, such variations did not occur.

**RESULTS**

**Variation of survivors with  $a_w$ .** In Fig. 1 are plots for a given heat treatment of log survivors versus water activity for each chemical state of the spores. Log survivors fall off very steeply on each side of the moderately broad maximum. Fortunately, this maximum is at about the same position for each chemical state. The optimal water activity (0.28) chosen for this study was taken from the central portion of these maxima. The position of the maximum was not significantly changed when the heating time for the resistant state (preparation 3) was heated for 50 min at 150 C rather than 30 min, although the height of the maximum was reduced by about 2.5 log units. The water activity at the maximum agrees closely with that reported by Murrell and Scott (10) for their bithermal method.

**Survivor curve characteristics for the sensitive and resistant states.** It is obvious from Fig. 2 and 3 that the log survivors versus heating time curves are indeed curves without the possibility of reasonable approximation by straight lines (log death). This curvilinearity holds for both chemical states but is most prominent in the log survivor curves for the resistant-state spores. Since the log survivor versus time plots are not linear, the  $D$  value concept is not applicable to heat inactivation

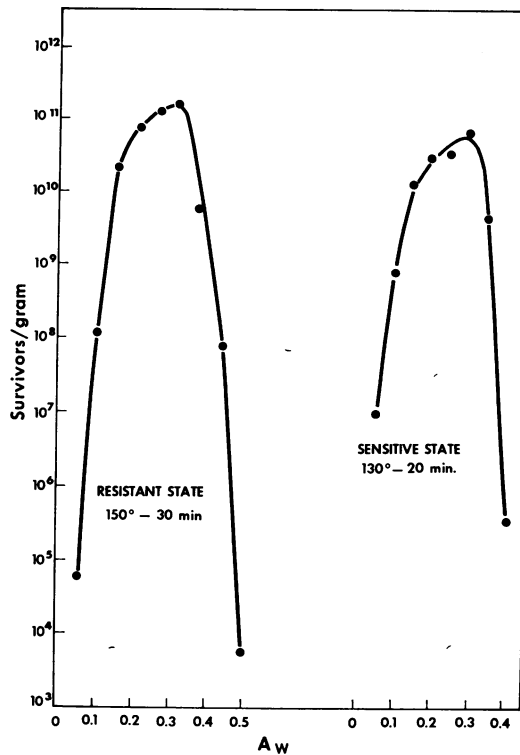


FIG. 1. Heat survival versus environmental water activity for the chemical forms of *B. stearothermophilus* spores.

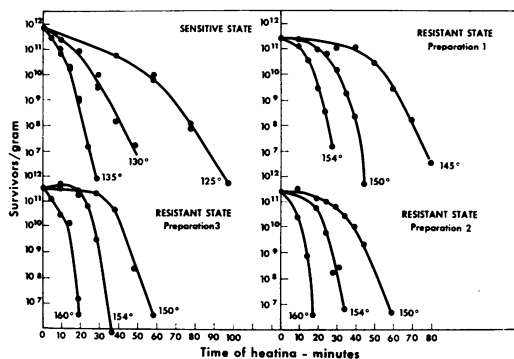


FIG. 2. Heat resistance of the chemical states of *B. stearothermophilus* spores at intermediate water activity ( $a_w = 0.28$ ).

tion of spores under these conditions of intermediate water activity.

For describing heat inactivation at  $a_w = 0.28$  under these conditions, an empirical expression,  $[(\log \text{ initial count/g}) - (\log \text{ survivors/g})]^a = kt_{\text{min}} + C$ , was chosen which gave a reasonable fit to the log survivor curves. The exponent  $a$  for

each chemical state was chosen from the least squares slope of plots of  $\log [(\log \text{ initial count/g}) - (\log \text{ survivors/g})]$  versus  $\log t_{\min}$ . For the sensitive-state spores, a value of the exponent  $a$  of 0.58 was selected from such slopes. For the resistant state,  $a$  was taken as 0.33. The log initial count/g was 11.47 for the original unheated resistant-state preparation and 11.85 for the sensitive-state preparation. Thus, the expressions used to describe heat inactivation at  $a_w = 0.28$  under these experimental conditions were

$$(11.47 - \log S)^{0.33} = k't_{\min} + C \text{ for resistant state} \quad (1)$$

$$(11.85 - \log S)^{0.58} = k't_{\min} + C \text{ for sensitive state} \quad (2)$$

where  $S$  is the number of survivors per gram of spores at heating time  $t$  in minutes, and  $k'$  and  $C$  are constants. As shown in Fig. 4, plotting  $(\log S_{\text{original}} - \log S)^{0.33}$  for the resistant state and  $(\log S_{\text{original}} - \log S)^{0.58}$  for the sensitive state versus time gave reasonably straight lines for several test temperatures. The least squares-determined slopes of these lines yielded coefficients of  $t$  in equations 1 and 2 for various temperatures. These coefficients of  $t$  were used as reaction velocity constants ( $k'$ ) in determining the temperature dependence of heat inactivation (Fig.

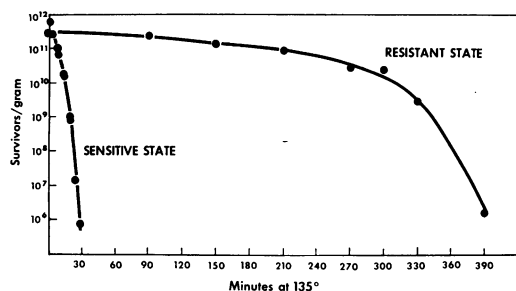


FIG. 3. Heat resistance at 135 C for the chemical states of *B. stearothermophilus* spores at intermediate water activity ( $a_w = 0.28$ ).

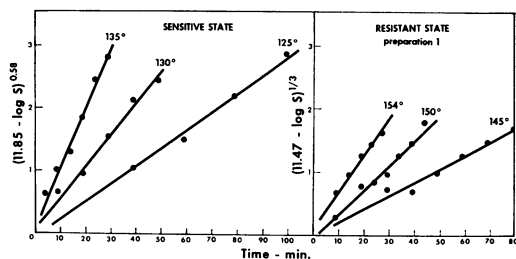


FIG. 4. Linearization of heat survivor rates at intermediate water activity ( $a_w = 0.28$ ).

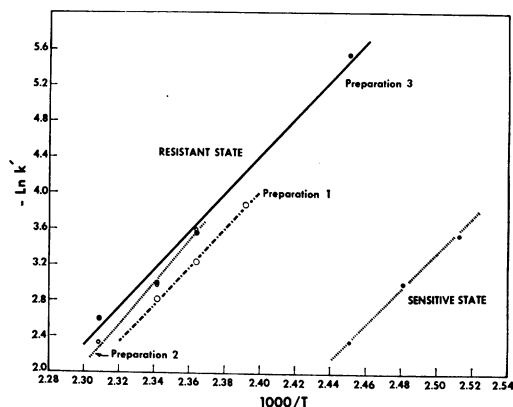


FIG. 5. Temperature dependence of heat resistance at intermediate water activity ( $a_w = 0.28$ ) for the sensitive and resistant states of *B. stearothermophilus* spores.

5, Table 1). For convenience, the Arrhenius plots for both states have been included in Fig. 5, but it should be noted that the rate expressions (equations 1 and 2) for the two states are different and so equal values of the rate constants ( $k'$ ) between states do not mean equal numbers of survivors will result from equal reaction times.

It is apparent that the temperature dependence of spore heat survival for each of the chemical states at  $a_w = 0.28$  is similar to that for strictly dry heat resistance (3), even though the survivor curve shapes and the general level of heat sensitivity are quite different. The activation energies for both chemical states at both environmental humidity conditions of heat challenge (strictly dry and  $a_w = 0.28$ ) are all about 40,000 cal. However, the log survivor versus time relations for strictly dry heat resistance are straight, that is, follow "logarithmic death," whereas these same relations for heat challenge at intermediate water activity of 0.28 are prominently curved. The general level of heat sensitivity of the chemical forms under strictly dry conditions is about like that for ordinary aqueous heat resistance. That for the same chemical forms at the intermediate water activity is much higher.

## DISCUSSION

It is evident from Fig. 2 and 3 that mature bacterial spores can be manipulated chemically into different states for the property of resistance to heat at intermediate water activity. Since, like heat resistance at both strictly dry and strictly wet environmental conditions, heat resistance at intermediate water activity is a spore property manipulatable by in vitro pretreatments, the same

TABLE 1. *Temperature-survival relations for the spore chemical states at a<sub>w</sub> 0.28*

| Spore state | Temp (C) | Survivors/g versus $t_{min}$              | $E_a$  |
|-------------|----------|---|--------|
| Resistant   |          | $(11.47 - \log S)^{0.33} = k't_{min} + C$ |        |
| Prepn 1     | 145      | $= 0.02120t - 0.009$                      | 41,500 |
|             | 150      | $= 0.03918t - 0.051$                      |        |
|             | 154      | $= 0.05965t + 0.072$                      |        |
| Prepn 2     | 150      | $= 0.02773t + 0.056$                      | 46,100 |
|             | 154      | $= 0.04935t - 0.016$                      |        |
|             | 160      | $= 0.0982t + 0.034$                       |        |
| Prepn 3     | 135      | $= 0.00396t - 0.003$                      | 42,300 |
|             | 150      | $= 0.02858t - 0.068$                      |        |
|             | 154      | $= 0.04572t - 0.164$                      |        |
|             | 160      | $= 0.07493t + 0.197$                      |        |
| Sensitive   |          | $(11.85 - \log S)^{0.58} = k't_{min} + C$ |        |
|             | 125      | $= 0.02851t - 0.055$                      | 39,200 |
|             | 130      | $= 0.04975t + 0.086$                      |        |
|             | 135      | $= 0.09431t + 0.103$                      |        |

implications for experimental strategy which we have stressed previously (2, 3) for the  $a_w = 0$  and  $a_w = 1$  conditions also hold for this intermediate humidity situation. The most important of these is that meaningful estimates of heat resistance potential cannot be made without knowing the chemical state of the spores. This knowledge of chemical state can be gained by converting deliberately a portion of the spore sample into each chemical state before measuring heat resistance at controlled water activity.

As pointed out previously (2), if artifacts are to be avoided in the measurement of the aqueous heat resistance of the different spore chemical states, special care must be taken to use a testing medium inert with respect to its capacity to induce change of chemical state during the test. In fact, aqueous conditions can be arranged deliberately for the adaptation of spores to heat (4) by using a noninert suspending medium for heating. Nonaqueous heat resistance of spores, both dry and at intermediate water activity, is generally not subject to these changes of chemical state effects during the lethal heat challenge itself. However, we have pointed out how inadvertent changes of spore resistance state could occur by interaction of spores and their supporting surface in the preparatory phases of nonaqueous heat resistance measurement (3).

Even in the case of aqueous heat resistance, it has been found possible to sensitize spores to heat in the presence of complex biological mixtures at their ordinary pH (2). It is not necessary to have acid conditions present during lethal heating of spores in such wet, complex biological mixtures,

probably because free calcium ion is absent by virtue of the complexing action of the organic materials.

Such sensitization to heat for facilitating spore killing in a practical situation should be straightforward in the case of nonaqueous heat resistance, providing the spores are or can be made physically available to the sensitizing reagent. Merely washing the sensitizing acid away before lethal heating would restore the original nonacid condition of an inert substrate on which the spores were supported. Conceivably, in some situations, the spores could be chemically sensitized before encapsulation in solid matrices.

From the survivor versus  $a_w$  plots of Fig. 1, some inferences may be drawn about the apparent temperature dependence of heat resistance of spores encapsulated within a given volume with a given *weight* (as contrasted with *activity*) of water which considerably exceeds the water-holding capacity of the enclosed spores, that is, where the encapsulating volume exceeds spore volume by several hundred-fold. In the absence of liquid water, specification of the water activity within an enclosed space requires knowledge of three terms: volume of space, weight of water, and temperature. Of course, if pure liquid water is known to be present at all temperatures of interest,  $a_w$  is 1 by definition. For spores encapsulated in solid matrices such as plastics, crystals, tightly joined surfaces, etc., neither the volume of the encapsulating space nor the weight of water is likely to be known, leaving temperature as the only measurable variable, and water activity unknown. In Fig. 1 it may be seen that the depend-

ence of survivors on water activity is quite sharp on either side of the maximum. This could make for an apparent unusual temperature dependence of heat resistance for spores encapsulated with a given weight of water since, when volume and weight of water (short of saturation) are fixed,  $a_w$  varies inversely with temperature. If, for example, the particular tube used for the last point on Fig. 1 (Resistant State) had been heated at a temperature  $10^\circ$  higher, the spores would tend to be protected against the higher temperature. Instead of 0.50, the water activity at the  $10^\circ$  higher temperature would be only 0.39 for these encapsulating conditions. Thus, on the right side of the maximum, the lethal effect of an increase in heating temperature would tend to be compensated by the temperature-induced lowering of water activity. On the left side of the maximum in the  $a_w$  versus survivor curve, the killing effect of raising the temperature would be reinforced by an  $a_w$  change toward a less favorable (for survival) value. If, on the other hand, the tubes mentioned were heated at a  $10^\circ$  lower temperature the situation would be reversed with survival for tubes on the left side of the maximum being reinforced, whereas survival on the right side would be opposed by such a lowering of heating temperature.

It is thus apparent that for such casually (with a given weight of water) encapsulated spores, it is not possible to determine temperature dependence of heat lethality in the usual sense. The temperature dependence of spore heat survival rate is commonly expressed as a  $z$  value, the temperature change required for a 10-fold change in survival rate. Such  $z$  values are considered to be a property of the spore in a given, presumably constant, environment. For example, in both aqueous and strictly dry heat environments water activity is essentially independent of temperature. However, as discussed above, when spores are encapsulated with a given weight of water, the kind of environment is also temperature dependent; water activity itself is a function of temperature. On the left side of the  $a_w$  versus survivor maximum, apparent  $z$  values would be low, on the right side, high. Even in cases where the encapsulating fit around a spore is close with the resulting expected buffering of environmental water activity by the spore itself, the required lowering of equilibrium water content with increasing temperature would be expected to give some environmental disturbance. Only when volume and water content of the encapsulating space are known and manipulatable is it possible directly to determine a  $z$  value in the usual sense of its being a spore property.

It appears that such considerations of environ-

mental water activity temperature dependence can explain the anomalous  $z$  values reported by Angelotti et al. (5) for their paper system and possibly also for their highly torqued stainless-steel-surface system. The cellulose moisture sorption isotherm and the geometry of the system used for drying the paper would indicate an appreciable final water content in the paper, probably at least 2%. Such a moisture content of 2%, when released by high temperature, would result in an  $a_w$  of about 0.06 at 125 C, about 0.04 at 140 C. Such values lie on the steep, left side of the curves of Fig. 1 at which point lethality of heat is reinforced by temperature-induced lowering of  $a_w$ , in other words, where apparent  $z$  values would be low. Doubling or halving, or more, the assumed value of 2% moisture content for the paper would not affect this qualitative argument for low  $z$  values since the  $a_w$  would still be on the steep, left side of the  $a_w$  versus survivor plot of Fig. 1. As we have reported (3), extremely low water activities still can elevate spore heat resistance over that at a strictly dry condition.

Thus, the abnormally low  $z$  value operationally observed for the paper system appears to have been due to lack of environmental constancy during its measurement rather than being an expression of a temperature characteristic which could be interpreted as a "wet kill mechanism." Although sufficient information on environmental water activity is not available for the other unusual  $z$  value in the highly torqued stainless-steel system, its direction (high) would be expected for wetter-than-optimal conditions on the right side of the curves of Fig. 1. The cavities provided by the roughness of the no. 4 stainless finish of the washers should not have been large enough to accommodate spore size particles. One possible source for sufficient water in the environment would be water released from mechanical disintegration of most of the spores.

Another recent report of an anomalously high (139 C)  $z$  value is that of Bruch and Smith (6) for spores on Teflon film. Here, the Kapton film interlayer was known to have an appreciable water-holding capacity and is a reasonable source for sufficient water to furnish a wetter-than-optimal environment with the tendency to appear high  $z$  values.

Some inferences can be drawn on the question of efficient heating conditions for killing spores encapsulated under conditions in which environmental water activity is highly temperature dependent. For random encapsulating conditions in which the water activities of the cavities are distributed over the whole of a curve like that of Fig. 1, temperature cycling should be useful in

successively moving spores off the optimum (for survival) of the curve down to the steep sides where heat sensitivity is much greater.

Murrell and Scott's comprehensive report (10) on the effect of water activity on spore heat resistance clearly established the large effect of environmental water activity on the heat resistance of several species of bacterial spores in the absence of liquid water. They showed that the optimal relative humidity for heat survival was in the range 0.2 to 0.4  $a_w$  and that in this optimum environmental  $a_w$  region the great interspecies differences in heat resistance largely disappeared.

There are several differences between our work here and that of Murrell and Scott (10). These differences fall in three principal categories: (i) experimental methods of water activity control, (ii) heat survivor curve shape, and (iii) knowledge and control of the chemical resistance state of the spore samples used.

For the bulk of their data, Murrell and Scott used several conventional humidity-controlling solutions packaged with, but separated from, the spores by various two-container arrangements. In all these arrangements, the whole system was evacuated prior to final sealing so that equilibration of water activity would be speeded during lethal heating. They also made less extensive use of a bithermal method. Each of these variations of the methods was more than adequate to show the striking maximum in the curve relating  $a_w$  and  $D$  value. However, the  $a_w$  at the maximum was somewhat variable, and at particular  $a_w$  values off the maximum,  $D$  value variations among the methods amounted to many-fold. It was recognized that the behavior of the controlling solutions at high temperature was somewhat uncertain and the water activity control was described as approximate.

We have chosen a simpler, direct method of controlling water activity which insures that the gaseous water concentration in the spore-encapsulating environment is known unequivocally once equilibration to the lethal temperature has been achieved. The usable range is not limited to low temperatures. So long as the spore weight is kept sufficiently low that any water uptake or emission by the spores is negligible by comparison with the total water content of the whole encapsulating environment, there appears to be no reason to use humidity-controlling solutions. Both with our proposed method and that of Murrell and Scott, temperature lags will occur during the come-up and come-down periods of the lethal heating operation. Such temperature lags should be less with our method because of its smaller mass and the fact of two containers in the system of Murrell

and Scott. We have made some measurements of the time required for disappearance of the water drop in our method and these times are measured in seconds. For example, in a 4-ml volume, 2.55 mg of water took 15 sec to disappear at 148° from one spot. When the 2.55 mg of water was divided into three roughly equal spots on the wall of the evacuated tube, disappearance took about 5 sec. Water spread over the upper 1 to 2 cm of the tube was more difficult to observe precisely but disappeared very quickly.

During the temperature come-up period with our method and also with arrangements 1 and 5 of Murrell and Scott (10), the spores temporarily would see a water activity lower than that intended. We did obtain increases of survival by spreading the water over the upper part of the tube as compared to letting it vaporize from a single spot, but the differences were not large.

The second major difference between our work and that of Murrell and Scott lies in the radically different log survivor curve shapes. They reported a linear log survivor versus time relation and expressed survival in the usual  $D$ -value terms. Our log survivor curves, on the other hand, are, as pointed out above, definitely and prominently curvilinear and not susceptible to even rough approximation by the single  $D$  value concept. The reasons for this difference in the survival versus time relation are not definitely known. The experimental physical arrangements and means of water activity control are quite different for the two methods. Another possibility for the origin of the curve shape discrepancy may lie in the fact that Murrell and Scott determined resistance at temperatures which are, at least for the resistant form of spores like *B. stearothermophilus*, quite low. Under the conditions of the resultant long survival times such as curve #2 of their Fig. 2, it would be difficult to distinguish between a curvilinear log survivor relation like our equation 1 and classic logarithmic death. Both expressions would have about the same appearance of linearity with low slope over the early portion of the log survivor drop. For example, in our Fig. 3, if only the data in the first three quarters of the time period were available, an interpretation of log death could easily be made. At such low temperatures, only in tests observing survival over several log units would the curvilinearity following the long lag period become evident.

The third main category of difference between this work and that of Murrell and Scott lies in the control of the chemical state of the spore sample. Here, we converted the spores to a known heat-resistance state before measuring heat resistance. Murrell and Scott used naturally occur-

ring spore crops which can be composed of various mixtures of the heat-resistance forms.

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