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Kinetics of Thermal Death of Bacteria

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Experimental observations on thermal injury and death of bacteria in the stationary phase can be explained by assuming that death results from inactivation of (X_L) of N critical sites. It is assumed: (i) that inactivation of individual sites occurs at random and follows first-order kinetics, (ii) that the critical sites are identical and of equal heat resistance, and (iii) the bacterial population is homogeneous in heat resistance. A method is described for calculating k (the rate constant for inactivation of individual sites), N (sites per cell), and X_L (the number which must be inactivated to cause death under the experimental conditions used) from experimental data. Theoretical curves calculated by using this model are identical with experimental curves, providing support for the assumptions used. Calculated values of N and X_L were 130 and 21.7 for *Pseudomonas viscosa* and 175 and 2.7 for *Salmonella anatum*. There is considerable uncertainty in the absolute values of N, but they are probably >100. It is predicted that X_L will vary depending on the recovery medium used after heating. This theory is consistent with all experimental observations on thermal injury and death of bacteria.

Heat treatment is one of the most widely used methods for destruction of spoilage and pathogenic bacteria. Mild-heat treatment, known as pasteurization, dates from the discoveries of Pasteur in 1860-64 (11), and is widely used for food products which are adversely affected by excessive heating. Despite this long history, no adequate explanation has been developed for the mechanism of thermal destruction of bacteria by heat. An understanding of the mechanism and kinetics of thermal death of bacteria would be helpful in the practical use of heat in processing heat-sensitive foods.

Most textbooks of microbiology state that, at constant temperature, thermal death of bacteria is exponential with time, and thermal process calculations used in food processing are based on this assumption (2, 29). However, examples of nonexponential survivor curves, too numerous to list, are found in the literature. They fall into four general types (Fig. 1) which were recognized as early as 1912 by Eikjmann (10). Curve A is the type commonly found by the most careful investigators and shows an initial lag in death rate followed by a logarithmic portion. Curve C is similar to curve A but tails. Wood (31) attributes the tailing to a small population of heat-resistant cells. Concave curves similar to curve D are generally interpreted as indicating that the population is heterogeneous with regard to heat resistance.

Rahn (25, 26) gives a good discussion of the early literature on survivor curves. He credits Madsen and Nyman (20) and Chick (6), working independently, with being the first to observe the apparently exponential nature of survivor curves, although Madsen and Nyman present few data to support their conclusions with regard to thermal death. Chick (7) also observed both concave and convex curves. She explained concave survivor curves as indicating a population heterogeneous in heat resistance; this remains the only satisfactory explanation. Rahn (25) replotted data of Reichenbach (28) obtained with "*Bact. paratyphosum*" to show that the shape of the survivor curve depends on the age of the culture, being concave at 5.5 hr, linear at 13.5 and 18 hr, and convex, with an initial lag in death rate, after 24 hr.

The theory that thermal death of bacteria follows the kinetics of a unimolecular reaction (first-order kinetics) has been discussed in detail by a number of authors, including Rahn (26, 27), Charm (5), and Stumbo (29). A first-order reaction is one in which the rate is proportional to the number of molecules present. If thermal death of bacteria truly follows first-order kinetics, death must then result from inactivation of a single molecule or site per bacterial cell. Furthermore, the death rate should be highest at the start when the number of bacteria is highest. This theory cannot, therefore, account for the initial lag in death rate which is frequently observed.

A further objection to the assumption of an

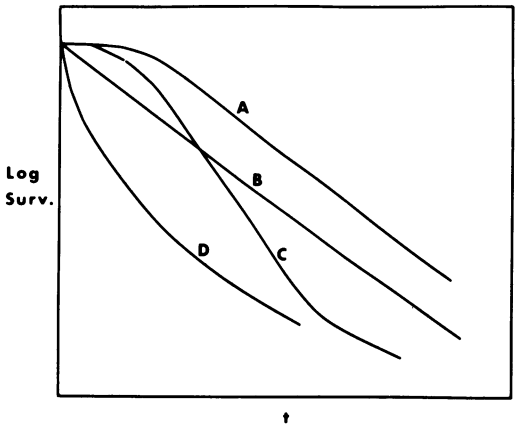

FIG. 1. *Nonexponential survivor curves. Curve A: convex survivor curve—initial lag in death rate followed by an approximately logarithmic death rate, commonly observed; curve B: logarithmic death rate as described in textbooks; curve C: similar to curve A but with tails; curve D: commonly observed with cells in logarithmic growth phase and considered to indicate a heterogeneous population.*

exponential death rate, discussed by Ball and Olson (2), is that this assumption cannot account for sublethal injury, a phenomenon which is generally recognized by bacteriologists. The usual criterion of death of a bacterial cell is loss of the ability to reproduce (29), and it is indeed difficult to conceive of any other means of reliably determining the viability of a bacterial cell. But reproduce on what? Nelson (22, 23) has observed that one of the first effects of heating is a change in nutritional requirements. Thus, if heated bacteria are grown on a minimal medium, some do not reproduce and so are dead, whereas in a richer medium, they reproduce and are alive. Similar results were reported by Heather and Vanderzant (12). Clark et al. (8) have shown that, with *Streptococcus faecalis*, more survivors are found on a nonselective than on a selective medium and that the resultant survivor curves are quite different. Similarly, differences in recovery of *Staphylococcus aureus* after heating were observed on different media (4). Yokoya and York (32) reported that the number of viable spores of *Bacillus coagulans* found after heating depended on the plating medium used. Dabbah et al. (9) found that, after bacteria (*Pseudomonas* sp.) were heated to the point at which no survivors were found on agar plates, some cells could recover and grow in a suitable liquid medium. Recovery times of up to 10 days were observed. If thermal death of bacteria results from inactivation of a single site per cell, how do we explain the above results? The bacteria should be unequivocally either dead or alive if the single-site theory is correct. Experimental results, however, indicate that mild-heat treatment does not instantaneously kill bacterial cells but instead causes varying degrees of injury. After heating, cells eventually will either die or recover, depending on the degree of injury and on the conditions under which they are held.

Several authors have advanced multiple-site or "multiple-target" theories of thermal death of bacteria although multiple-target theories have been more widely discussed in connection with the effects of ionizing radiation. These have been approached on two bases. The equations of Atwood and Norman (1) based on the premise that death occurs when all of N critical sites are inactivated were developed to describe the effects of radiation, but have been discussed by Meynell and Meynell (21) and by Van Uden et al. (30) with regard to thermal death of microorganisms. This theory accounts for the initial lag in death rate, but does not satisfactorily explain variations in recoveries on different media. Rahn (26), Johnson et al. (14), and Wood (31) described multiple-site theories based on the assumption that death occurs when some fraction of N critical sites are inactivated. Rahn (26) pointed out that, by the laws of chance, some bacterial cells will have more sites inactivated and therefore be killed sooner than others even if the population is homogeneous in regard to heat resistance, but he ultimately rejected the multiple-site theory and, in 1945, stated that thermal death of bacteria is strictly logarithmic (27). Kiga (17), Johnson et al. (14), and Wood (31) showed that the number of critical sites inactivated per cell would follow the binomial distribution if death results from inactivation of some fraction of N critical sites. The calculation is unwieldy, however, when N is large. The above authors were successful in predicting the general shape of the experimental curves (an initial lag followed by a logarithmic portion) but they were not able to fit their theoretical curves to actual experimental data, presumably because they had no means of determining the number of critical sites per cell and the number which must be inactivated to cause death. Other variations of multiple-site or multiple-hit theories have been proposed, particularly in connection with the effects of radiation (19, 33). Zimmer (33) discusses models of multiple hits on a single target. Critical sites might also be of different heat resistance (1, 21).

Although the same kinetic models have been proposed to explain survivor curves from heat and from ionizing radiation, the effects of ionizing radiation are quite different from heat. High-

energy radiation can be expected to cause localized indiscriminate damage to the cell along its track, either directly or from the formation of ions or free radicals which cause secondary effects (33). Heat, on the other hand, will be more or less uniformly distributed in the cell and may be expected to selectively damage only the most sensitive molecules within the cell. For example, ionizing radiation is mutagenic, whereas heat is not known to be, indicating quite different types of damage to the cell.

In this paper, I will consider the application to the kinetics of thermal death of bacteria of the model where inactivation of a portion of multiple critical sites causes death and present evidence that, with homogeneous bacterial populations, a curve calculated on this assumption fits experimental data to very high probabilities of kill.

MATERIALS AND METHODS

Preparation of cultures. The culture of *Salmonella anatum* (ATCC 9270) was carried on Trypticase Soy Agar (TSA; BBL) slants and then passed once in Trypticase Soy Broth (TSB; BBL) before being inoculated in the growth medium (TSB) or basal minimal medium [formula of Heather and Vanderzant (12)]. Cultures were grown at 35 C for 24 hr in TSB or 48 hr in basal medium, centrifuged, washed twice in 0.1 N phosphate buffer (*p*H 7.0), and suspended in the heating medium.

Heating procedure. Heating was carried out in sealed 1-ml ampoules completely immersed in the water bath. Temperatures in the ampoules were measured in an identical ampoule with a thermocouple sealed inside. Holding times were recorded from the time the temperature reached 5 C below the holding temperature to removal from the water bath which gives a reasonable correction for come-up and cooling time. Ampoules were removed at intervals and immediately cooled in an ice-water bath.

Enumeration of survivors. The cooled ampoules were opened, and appropriate dilutions in peptone (BBL) water were plated in triplicate on the indicated agar.

Theoretical considerations. According to the model used, the probability that a cell survives is equal to the probability that the cell has less than X_L of N critical sites inactivated. The binomial solution may be expressed as the following equation.

$$P(\text{survivors}) = \sum_{X=0}^{X_L-1} (N/X)e^{-kt(N-X)}(1 - e^{-kt})^X \quad (1)$$

If $X_1 = N$, the expression reduces to the equation of Atwood and Norman (1).

When N is large, the normal distribution approximates the binomial distribution (24). I have derived a method of determining the rate constant (k) for inactivation of individual sites, the number of critical sites (N), and the number which must be inactivated to cause death (X_L) from experimental data, by using the normal distribution. I am considering "sites" at the molecular level where a single event is required to change or inactivate a site. It does not appear useful, in the present context, to define sites in a manner where more than one "hit" is required to inactivate a "target" as proposed by Zimmer (33). For the derivation, the following assumptions are made. (i) Inactivation of individual sites follows the first-order rate equation and occurs at random, (ii) all sites are identical and of equal heat resistance, and (iii) the bacterial population is homogeneous with regard to heat resistance.

The first-order rate equation may be written (18):

$$\ln N/(N - X) = kt \quad (2)$$

or

$$(N - X)/N = e^{-kt} \quad (3)$$

or

$$X = N(1 - e^{-kt}) \quad (4)$$

where t is the time, k the rate constant, N the initial number of critical sites, and X the number of critical sites inactivated at any time (t). N and X are concentration terms, which may be expressed as sites per bacterium. Figure 2 shows the normal frequency distribution of sites inactivated in individual bacterial cells for various mean values of X (sites inactivated per bacterium). The curves are much broader toward the center of the range, the spread being greatest when 50% of the sites are inactivated, and the standard deviation (σ) is a function of X.

$$\sigma = \sqrt{Npq} \quad (5)$$

where p = the probability that a site is inactivated

$$= X/N \quad (6)$$

and q = the probability that a site is not inactivated

$$= (N - X)/N \quad (7)$$

Thus, if 10% of the sites are inactivated, $\sigma = \sqrt{0.09N}$; if 50% are inactivated, $\sigma = \sqrt{0.25N}$.

The fraction of bacteria which have greater than X_d sites inactivated are considered to be dead. Setting the total area under the normal distribution curve as being equal to 1, the area of the curve for each mean value of X which lies to the right of X_L equals the fraction of bacteria killed; the area to the left is the fraction of survivors. The problem then is to determine the area of the curve to the left of X_L if one wishes to calculate

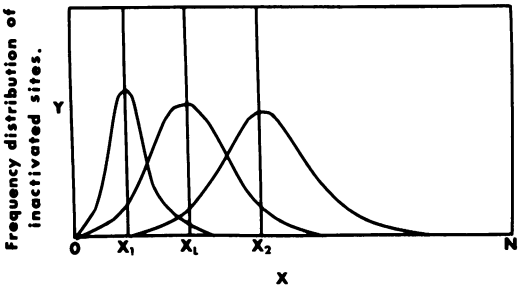

FIG. 2. *Illustration of the broadening of the frequency distribution of sites inactivated per cell as the mean number of sites inactivated changes. X_L represents the number of sites which must be inactivated to cause death under the recovery conditions used. That part of the frequency distribution for each mean value of X which lies to the left of X_L represents the fraction of survivors.*

the fraction of survivors. Tables of the normal probability integral are available in terms of the area under the curve from various ratios of d/σ to infinity. The deviation (d) from the mean is

$$d = X - X_L \quad (8)$$

From equations 5 to 7

$$\sigma = \sqrt{(X)(N - X)/N} \quad (9)$$

When 50% of the bacteria are killed, $X = X_L$.

$$X_L = N(1 - e^{-kt_{50}}) \quad (10)$$

Then $$d/\sigma = (X - X_L)/\sqrt{X[(N - X)/N]} \quad (11)$$

Substituting from equations 3, 4, and 10, squaring both sides, and rearranging, we obtain

$$(d/\sigma)^2 = N(e^{-2kt} - 2e^{-(kt+kt_{50})} + e^{-2kt_{50}})/(e^{-kt} - e^{-2kt}) \quad (12)$$

This is an equation with two unknowns, k and N. The times (t and t_{50}) are determined experimentally and d/σ can be determined from the fraction surviving at time (t). From the fraction surviving at two different times, equation 12 can be solved simultaneously for k. There is no direct way to solve for k; solution requires substitution of values of k until the equality is satisfied. This is tedious if done by hand but can be done easily be computer.

Sample calculation. *Salmonella anatum* was heated at 55 C for various lengths of time, plated on Trypticase Soy Agar, and incubated at 35 to 37 C for 48 hr. The survivor curve was plotted, and the time that 50% of the population was killed (t_{50}) was determined from the graph to be 1.2 min. Two experimental points which lay on the curve were selected and were used to solve equation 12 simultaneously for k.

At 2.5 min, the fraction is 0.108. From a table of the normal probability function, it can be determined the d/σ is 1.24 when the area of the tail of the normal probability function is 0.108.

Similarly, at 8.5 min, the fraction surviving is 0.000059 and d/σ is 3.85.

Substituting these values into equation 12

$$(1.24)^2 = N(e^{-5.0k} - 2e^{-3.7k} + e^{-2.4k})/(e^{-2.5} - e^{-5.0k})(2.5 \text{ min}) \quad (13)$$

$$(3.85)^2 = N(e^{-17k} - 2e^{-9.7k} + e^{-2.4k})/(e^{-8.5k} - e^{-17k})(8.5 \text{ min}) \quad (14)$$

Solving simultaneously for k

$$(3.85)^2/(1.24)^2 = (e^{-17k} - 2e^{-9.7k} + e^{-2.4k}) \cdot (e^{-2.5k} - e^{-5.0k})/(e^{-5.0k} - 2e^{-3.7k} + e^{-2.4k}) \cdot (e^{-8.5} - e^{-17k}) = 9.65 \quad (15)$$

This equation can be solved by substituting values of k until the equality is satisfied. A good set of tables of the exponential function is necessary. A value of k = 0.013/min satisfies the equality. It should be emphasized that k is the rate at which individual sites in bacteria are being inactivated, not the rate at which the entire bacterium is inactivated.

N can then be determined from equation 13 or 14.

$$N = 175$$

$$X_L = N(1 - e^{-kt_{50}})$$

$$= 175\ (1 = e^{(0.013)(1.2)})$$

$$= 2.7$$

To determine the fraction surviving at any time (t), $X = N(1 - e^{-kt})$. For t = 4.5 min, X = 175 ($1 - e^{-(0.013)(4.5)}$) = 9.9.

Thus, $d/\sigma = (X - X_L)/\sqrt{X[(N - X)/N]}$ = (9.9 − 2.7)/(9.9) [(175 − 9.9] = 2.35.

From the tables, the area of the tail of the normal distribution for d/σ = 2.35 is 0.0094 = fraction of survivors as 4.5 min.

From Table 1, the observed fraction of survivors is 0.0092.

RESULTS AND DISCUSSION

Tables 1 to 3 show the theoretical and calculated fraction of survivors of *S. anatum* (data from our own laboratory), *Pseudomonas viscosa* [data from Kaufmann and Andrews (15)], and *Streptococcus faecalis* (A. J. Mercuri, *unpublished data*), respectively. For the data of Kauf-

TABLE 1. *Experimental versus calculated fraction surviving Salmonella anatum heated at 55 C*[a]

Time (min)	Fraction survivors	
	Experimental	Calculated
0	1.00	1.00
2.5	0.108	0.109
4.5	0.0092	0.0094
6.5	0.00083	0.00074
8.5	0.000059	0.000057
10.5	0.0000053	0.0000046

[a] Heated in Trypticase Soy Broth and plated on Trypticase Soy Agar; k = 0.013/min, N = 175, X_L = 2.7, t_{50} = 1.2 min.

TABLE 2. *Experimental versus calculated fraction surviving Pseudomonas viscosa heated at 118.6 F*[a]

Time (min)[b]	Fraction survivors	
	Experimental	Calculated
0	1.00	1.00
4.8	0.85	0.86
6.8	0.33	0.31
7.8	0.124	0.125
9.8	0.0098	0.0104
11.8	0.00055	0.00056
13.8	0.000037	0.000018
16.0	—	0.00000026

[a] Data of Kaufmann and Andrews (15); k = 0.030/min, N = 130, X_L = 21.7, t_{50} = 6.1 min.

[b] Corrected for heating lag.

mann and Andrews (15), a correction of 0.2 min was subtracted from the published holding times. The authors injected the bacteria into the heating medium and assumed no lag in heating. Later findings by Kaufmann et al. (16) showed that, when bacteria are injected into the heating medium, the effective temperature is lower than that of the medium for the first few tenths of a minute so that the correction is justified.

I have applied this method of calculation to a number of survivor curves of type A or C, and agreement between calculated and experimental curves was excellent in all cases. With curves of type C, it is necessary to disregard the tail which apparently results from a small heat-resistant population as suggested by Wood (31). Many of our experimental curves are more complex and these will be discussed in another paper.

The values calculated for k and N are markedly affected by small changes in the value selected for t_{50}. A change by ± 0.1 min in t_{50} may change the values of k and N several fold, the two being roughly inversely proportional. This means that the absolute values of k and N are quite uncertain since it is difficult to estimate t_{50} more accurately than ± 0.1 min. However, the accuracy of the parameters N, k, and X_L calculated from various estimates of t_{50} can be checked to some extent by comparing theoretical curves calculated by using these parameters with the experimental curves. If N is <100, the theoretical curve falls decidedly below the extrapolated logarithmic portion of the experimental curve after a 10^5- to 10^6-fold reduction in survivors. We have carried many survivor curves through a 10^8- to 10^9-fold reduction in survivors, and, in all cases, they continue to be nearly logarithmic or tail to the right. Therefore, N is probably >100 and values below 100 are questionable. Calculated values of N from a number of experiments have generally been in the 100 to 200 range.

In the example shown in Table 1, the value of 1.2 min was used for t_{50}. If 1.3 min is used, k is negative which cannot be; if a value of 1.1 min is used, k = 0.052/min, N = 37.8, and X_L = 2.1. The curves are compared in Table 4. The deviation between the calculated curves is slight up to 8.5 min, but is very marked at 16.5 min. The experimental number of survivors at 16.5 min is too few (ten) for a precise estimate but is certainly closer to the curve calculated by using N = 175.

Despite the large uncertainty in the absolute values of k, N, and X_L, the results are consistent with a model where death results from inactivation of a small fraction of a large number of critical sites. Precise computation of N will require either very precise data or a method of calculation which is not so critically dependent on estimation of t_{50}.

TABLE 3. *Experimental versus calculated fraction surviving Streptococcus faecalis heated at 60 C in liquid whole egg*[a]

Time (min)	Fraction survivors[b]	
	Experimental	Calculated
0	1.00	1.00
0.7	0.69	0.998
3.7	0.71	0.63
6.7	0.19	0.15
9.7	0.032	0.026
12.7	0.0035	0.0043
15.7	0.00043	0.00062
18.7	0.00011	0.000092
21.7	0.000009	0.000011

[a] Unpublished data of A. J. Mercuri; k = 0.008/min, N = 152, X_L = 5.1, t_{50} = 4.3 min.
[b] Enumerated on Trypticase Soy Agar.

If clumping occurs, X_L and N will be units per clump rather than units per bacterium. Stumbo (29) has attributed the initial lag solely to clumping. If this were the case, X_L would equal N and the equation of Atwood and Norman (1) would apply, with the sites (N) per clump equaling the number of cells per clump. The assumption that death occurs only when all of N sites are inactivated or that the lag in death rate occurs solely from clumping does not explain differences in survival on different media.

Kinetics data of the type shown here actually measure a degree of injury as shown by loss of ability to reproduce on a given medium. This gives a reproducible end point and is therefore a valid method for kinetic studies. The bacterial cells are not necessarily dead, however, since some of them may be able to recover and reproduce after heating if placed in a medium more favorable than the experimental one. Bacterial cells which do not recover must eventually die. As heating is continued, a point will be reached where the cells in a given population are unable to reproduce even on the most favorable media.

Table 4 shows the results obtained in an experiment in which *S. anatum* was heated and portions were plated on two different media. For the calculations, k was assumed to be the same for each curve (which it must be since the same heated cells were used), and N and X_L were calculated. The data are somewhat limited but clearly show a difference of greater than twofold in X_L. Agreement between the values of N is not bad considering the sketchy data. These data support the hypothesis that cells can survive with more critical sites inactivated in one medium than in another.

TABLE 4. *Comparison of calculated and experimental survivor curves for two values of t_{50} for Salmonella anatum heated at 55 C*

Time (min)	Fraction survivors			
	Experimental	Extrapolated logarithmic curve	Calculated	
			t_{50} = 1.1 min	t_{50} = 1.2 min
0	1.00		1.00	1.00
2.5	0.108		0.108	0.109
6.5	8.3×10^{-4}	8.3×10^{-4}	8.7×10^{-4}	7.4×10^{-4}
10.5	5.3×10^{-6}	5.3×10^{-6}	2.7×10^{-6}	4.6×10^{-6}
16.5	1.3×10^{-8}	2.5×10^{-9}	4×10^{-11}	1.3×10^{-9}
N			37.8	175
k			0.052 min	0.013 min
X_L			2.1	2.7

It is possible that truly logarithmic survivor curves involving inactivation of only a single site per cell occur since X_L calculated in the one experiment with *Salmonella anatum* was only 2.7. However, a bacterial population which is heterogeneous with regard to heat resistance could also produce a death rate which is approximately logarithmic. Figure 1 shows that if curve A is the survivor curve for a homogeneous population, survivor curves for a heterogeneous population could lie anywhere between A and D depending on the proportion of cells of different heat resistances.

The model used makes the simplifying assumptions that N and X_L are the same in each cell in the population and that each site is of the same heat resistance. The assumptions are not unreasonable for bacteria in the stationary phase giving a curve of type A, and this model is consistent with the experimental data under these conditions. For bacteria in the logarithmic growth phase which typically give curves of type D, a more complicated model will be necessary allowing for variability in the heat resistance of the population.

The nature of the critical sites is unknown. They are probably not enzymes since "death" usually occurs when only a small per cent of the sites are inactivated. It seems improbable that inactivation of only a small per cent of an enzyme would seriously affect the viability of a bacterium. Parts of the bacterial cell other than critical sites might be damaged during heating. Unless this damage affects the viability of the cell, it would not be reflected in the kinetics data.

Iandolo and Ordal (13) presented evidence that the primary injury to heated bacterial cells is damage to the cell membrane as evidenced by leakage of potassium, amino acids, and nucleotides or nucleic acids, and the loss of salt tolerance. The kinetics data are not inconsistent with the hypothesis that the critical sites are structures contained in or making up the cell membrane. Inactivation of critical sites in the cell membrane might create holes in the membrane. Substances which could not pass through the intact membrane could then diffuse in or out of the cell. Inactivation of only one site could allow some leakage; the more sites which were inactivated, the more the leakage. In a rich medium, the cell might survive a loss of constituents which would be fatal in a lean medium. Damage to the cell membrane would allow penetration of toxic agents which cannot pass through an intact cell membrane. This would account for the lower recoveries of heated *S. faecalis* noted by Clark et al. (8), on selective agar plates (which contain toxic agents) as compared with a nonselective agar.

From a practical standpoint, these results support the assumption, made by many authors, that the survivor curve after the initial lag is essentially logarithmic if the population of bacteria is homogeneous with regard to heat resistance. We will present data in a subsequent paper showing that bacterial populations are frequently heterogeneous in heat resistance. Furthermore, the curves will vary depending on the heating and recovery media used. Clark et al. (8) showed that both lag time and slope varied depending on the type of agar used for plating. Dabbah et al. (9) showed that recovery may occur in liquid media when no survivors are noted on agar plates. Therefore, extreme caution is advisable in using survivor curves to determine conditions required to sterilize a bacterial population.

The approach to the kinetics of thermal death of bacteria described in this paper should be a useful tool in understanding the mechanism of heat injury and death, since it provides a method of calculating parameters probably related to actual physical structures in the cells. The model used is consistent with experimental kinetics data and satisfactorily explains degrees of sublethal injury observed which cannot be explained by other proposed models of thermal death.

TABLE 5. *Recovery of Salmonella anatum on different media after heating*[a]

Time (min)	Trypticase Soy Agar		Basal minimal agar	
	Experimental	Calculated	Experimental	Calculated
0	2.8×10^{7}		2.4×10^{7}	
2.6	2.3×10^{6}	2.5×10^{6}	2.5×10^{6}	2.5×10^{6}
4.6	1.7×10^{4}	1.7×10^{4}	2.1×10^{3}	2.0×10^{3}
6.6	2.4×10^{2}	1.1×10^{2}	5	9.6
X_L	7.8		3.3	
N	93		76	
k	0.055/min		0.055/min	
t_{50}	1.6 min		0.8 min	

[a] Heated at 55 C in basal minimal medium.

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