MICROBIAL DEATH
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I. INTRODUCTION

Microbial inactivation studies seem to be dominated by the exponential-single hit and multitarget theories, the former being applied mostly to heat inactivation kinetics and the latter to microbial inactivation by radiations. However, both theories are unable to satisfactorily explain some relevant experimental results, although they are valid enough to be of some predictive value in sterilization technology. Several satellite theories of lesser relevance do not add significantly to the understanding of inactivation phenomena. In this chapter an attempt is made to avoid interpreting experimental data in too simplistic a fashion, and a general theory is presented which provides a unifying description of microbial inactivation kinetics.

A. Microorganisms and Lethal Agents

Microorganisms can be regarded as elementary biological particles able to undertake functional relationships within aqueous surroundings, whether provided with levels of autonomous functional organization (bacteria, yeasts, molds, etc.) or not (viruses). Characteristically, the primary function of microbial interaction with the environment is the production of progeny. Hence, the single practical criterion of death of microorganisms is the failure to reproduce in suitable environmental conditions.

Physical and chemical agents affecting microbial activities to such an extent as to deprive microbial particles of the expected reproductive capacity can be regarded as lethal agents. Heat, ionizing, and UV radiation are the most relevant physical lethal agents. Ultrasonic frequencies, pressure, surface tension, etc. are mostly employed as cell disrupting agents in studying subcellular components.

Chemical lethal agents compose a wide range of compounds employed in the microbial inactivation process called disinfection. The most important are oxygen, hydrogen peroxide, halogens, acids, alkalis, phenol, ethylene oxide, formaldehyde, and glutaraldehyde.

B. Shape of Survivor Curves

Survivor curves are usually described by plotting the logarithm of the number of microorganisms surviving against the size of treatment (time, dose of radiation, concentration of the chemical lethal agent).

Figure 1. Shape of survivor curves of microorganisms treated with lethal agents: convex curve [A], sigmoid [B], concave or continuously decreasing rate curve [C & D]. Cs is the concentration of survivors after the lethal treatment of size S.
The semilogarithmic plot of survivor curves may have various shapes such as convex, sigmoid, concave, or linear (Figure 1).

Different shapes can be obtained: (1) under identical experimental conditions with different microorganisms or with the same organism in a different physiological condition (i.e., vegetative cell or spore, the former being in the lag or log phase, the latter being activated or dormant, etc.); 2) using the same population of organisms while changing the destructive potential of the applied lethal agent (viz., changing the treatment temperature or the concentration of the chemical lethal agent); (3) using the same population of organisms while changing the environment in which the microorganisms are suspended or the culture medium employed to detect surviving fractions, etc.

Radiation inactivation studies show the typical convex survivor curve (often erroneously called sigmoid) characterized by a more or less extended lag in inactivation at lower doses (shoulder), followed by a nearly exponential decay phase (Figure 1(A)). Such a shape has also been found in heat inactivation experiments, although less frequently, and in microbial inactivation by chemical compounds.

Often disregarded, though not uncommon, is the true sigmoid shape characterized by a more or less pronounced tail occurring after initial shoulder and exponential decay phases (Figure 1(B)). Concave survivor curves characterized by a continuously decreasing death rate (CDDR) with increasing treatment time or size, and often described as bi- or multiphasic, can be produced by many physical and chemical lethal agents.

The complexity of the situation was recognized about 70 years ago. Nevertheless, most of the early authors attempted to describe semilog survivor curves by a straight line (exponential inactivation) regardless of shape, claiming that the direct utilization of the data would have been otherwise quite difficult. The most relevant practical application of the exponential simplification has been in heat sterilization technology. Reports of Bigelow and Esty and Esty and Meyer on the destruction rate of bacteria subjected to moist heat aided greatly in strengthening the belief in exponential inactivation, although counter to experimental evidence from their own results. Fundamental books on heat sterilization technology perpetuated the belief in this exponential relationship, particularly the book by Stumbo, who tried to explain any deviation from exponential decay with a series of conjectures, rather than with experimental evidence. Meanwhile, Pflug and Schmidt claimed that "the majority of survivor curves are not straight lines on semilog plot", but did not propose an acceptable alternative theory.

II. SINGLE HIT THEORY

According to the single hit theory, the death of microorganisms results from the inactivation of a single molecule or site per cell; the death rate is expected to be proportional to the number of organisms remaining alive and follows first-order kinetics.

A. Heat Inactivation Kinetics

Plotting logarithm of surviving cell concentration, \( N_t \) (\( N_t / g \), \( N_t / m^3 \) or \( N_t / c^2 \)) against the time, \( t \), of treatment at temperature \( T \), a linear relationship is expected to occur:

\[
\log_{10} N_t = \log_{10} N_0 - k' * t
\]  

(1)

where \( N_0 \) is the concentration of the untreated population and \( k' \) is the inactivation rate constant. This type of plot is regarded as very convenient if the resistance of different microorganisms is to be compared. In fact, a parameter called the decimal reduction time is easily obtained from the regression coefficient \( k' \) and is denoted by \( D_{90}, D_{10}, D_T \):
\[ D_T = 1 / k' \] (2)

The use of \( D_{90} \) or \( D_{10} \) refers to the fact that after a treatment time \( t = 1 / k' \), 90% of the microbial population is destroyed or, alternatively, 10% of the population survives. In heat inactivation studies \( D_T \) is preferred, where \( T \) is the treatment temperature.

In exponential form, Equation 1 may be written:

\[ N_t = N_0 \times 10^{-k' \times t} \] (3)

so that after a treatment time \( t = t_1 \) Equation 3 becomes:

\[ N_1 = N_0 \times 10^{-k' \times t_1} \] (4)

and after treatment time \( t = t_2 \):

\[ N_2 = N_0 \times 10^{-k' \times t_2} \] (5)

Solving for \( k' \):

\[ \frac{N_2}{N_1} = 10^{-k' \times (t_2 - t_1)} \] (6)

which in logarithmic form yields:

\[ \log_{10} \left(\frac{N_2}{N_1}\right) = -k' \times (t_2 - t_1) \] (7)

so that:

\[ -k' = \frac{\log_{10} N_2 - \log_{10} N_1}{t_2 - t_1} \] (8)

and

\[ k' = \frac{\log_{10} N_1 - \log_{10} N_2}{t_2 - t_1} \] (9)

From Equation 2:

\[ D_T = 1 / k' = \frac{t_2 - t_1}{\log_{10} N_1 - \log_{10} N_2} \] (10)

and, as expected, if the treatment time is increased from \( t_1 \) to \( t_2 \), 90% of the population is destroyed, \( \log_{10} N_1 - \log_{10} N_2 \) equals 1 and the decimal reduction time at temperature \( T \) is:

\[ D_T = t_2 - t_1 \] (11)

Each type of microorganism (virus, bacterial vegetative cell or spore, yeast vegetative cell or spore, fungal cell or spore), as well as each species and strain of the same group of organisms, has its own resistance at a particular temperature \( T \) under defined environmental conditions, that is, its own \( D_T \). On changing the environment, the microbial resistance changes accordingly.

The most relevant physicochemical factors affecting heat resistance are the water content in the environment, \( pH \), and temperature. Changing the solute concentration in the medium also changes the osmotic pressure or, referring to the usual parameter employed in food microbiology, the water activity \( a (aw = p/p_0 \) where \( p \) and \( p_0 \) are the water pressures of the medium and of pure water,
respectively, in isothermal and isobaric conditions). The water activity is described by Raoult's law, so that:

\[ a_w = f \left( \frac{n_w}{n_w + n_s} \right) \]

(12)

where \( n_w \) and \( n_s \) are the concentrations of water and solute, respectively. It follows that by increasing the solute concentration, the \( a_w \) of the environment decreases. As \( a_w \) decreases, the thermal resistance of microorganisms increases. The \( D_T \) value in dry conditions may be more than 100 times higher than that in moist (high \( a_w \)) conditions. An exact relationship between \( D_T \) and \( a_w \) has not been developed.

Thermal resistance is usually higher at neutral pH and it decreases when the pH is increased or decreased. A defined relationship between \( D_T \) and pH is not known, though a tenfold change in \( D_T \) is often observed for each 2 pH units.

With respect to chemical reactions, a defined relationship between \( D_T \) and temperature can be established. Plotting Log D against temperature, a linear relationship is usually obtained:

\[ \log_{10} D_T = \log_{10} U - b \times T \]

(13)

where \( U \) is a proportionality constant and \( b \) is the rate at which D changes with temperature. Equation 13 may be written:

\[ D_T = U \times 10^{-b \times T} \]

(14)

The above equations yield the parameter \( z \):

\[ z = \frac{1}{b} \]

(15)

which is related to the temperature coefficient \( Q_{10} \) by:

\[ z = 10 / \log_{10} Q_{10} \]

(16)

where

\[ Q_{10} = \frac{K_{(T+10)}}{K_T} \]

(17)

\( z \) is the number of degrees required to achieve a tenfold change in \( D_T \). It follows that:

\[ 0.1 \times D_{T-z} = D_T = 10^* D_{T+z} \]

(18)

If we let \( D_1 \) and \( D_2 \) be the \( D_T \) values at the temperature \( T1 < T2 \) respectively, it follows that:

\[ D_1 = U \times 10^{-b \times T1} \]

(19)

and

\[ D_2 = U \times 10^{-b \times T2} \]

(20)

so that, solving for \( b \):

\[ D_2 / D_1 = 10^{-b \times (T2 - T1)} \]

(21)

and in logarithmic form:

\[ \log_{10} (D_2 / D_1) = -b \times (T2 - T1) \]

(22)

and then:

\[ b = (\log_{10} D_1 - \log_{10} D_2) / (T2 - T1) \]

(23)
and
\[ z = 1 / b = (T2 - T1) / (\log_{10} D1 - \log_{10} D2) \]  \hspace{1cm} (24)

As expected, when \( D1 = 10^* D2 \), the z value will be equal to \( T2 - T1 \).
Knowledge of the DT and z values for microorganisms in given environmental conditions is very useful in practice, when heat sterilization times at an unknown temperature \( Tu \) must be determined when \( DTu \) is not known. In such a case,

\[ Tu - T = n*z \]  \hspace{1cm} (25)

\[ DTu = DT * 10^{-(Tu - T)/z} \]  \hspace{1cm} (26)

\[ DTu = DT * 10^{-n} \]  \hspace{1cm} (27)

\[ D(T + n*z) = DT / 10^n \]  \hspace{1cm} (28)

and so:

\[ t_{(T + n*z)} = t_{T} / 10^n \]  \hspace{1cm} (29)

where \( t_{(T + n*z)} \) is the treatment time at the temperature \( T + n*z \) equivalent to the time \( t_{T} \) at the reference temperature \( T \).
Sterilization cycles are usually based on a minimum time required to obtain 12 decimal reductions of a particular microorganism (Clostridium botulinum spores in food sterilization technology, for instance). Usually Equation 29 is employed, although using the symbolism:

\[ \tau_{(T + n*z)} = \tau_T / 10^n \]  \hspace{1cm} (30)

where \( \tau \) is the number of minutes required to obtain the expected number, i, of decimal reductions (\( \tau = i * D \)). In heat sterilization technology, at the reference temperature \( T = 121.1°C = 250°F \), the \( \tau_{121.1°C} = \tau_{250°F} \) value is \( Fo \) and \( z = 10°C = 18°F \), so that Equation (30) becomes:

\[ \tau_{121.1°C} / \tau_{(T + n * 10)} = 10^n \]  \hspace{1cm} (31)

or in a better known form, putting \( T\alpha = T_{(T+n*10)} \)

\[ F / \tau = 10^{(T\alpha - 121.1°C) / 10} \]  \hspace{1cm} (32)

Equation 32 is currently employed to evaluate the efficiency of sterilization cycles.7,8

By analogy with chemical kinetics, the exponential heat inactivation curve can be described by the relationship:

\[ - dN_t / dt = k * N_t \]  \hspace{1cm} (33)

yielding, by integration:

\[ \log ( N_t / N_o ) = - k* t \]  \hspace{1cm} (34)

and:

\[ N_t / N_o = \exp ( - k*t) \]  \hspace{1cm} (35)
It follows that $D_T$ as defined by Equation 10 is

$$D_T = 2.303 / k$$

(36)

The analogy can be extended to the relationship between $k$ and temperature. According to the chemical kinetics:

$$k = A \times \exp(-E_a / R \times T)$$

(37)

where $A$ is a frequency factor, $E_a$ is the Arrhenius activation energy, $R$ is the gas constant (1.987 cal/mol), and $T$ is the absolute temperature (degrees Kelvin).

From Equation 37:

$$k_1 = A \times \exp(-E_a / R \times T_1)$$

(38)

and

$$k_2 = A \times \exp(-E_a / R \times T_2)$$

(39)

so that:

$$k_1 / k_2 = \exp \left[ -E_a (1/T_1 - 1/T_2) / R \right]$$

(40)

From Equation 40,

$$\log (k_1 / k_2) = \frac{-E_a}{R} \left( T_2 - T_1 \right) / (T_2 \times T_1)$$

(41)

and since $k = 2.303 / D_T$, from Equation 36,

$$\log (D_2 / D_1) = \frac{-E_a}{R} \left( T_2 - T_1 \right) / (T_2 \times T_1)$$

(42)

And

$$\log_{10} (D_2 / D_1) = \frac{-E_a}{2.303 \times R} \left( T_2 - T_1 \right) / (T_2 \times T_1)$$

(43)

Taking into account Equation 24,

$$z = 2.303 \times R \times T_2 \times T_1 / E_a$$

(44)

It follows that the $z$ value cannot be regarded as being constant, but varies with temperature. It follows that a $z$ value of 5°C obtained between 60 and 65°C increases to 5.23 between 65 and 75°C, and to 5.53 between 75 and 85°C. Analogously, a $z$ value of 10°C obtained in the temperature range 100 to 110°C will reach the value of 11.09 in the range 120 to 130°C.

According to Equation 44, the Arrhenius relationship requires that $z$ increases as the temperature increases. The constancy of $z$ values obtained by plotting $\log_{10} D_T$ against temperature is difficult to ascertain in practice, as the evaluation of $D$ is not sufficiently accurate. In some instances, a trend toward an increase of $z$ with increasing temperature has been shown. Following chemical kinetics, the Arrhenius activation energy $E_a$ may be obtained using Equation 37, plotting $\log k$ against $T^{-1}$. Alternatively, $E_a$ can be obtained from:

$$E_a = a \times \log Q_{10}$$

(45)

or

$$E_a = c / z$$

(46)

where

$$a = 0.1 \times R \times T_1 \times T_2$$

and

$$c = 2.303 \times R \times T_1 \times T_2$$
since, according to Equation 43:

\[ E_a = 2.303 \log_{10} \left( \frac{D_1}{D_2} \ast R \ast T_2 \ast T_1 \right) / (T_2 - T_1) \] (47)

Nevertheless, \( z \) is not constant, according to Equation 44, so \( E_a \) values obtained from Equation 46 differ from those from the Arrhenius equation (Equation 37) by about 1 kcal / mol (4.18 kJ/mol). Figure 2 shows the expected value of \( E_a \) as a function of \( Q_{10} \) and ‘\( z \)’ in temperature ranges resulting in the thermal destruction of microorganisms.

The standard energy of activation, \( \Delta G \), the standard enthalpy of activation, \( \Delta H \), and the standard entropy of activation, \( \Delta S \), are sometimes reported for the thermal death of microorganisms. According to Eyring’s relationship:

\[ k = k' \left( \frac{K \ast T}{h} \right) \ast k_c \] (48)

where \( k \) is the reaction rate constant observed at the absolute temperature \( T \), \( k' \) is the transmission coefficient (usually considered = 1), \( K \) is Boltzmann's constant (1.38*10^{-16} erg/degree), \( h \) is Planck's constant (6.624 *10^{-27} erg/sec), and \( k_c \) is the equilibrium constant; \( k_c \) may be expressed in terms of the standard energy of activation:

\[ k_c = \exp \left( -\frac{\Delta G}{R \ast T} \right) \] (49)

and since:

\[ \Delta G = \Delta H - T \ast \Delta S \] (50)

Equation 48 becomes:

\[ k = \left( \frac{K \ast T}{h} \right) \ast \exp \left( \frac{\Delta S}{R} \right) \ast \exp \left( -\frac{\Delta H}{R \ast T} \right) \] (51)

FIGURE 2. Relationship between \( Q_{10} \), corresponding ‘\( z \)’ values, and Arrhenius activation energy \( E_a \), predicted by thermodynamic treatment of first-order inactivation kinetics of microorganisms subjected to low temperatures (50 to 60°C) (heat sensitive organisms, HSO, with an average \( Q_{10} = 100 \)), or to high temperatures (\( \geq 100°C \); heat resistant bacterial spores, HRBS, with an average \( Q_{10} = 10 \)).

Equation 51 is very similar to the Arrhenius relationship, taking into account that:

\[ \exp( -\frac{\Delta H}{R \ast T}) = \exp( -\frac{E_a}{R \ast T}) \] (52)
when \( E_a \gg R*T \), since:

\[
\Delta H = E_a - R*T
\]  
(53)

and from Equations 37 and 51:

\[
A = (K*T / h) \times \exp(\Delta S / R)
\]  
(54)

Because, in microbial heat inactivation kinetics \( R*T \) is more than 100 times lower than \( E_a \), Equation 51 can be written:

\[
k = (K T / h)\times \exp(\Delta S / R)\times \exp(- E_a / R*T)
\]  
(55)

after which:

\[
\Delta S = R*(\log k - \log (K*T / h) + E_a / R*T)
\]  
(56)

or, taking into account that \( K = R / N \), where \( N \) is Avogadro's number and \( R = 8.32*10^7 \) erg/mol/degree:

\[
\Delta S = R*(\log k - \log (R*T / N*h) + E_a / R*T)
\]  
(57)

![FIGURE 3. Relationship between Arrhenius activation energy \( E_a \) (i.e., the activation enthalpy minus RT) and activation entropy \( \Delta S \), predicted by thermodynamic treatment of first-order inactivation kinetics of microorganisms subjected to 50°C (heat sensitive organisms, HSO) or to higher temperatures (heat resistant bacterial spores, HRBS).](image)

As shown in Figure 3, there is a linear relationship between \( E \), or \( \Delta H \) and \( \Delta S \) at any given temperature. It follows that Equation 57 (or Equation 56) can be reduced to:

\[
\Delta S = (E_a / T) + B
\]  
(58)

where \( B \) equals \( R*(\log k + \log (R*T / N*h)) \) or \( R*(\log k + \log (K*T / h)) \).

Equation 58 is often called the "isokinetic relationship" or the "compensation law", since an increase in the activation energy or enthalpy is exactly compensated by an increase in entropy.\(^{15-17}\)

Nevertheless, when activation energy and activation entropy values are obtained by a series of computations based on Equations 37 to 58, the compensation law is not such a mysterious
relationship as it is sometimes regarded, but follows directly from the premises (Equation 48).\textsuperscript{18,19}

At this point, the analogy between microbial heat inactivation kinetics and chemical reaction kinetics should be examined closely.

1. Thermodynamic Inconsistencies

Figure 4 shows the frequency distribution of 231 $z$ values collected from the literature. As can be seen, the spread is very large. After grouping collected data into 5°C classes the most frequent $z$ value obtained for viruses and microbial vegetative cells is 5°C ($Q_{10} = 100$) and for bacterial spores is 10°C ($Q_{10} = 10$). It must be remembered that viruses and vegetative microbial cells are usually $10^2$ to $10^9$ times less heat resistant than bacterial spores. Vegetative cells are destroyed at temperatures ranging from 50 to 60°C at a rate $k = 3.838 \times 10^{-3}$ (D$_T \approx$10 min). Bacterial spores are destroyed at a similar rate at temperatures ranging from 100 to 120°C.

As shown in Figure 2, the activation energy obtained for less resistant vegetative cells (mean $z = 5°C = 9°F$) is much higher than the $E_a$ value of more resistant bacterial spores (mean $z = 10°C = 18°F$), the former being about 90 kcal/mol (380 kJ/mol) and the latter about 60 kcal/mol (250 kJ/mol).

Disregarding the parameter $A$ in Equation 37, reaction rates of analogous reactions are expected to be inversely related to the activation energy value required for the occurrence of single reactions. Contrariwise, the heat inactivation of bacterial spores has a lower activation energy, while occurring at a rate very much slower than that of heat-sensitive viruses or vegetative microbial cells. Consequently, it can be argued, the $A$ value in the Arrhenius equation cannot be disregarded and Eyring’s relationship must be taken into account.

As previously shown, the so-called compensation law requires that high $E_a$ (or $\Delta H$) values necessarily follow high $\Delta S$ values. In fact, as shown in Figure 3, $\Delta S$ for vegetative cells is about 210 cal/mol/degree (880 J/mol/degree) and $\Delta S$ for bacterial spores is about 90 cal/mol/degree (380 J/mol/degree). Accordingly, higher activation energies required to inactivate vegetative cells would
be justified on the basis of the greater activation entropy involved in the process. Disregarding uncertainties associated with the rigid application of the compensation law, it seems hard to envisage a greater $\Delta S$ for the heat inactivation of vegetative cells rather than of spores, since it is reasonable to assume that the entropy level of the former is higher than that of the more orderly assembled molecular structures of bacterial spores; however, this may not be the case. Nevertheless, the reservations regarding what results merely from a thermodynamic treatment of exponential heat inactivation kinetics are further strengthened by the very high values of the ensuing $E_a$ or $\Delta H$. Using the Maxwell-Boltzmann's law for the distribution of velocities among molecules:

$$n_E/n_0 = 2\pi^{-1/2} \left( E_a / R^*T \right)^{1/2} \exp\left( -E_a / R^*T \right)$$

(59)

where $n_E$ and $n_0$ are the number of molecules having energy $E_a$ and the total number of molecules, respectively; or in the simplified form (i.e., on the basis of two axes):

$$n_E/n_0 = \exp\left( -E_a / R^*T \right)$$

(60)

For values of $2\pi^{-1/2} \left( E_a / R^*T \right)^{1/2}$ ranging between about 5 and 15 at temperatures $\geq 273.15^\circ$K and $E_a$ values lower than about 100 kcal/mol, it can be computed that the probability of finding $E_a$ values as high as those obtained for vegetative cells exposed to lethal temperatures is so low as to be unreasonable. The probability $P(E) = n_E/n_0$ of occurrence of molecules having $E_a = 90$ kcal/mol at 60°C is expected to be:

$$n_{90,000} / 6*10^{23} = \exp\left( -90,000 / ((60 + 273.15)* 1.987) \right) = 8.99*10^{-60}$$

which means that a single molecule carrying more than 90 kcal could occur, at 60°C, in about $10^{36}$ mol of a substance (i.e., a mass of hydrogen about $10^3$ times that of the sun).

A mole of cells weighs about $10^{11}$ g; therefore, we would find a single molecule carrying the above energy in $10^{37}$ g of cells, a weight about $10^3$ times that predicted for our galaxy.

Summing the number of intermonomer chemical bonds of about $10^6$ protein molecules (average mol wt, $2*10^4$) and of DNA (mol wt about $10^9$) occurring in a microbial cell, we obtain about $10^{10}$ chemical bonds per cell. This implies that we would expect to find a single one of the above $10^{10}$ bonds with about 100 kcal in a mass of cells approximately $10^4$ times that of the sun. The above results seem to suggest that $E_a$ values as high as those obtained by the thermodynamic treatment usually applied to microbial heat inactivation kinetics can be regarded to be of doubtful meaning.

**a. Maximum Allowable $Q_{10}$**

$Q_{10}$ values obtained in microbial heat inactivation studies are usually much higher than those expected to occur in chemical kinetics. The maximum $Q_{10}$ ($MQ_{10}$), the value above which thermodynamic treatment of reaction rate loses any meaning, can be evaluated. According to Equations 45 and 60, and letting $P(E)$ be the probability of occurrence of molecules carrying more than $E_a$ energy, it follows that:

$$P(E) = \exp\left( -a \times \log Q_{10} / R^*T \right)$$

(61)

The minimum value of the probability ($mP(E)$) required for a reaction to occur at the temperature $T_i = T + 10^\delta$ can be regarded to be 1, that is, at least one $n_i$ molecule can be expected to be present in the total amount of available reactants, or one $n_i$ per total number of available molecules; so that:
\[ mP(E) = \frac{1}{i*N} \quad (62) \]

where \( i \) is the number of moles of available reagents and \( N \) is the Avogadro’s number. If the above probability is higher at \( T_2 \) than at \( T_1 \) Equation 61 can be written:

\[ mP(E) = \exp (-a \cdot \log Q_{10} / R \cdot T_2) \quad (63) \]

so that combining Equations 62 and 63:

\[ (i*N)^{-1} = \exp (-a \cdot \log Q_{10} / R \cdot T_2) \quad (64) \]

As \( a = 0.1 \cdot R \cdot T_1 \cdot T_2 \), as previously shown, and rearranging:

\[ MQ_{10} = \frac{(i*N)^{10/T_1}}{} \quad (65) \]

it follows that, letting the highest conceivable value of \( i = 10^3 \), at \( T_1 = 50 \) or \( 100^\circ \text{C} \) the maximum allowable \( Q_{10} \) would be 6.74 or 5.22, respectively.

A value of \( Q_{10} = 2 \) to 3, as is usually found in chemical kinetics, is expected to be obtained at about \( 100^\circ \text{C} = 212^\circ \text{F} \) in an amount of solution containing about \( 10^{-12} \) to \( 10^{-6} \) mol of reagents, that is, about \( 10^{11} \) or \( 10^{18} \) molecules, respectively (\( i = (M \cdot Q_{10})^{T_1/10 \cdot \log N^{-1}} \)).

**Table 1**

**MAXIMUM ALLOWABLE QUE AND MINIMUM ALLOWABLE Z**

**EXPECTED FROM THERMODYNAMIC TREATMENT OF**

**FIRST-ORDER INACTIVATION KINETICS OF 1 g (A) OR 1 kg**

**B (B) OF MICROBIAL PARTICLES HEATED AT THE**

**TEMPERATURE \( T_2 = T_1 + 10^\circ \text{C} \)**

<table>
<thead>
<tr>
<th>Viruses ((g \cdot 10^{11}))</th>
<th>Bacteria ((g \cdot 10^{10}))</th>
<th>Yeasts ((g \cdot 10^{10}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vegetative</td>
<td>Spores</td>
</tr>
<tr>
<td>( T_1 )</td>
<td>( Q_u )</td>
<td>( z' )</td>
</tr>
<tr>
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<td>2.91</td>
</tr>
<tr>
<td></td>
<td>B</td>
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<td>c</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>c</td>
</tr>
</tbody>
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*Note:* \( T_1 = ^\circ \text{C}; \) \( Q_u = \) maximum allowable \( Q_{10} \); \( z' = \) minimum allowable \( z \).

Table 1 shows the \( M \cdot Q_{10} \) values expected from a thermodynamic treatment of microbial heat inactivation, according to Equation 65, for two amounts of cells (1 g and 1 kg), exceedingly higher than those employed in practice. By comparing values reported in Table 1 with those in Figure 4, it can be seen that all \( Q_{10} \) values obtained by treating microbial heat inactivation as a first-order reaction, can be regarded as unreasonably high.
b. Minimum Allowable z
According to Equation 17, if \( Q_{10} \) increases, \( z \) decreases. The minimum allowable \( z \) value, \( z_m \), under which \( P(E) \) must be regarded as being unreasonably low, can also be obtained. From Equations 17 and 65:

\[
(i \times N)^{10/T_1} = 10^{10/Z_m}
\]  

(66)

Taking logarithms and rearranging:

\[
z_m = T_1 / \log_{10}(i \times N)
\]  

(67)

Microbial heat inactivation experiments are usually done employing less than \( 10^{12} \) particles per liter of medium. Therefore, while taking into account the maximum allowable concentration of cells that could be employed in practice, the minimum allowable \( z \) values following Equation 67 are two to six times higher than those found in the literature (Table 1). We could assume ah absurdo that a microorganism is inactivated if a single chemical bond in the cell is broken, so that the quantity \( i^*N \) in Equation 67 could be regarded to be about \( 10^{25} \); the resulting \( z \), will be 13.33, 14.13, 14.93, or 15.73 at \( T_1 = 60, 80, 100, \) or \( 120^\circ C \), respectively. As a consequence, all \( z \) values reported in the literature (Figure 4) can be regarded as unreasonably low.

c. The Highest Allowable Energy Value
It would be interesting to know the highest reasonable \( E_a \) value. To this end, Equation 63 can be written:

\[
mP(E) = \exp(-ME_a / R*T_2)
\]  

(68)

where \( ME_a \) is the maximum value of the energy of activation at the temperature \( T_2 = T_1 + 10^\circ C \), and thus the \( E_a \) value above which \( P(E) \) is too low to be acceptable. From Equation 68:

\[
ME_a = R*T_2*(- \log mP(E))
\]  

(69)

and then:

\[
ME_a = R*T_2*( - \log (1/(i*N)))
\]  

(70)

\[
= R*T_2*( \log (i*N))
\]

It follows that at \( T_2 = 50 \) or \( 100^\circ C \) the maximum allowable \( E_a \) value is expected to be 39,591 or 45,716 cal/mol (165,708 or 191,348 J/mol).

Table 2 shows expected values of \( ME_a \) according to Equation 70 for first-order kinetics of microbial heat inactivation. As can be seen, all \( E_a \) values reported in the literature are unreasonably high. Perhaps, as a provisional conclusion, we may say that the thermodynamic treatment of reaction kinetics on an exponential basis is not readily applicable to the heat inactivation of microorganisms. It can be rather useful to retrace our steps in order to answer, first, the most relevant question: could microbial heat inactivation kinetics be regarded as an exponential phenomenon? Several observations seem to suggest that this is not always the case. The question will be examined later.
2. Mechanisms and Models

The greatest insight into heat inactivation mechanisms was obtained by Ball and Olson, as presented in the book Sterilization in Food Technology, still regarded as the bible of the field. Through an analysis of the concepts of heat and temperature on a macroscopic and microscopic scale, they pointed out that "whenever Brownian movement is observed, our macroscopic concepts of temperature and heat transfer break down and must be replaced by energy considerations involving molecules in the discrete, and not in the statistical sense" (see also Tischer and Hurwicz). Soon after "... it is not something within the cell (such as temperature) which is the cause of death. The cause must be outside the cell. It must be in the medium." Accordingly, Ball and Olson suggested a mechanism of microbial death brought about by "... one or more molecules in the surrounding medium", having "the greater mean velocity ... according to the velocity distribution curve". Nevertheless, they did not develop a mathematical model for microbial death.

Charm presented a model referring to the discrete nature of molecules involved in heat inactivation mechanisms, although diverging somewhat from the work of Ball and Olson. Charm's model leads to exponential inactivation, but some uncertainties cast doubts on its reliability. Charm regards the microbial cell as "... composed of a number of sensitive volumes surrounded by a number N of water molecules; when the water molecule, in contact with a sensitive volume, is able to impart sufficient energy to the sensitive volume inside the cell, it is thought to cause inactivation of the cell". The model yields the equation:

$$\log(N_t/No) = - S * t * \exp(- E/RT)$$ (71)

where $S$ (min$^{-1}$) is the frequency with which water molecules exchange the energy, $E$, with the sensitive volume.

Some inconsistencies arising from the model can be pointed out: (1) Charm defined $E$ as energy / cell, while energy/mol was considered, since $R = 1.987$ cal/mol/degree was seemingly taken...
throughout for computation purposes; (2) reported E values seem to be too high (for C. botulinum spores heated in distilled water, for instance, E = 73,500 Btu/spore, equivalent to about 1.85 *10^7 cal or 7.35 * 10^7 J); (3) the reported values of the frequency factor S are much too high to be reasonable (S ≥ 10^20), being equal to or greater than the frequency of X-rays produced by a potential difference of about 1.7 * 10^3 V. In addition, Charm's model contains the same inconsistencies pointed out previously regarding the thermodynamic treatment of exponential kinetics (i.e., very high energy values, energy values greater for less than for more resistant organisms, etc.) and substantially, it leaves inactivation kinetics still unresolved.

B. Radiation Inactivation

At present, studies of microbial inactivation by UV radiation are usually carried out using low pressure mercury lamps which emit about 95% of their light at a wavelength very close to 253 nm. The plot of the logarithm of surviving organisms against UV dose yields, very frequently, nonlinear curves, with a pronounced tendency to tail off. A quantum of UV radiation has an energy level of about 5 eV, insufficient to eject electrons from atoms or molecules, and able to cause only excitation processes. High energy radiations (y and X-rays, n, p, and neutron particles) have, characteristically, the property of causing ionization in the absorbing material; many atoms and molecules are excited and converted to free radicals. The density of ionization and excitation events produced along the path of radiation depend upon the photon energy and the type of absorbing material, and is usually expressed as a linear energy transfer (keV/p,m). Linear energy transfer (LET) increases with the square of the charge carried by the particle and decreases as its speed increases. The relative biological effectiveness usually increases as the LET of the employed radiation increases, but this is not always the case. A very limited number of experimental survival curves of irradiated microorganisms can be safely described by the law of exponential decay:

\[
\frac{N_d}{N_0} = \exp(-a \cdot d)
\]  

(72)

where \(N_d\) is the number of microorganisms surviving the dose \(d\) of radiation and \(a\) is the rate constant of inactivation. The coefficient \(a\) in Equation 72 is expected to represent the probability of inactivation of microorganisms per unit dose (rad or gray) of radiation. Inactivation is expected to result from a single hit, that is, through excitations and ionizations occurring along the track of photons crossing the cell. Hence, the concept of "direct action" arises, according to which the target is a molecule or a narrow group of molecules within the cell, in which the primary event (i.e., an ionization) has to occur, or through which or near which a unit of ionizing radiation must pass. On the other hand, the "indirect action" theory assumes that the whole solution, in which microorganisms are suspended, is the true target, lethal events being produced by reactions of chemical active agents induced by radiation in water, outside, and/or inside the cell. In fact, a number of reactions do occur in irradiated water:

\[
\begin{align*}
\text{H}_2\text{O} & \rightarrow \text{H}_2\text{O}^+ + \text{e}^- \\
\text{H}_2\text{O}^+ & \rightarrow \text{OH}^- + \text{H}^+ \\
\text{e}^- + \text{H}_2\text{O} & \rightarrow \text{OH}^- + \text{H}^+
\end{align*}
\]
\[
2H^+ \rightarrow H_2 \\
2OH^- \rightarrow H_2O_2 \\
H^+ + OH^- \rightarrow H_2O \\
H^+ + O_2 \rightarrow \cdot HO_2 \\
2 \cdot HO_2 \rightarrow H_2O_2 + O_2
\]

The most active and long-living radicals are regarded to be OH\(^-\). For the single-hit theory to be tenable, direct interaction between the radiation and a single sensitive target inside the cell must occur.\(^{30}\) However, many environmental factors can modify the sensitivity of microorganisms to ionizing radiations such as oxygen, moisture, the presence of sulfydryl compounds and/or -SH reagents in the medium, previous treatments of the microorganisms, physiological age of the cells, etc.\(^{31-36}\) Actually, exponential survival curves of irradiated microorganisms usually result from experiments carried out either using very sensitive organisms or are not prolonged enough to determine the shape of survivor curves at very low surviving fractions. Nevertheless, it is well known that survivor curves of irradiated microorganisms are not usually exponential, but are typically sigmoid.

C. Chemical Inactivation

The concept of "effective concentration" requires that a lethal chemical compound must possess the ability to reach, and to accumulate at, the site(s) of action, whether on the surface of, or within, the microbial cell. Thus, a dose-effect curve is expected to be characteristically sigmoid. At very low concentrations of the lethal compound the microbial inactivation rate will be very low and approaches zero; at higher doses, the death rate is expected to increase as the dose itself is increased; at the highest doses, the rate of the process would not increase further, since it would be sorption-limited. In spite of these expectations, Madsen and Nyman\(^{37}\) and Chick\(^{38}\) established a mathematical model for chemical disinfection on the basis of an analogy between microbial inactivation process and first-order reaction kinetics. This model has formed the basis of most subsequent investigations.

According to the model, the relationship between the surviving organisms and the contact time, \(t\), with a given concentration of a lethal chemical compound is expected to be

\[
\frac{N_t}{N_0} = \exp(-k_t * t) \quad (73)
\]

where \(k_t\) is the rate constant. The inactivation rate constant \(k_t\) changes as a function of the concentration of the disinfectant. The relationship between the rate constant \(k_t\) and the concentration \(C\) of the lethal agent is expected to be:

\[
k_t = B * \exp(k_c * C) \quad (74)
\]

where \(k_c\) is the rate of change of inactivation rate per unit change in the concentration \(C\) of the lethal compound. Usually Equation 75 is used instead of Equation 74:

\[
k'_t = B' * 10^{k_c' * C} \quad (75)
\]
and $1/k_c$ is the concentration required to achieve a tenfold change in the inactivation rate. However, subjecting microbial populations to increasing concentrations of lethal compounds for a fixed contact time seldom produces survival curves described by a function of the type:

$$\frac{N_d}{N_0} = \exp (-k_d \cdot D)$$  \hspace{1cm} (76)

where $D$ is the concentration of the lethal compound. Usually Equation 77 is used instead of Equation 76:

$$\frac{N_d}{N_0} = 10^{-k_d' \cdot D}$$  \hspace{1cm} (77)

and $1/k_d'$ equals the change in concentration required for the survival probability $N_d/N_0$ to change ten times.

Reaction rate constants $k_t$, $k_c$ and $k_d$ are expected to increase as temperature increases, following the Arrhenius law:

$$k_{t,c,d} = F \cdot \exp\left(-\frac{E_a}{R \cdot T}\right)$$  \hspace{1cm} (78)

where $F$ is a frequency factor whose value is linked to the rate constant considered ($k_t$, $k_c$ or $k_d$). Actually, a relationship of the above type is expected to occur in disinfection processes at temperatures lower than about 45 or 100°C for vegetative cells (heat sensitive) or bacterial spores (heat resistant), respectively. In a range of temperatures sufficiently high for heat inactivation, a relationship more complex than Equation 78 is expected to be more appropriate. A strikingly limited number of Arrhenius plots is reported in the literature, notwithstanding the relevance of temperature coefficients of chemical lethal compounds to the practice of disinfection, especially with respect to the choice of suitable agents of disinfection.

$Q_{10}$ values for microbial inactivation by chemical compounds reported in the literature range between about 1.6 and 3.3 and have been obtained by treating bacterial spores with hydrogen peroxide, formaldehyde, glutaraldehyde, β-propiolactone, ethylene oxide, chlorine, and iodine, at temperatures ranging from -10 to 95°C.\textsuperscript{37-42} Therefore, Arrhenius activation energies are expected between about 10 and 25 kcal/mol. Recently, Gelinas et al.\textsuperscript{43} reported $E_a$ values ranging from 0 to about 37 kcal/mol, so that $Q_{10}$ fell between about 1 and 7.7, although, the method used by the authors to assay the sensitivity of vegetative bacterial cells cannot be regarded as being reliable. Higher $E_a$ values are reported for \textit{Escherichia coli} treated with phenol at temperatures from 30 to 42°C: 52 kcal/mol;\textsuperscript{44} a $Q_{10} = 10$ was reported using peracetic acid.\textsuperscript{45} Nevertheless, a disinfection model is difficult to develop on the basis of these sorts of results, since several observations suggest that survivor curves may have different shapes (mostly convex, sigmoid, and concave, with a more or less pronounced tail), according to the experimental conditions employed.\textsuperscript{37, 40-42, 45-49} A comprehensive theory of the disinfection process is, unfortunately, still lacking.

### D. Theoretical Uncertainties

Characteristically, the single-hit theory is applied to heat inactivation kinetics. Heat sterilization technology is based on the tenet of exponential inactivation. If this order of death should be found to be invalid, the efficiency of the sterilization process becomes questionable.

As pointed out by several authors, first-order kinetics are expected to be produced by unimolecular reactions, without regard to the underlying reasons (pseudo-first-order reaction, for instance). As far as microbial inactivation is concerned, a unimolecular reaction would comply with the single-hit or single-site theory, after which a single damage produced in the cell would
unequivocally lead to the death of the cell, whether affecting an enzyme, the DNA, or a different molecule. The concept is quite limiting, since microorganisms subjected to a lethal agent are not unequivocally dead or alive, but they either die or recover, depending on the environmental conditions applied after the treatment. The phenomenon is well known to microbiologists and is called "sublethal injury or damage". Usually, microorganisms treated with lethal agents become more exacting about their environmental conditions than untreated organisms. As a consequence, survivor curves obtained using a population subjected to a lethal treatment might differ depending on the experimental conditions applied after the treatment. As a rule, the extent of the damage becomes increasingly difficult to demonstrate as the intensity of the applied lethal condition increases. The sublethal injury phenomenon can hardly be reconciled with the single-hit theory. On the contrary, it seems to suggest that microbial death can be rather more satisfactorily envisioned as an end point of a gradual, damaging process.

III. TARGET THEORY

A. Inactivation by Radiation

The effects of ionizing radiation upon microorganisms have led to the development of some very interesting models, usually described under the name of "target theory". The concept of microorganisms, or something(s) inside microorganisms, as targets being hit by photons or particles, reflects quite closely what is believed to occur in radiological phenomena. The basic assumption of the "multiple hit" theory was that a single target must be hit n times before the organism is destroyed. Let a be the sensitive volume of the cell and h the average number of hits per unit volume of the microbial suspension; then, ‘ah’ is the average number of hits within the sensitive volume a. If the hits occur independently and at random, the probability that f hits fall within the volume a is given by the Poisson distribution:

\[ P(f) = \exp (-a*h) \left( \frac{(a*h)^f}{f!} \right) \]  

(79)

If n is the number of hits required to inactivate a microorganism, then all cells receiving less than n hits will survive. The probability \( N_h/No \) that after the dose h only a fraction \( f < n \) of hits had occurred, i.e., the probability of survival, is given by:

\[ \frac{N_h}{No} = \exp (-a*h) \sum_{o}^{n-1} \left( \frac{(a*h)^f}{f!} \right) \]  

(80)

The second multiple-hit hypothesis of the target theory follows directly from the single-hit theory. If a population of organisms contains n sensitive targets that are inactivated exponentially, the viability of the organism is ensured if fewer than n targets are hit. The probability that all the targets in such a group of n becomes inactivated is given by:

\[ P(n) = (1 - \exp (-k*d))^n \]  

(81)

since \( \exp(-k*d) \) is the probability that targets are not hit. This assumes that the rate ‘k’ of occurrence of hits is the same for all the targets. Therefore, the assumption is not simply that n hits per organism are required, but that each of n particles or targets within the cell must be hit at least once.
Both Equations 80 and 81 describe convex curves, characterized by an initial shoulder followed by a nearly exponential behavior at higher doses. The fitting procedures of experimental dose-effect curves by one or other of either equations are not critical. Discrimination between the two models would require a level of experimental accuracy that is not attainable in practice. It has been suggested that the n value should be referred to as the "extrapolation number", whether the multi-hit or the multi-target theory is considered. The multi-target theory suggests a likely explanation for the observed variations in recovery rate in different environmental conditions. In fact, it envisages microbial death as an end point of a process of gradually increasing damage as the applied dose increases, since the cell dies only when all the n vital sites are hit. Under suitable environmental conditions the cell can recover.

B. Heat Inactivation

As indicated above, convex survival curves are seldom reported in heat inactivation studies. Moats\(^{57}\) succeeded in fitting convex survival curves based on a multi-target model he developed for heat-treated bacteria. However, according to his model the survival curve is expected to be characterized, as in the multi-target theory developed by Atwood and Norman,\(^{61}\) by an initial lag (shoulder) followed by essentially exponential behavior over a wide range of intensities. The fundamental equation developed by Moats\(^{57}\) was

\[
P(S) = \sum_{X=0}^{X_p-1} \left( \frac{N}{X} \right) \exp(-k*t*(N - X)) \left(1- \exp(-kt)\right)^X
\]

(82)

where \(P(S)\) is the survival probability, \(X\) is the number of critical targets inactivated at any time \(t\), \(X_p\) is the number of critical sites that must be inactivated to cause death, \(N\) is the total number of targets, and \(k\) is the rate constant for inactivation of individual targets. The values of \(k\), \(N\), and \(X_p\) can be obtained from experimental data. Solving simultaneously for \(k\):

\[
d / s = N(\exp(-k*t) - 2 * \exp(-k*t + k*t_{50}) + \exp(-2*k*t^2)) / (\exp(-k*t) - \exp(-2*k*t))
\]

(83)

where \(d\) is deviation from the mean (i.e., \(X - X_L\)), \(s = (Npq)^{1/2}\) where \(p = X/N\) and \(q = (N - X)/N\), and \(t_{50}\) is the time at which 50% of the population is killed.\(^{54}\)

According to the model, cells of \emph{Salmonella anatum} heated at 55°C can survive if plated on trypticase soy agar (a relatively rich medium) having about 7.8 critical sites inactivated, while if plated on basal medium (a less rich medium), only about 3.3 sites need to be inactivated to cause death. Estimation of \(N\) and the procedure for calculating the rate constants is statistically difficult.\(^{63}\) In the example quoted above, \(N\) ranges between 38 and 173 (see Reference 57). As pointed out by Moats himself, the model is unable to explain all survival curves and especially those with a tail.\(^{56,57}\)

Alderton and Snell\(^{64}\) developed an empirical expression which gave a reasonable fit to heat-treated bacterial spore survivor curves, showing a shoulder followed by an exponential decay:

\[
\log_{10} \left( \frac{N_0}{N_t} \right)^a = k' * t + C
\]

(84)

where “\(a\)” is a constant characterizing the degree of resistance of the microorganism and \(C\) is a constant whose value increases with both treatment temperature and the sensitivity of the
microorganism. Equation 84 allows the linearization of survivor curve obtained following treatment with heat, radiation, and disinfectants.63, 65 Alderton and Snell64 did not supply an explanation of the parameters “a” and C, nor of the inactivation mechanism.

C. Limitations of the Theory
The target theory was developed to explain the shoulder in survivor curves of irradiated microorganisms. The single-hit survivor curve is a special case of the theory. Nevertheless, the target theory does not explain other types of survival curves occurring in many radiation inactivation experiments, such as true sigmoid curves, continuously decreasing death rate curves, and curves with long tails. The merit of the theory is that it suggests a multiplicity of events leading to death as a possible general mechanism of microbial inactivation.

IV. CONTINUOUSLY DECREASING DEATH RATE CURVES

A. Experimental Evidence
Moats et al.56 stated that "... examples of non-exponential survivor curves found in the literature are too numerous to list". Actually, as shown in Table 3, the list of only more prominent sigmoid or concave survivor curves reported in the literature is long. Nevertheless, some authors8, 50, 66 seem to ignore factual evidence, proposing hypothetical biological or experimental reasons for deviation from exponential behavior, although their proposals are not convincing and they provide little in the way of experimental evidence. The early experimental evidence of Bigelow and Esty5 showing many data incompatible with the exponential tenet and by Esty and Meyer's6 data showing a Clostridium botulinum spores heat destruction curve with a tail lasting about 40 min seem to be disregarded. The experimental evidence of deviations from exponential kinetics by many lethal agents is too widespread to be ignored.

B. Theoretical Aspects
A concave or biphasic survival curve suggests a phenomenon brought about by population heterogeneity. Chick's4 proposal that heterogeneity in heat resistance could be responsible for a concave survivor curve seemed to be the only explanation for more than 70 years, although based on very little experimental evidence. Microbial heterogeneity can explain biphasic survivor curves.7, 133-136 A mixture of two populations of organisms having different resistances to a lethal agent yields a biphasic survival curve. For example, mixing 10⁷ spores of C. botulinum with 103 spores of PA 3679 and then subjecting the suspension to a temperature of 110°C, results in two survivor curves which intersect after about 15 min of treatment. Biphasic survivor curves could also occur when cells of a single species are composed of two populations with respect to their resistance to the lethal agent employed. Based on the same reasoning, multiphasic survivor curves, or continuously decreasing death rate curves (CDDRC), would be expected when heterogeneous populations are treated.137 The first problem raised by several authors was whether the distribution of resistance among individuals in a population was permanent ("innate heterogeneity" theory)7, 8, 138-140 or if it was acquired during the treatment ("adaptation model").141-143 The second problem was which type of probability distribution could explain different shapes of the survivor curves.
C. Mathematical Models

Han et al. developed a model for both the innate heterogeneity hypothesis and the adaptation hypothesis. The following equation was derived for the former:

\[
\log_{10} \left( \frac{N_t}{N_0} \right) = -Kt + \left( \frac{s^2}{2} \right) t^2
\]  

(85)

where \( K \) is the most probable value of the destruction rate, "s" is the standard deviation, and "t" is the treatment time. The heat adaptation approach leads to the following equation:

\[
\log_{10} \left( \frac{N_t}{N_0} \right) = -K_0 \cdot X \left( (1 - a) t - a b \left( \exp \left( -\frac{t}{b} \right) - 1 \right) \right)
\]  

(86)

where \( K_0 \) is the initial rate of destruction, "a" is a constant representing the maximum amount of resistance attainable for a unit amount of destructive power, "b" is a constant representing the rate of development of resistance, and "t" is the time. By applying the two models to some bacterial
inactivation curves, the authors concluded that curvilinearity in the survival curves resulted from the development of resistance during the treatment, rather than from innate heterogeneity.

Sharpe and Bektash\textsuperscript{136} modified the models developed by Han et al.\textsuperscript{135} by utilizing other types of probability distributions of resistance in the population, including the normal, $\gamma$, shifted $\gamma$, and a modified Poisson distribution, and suggested that a combination of the innate heterogeneity and adaptation models might be appropriate. They proposed that the distribution of the initial rate of destruction ($K_0$) could be represented by any distribution having a probability $P(K < 0) = 0$, that the life of a cell follows an exponential distribution with mean $1/K$, and that the state of the cells (i.e., living or dead) is independent. These considerations lead to a probability of survival $S(t)$ at time $t$ of the following form:

$$S(t) = \log \left( \frac{N_t}{N_0} \right) = \log L_4(t)$$

where $L_4(t)$ is the Laplace transform of the density function of $K$ at time zero, that is for a normal distribution,

$$L_4(t) = \exp(-K_0 t + (s^2 t^2)/2)$$

for a $\gamma$ distribution,

$$L_4(t) = (1 + t / \lambda)^{-r}$$

for a shifted $\gamma$ distribution,

$$L_4(t) = \exp(-a^*t)(1 + t / \lambda)^{-r}$$

where “$a$” is the minimum value of the rate of destruction and for a modified Poisson distribution,

$$L_4(t) = \exp(-K_0 (1 - a)^*t + K_0 * a^*b*(\exp(t/b) - 1))$$

Sharpe and Bektash\textsuperscript{136} concluded that it is not possible to distinguish between the two possibilities (innate heterogeneity or development of resistance) on the basis of the survival data alone; the heat adaptation model of Han et al.,\textsuperscript{135} for instance, has been shown to be equivalent to an innate heterogeneity model with a modified Poisson distribution. Nevertheless, from the mathematical analysis carried out by the authors, it can be argued that all concave survivor curves can be reasonably explained by the innate heterogeneity theory.

Two objections can be made to the purely phenomenological models described above:

first, the adaptation model cannot be established if the acquisition of resistance during the treatment is not permanent; second, the distribution of resistance in an untreated population can be demonstrated experimentally. Furthermore, none of the models examined is able to explain the tailing phenomenon. As shown later, the experimental evidence is contrary to both models.

Brannen\textsuperscript{144} developed a model based on the assumption that survival depends on a number of subsystems, whose functionality is affected by heat. The model yields the four classical types of survivor curves, although "testing of the model is extremely difficult"\textsuperscript{144} and the relationship between heat resistance and water content of the environment is not explained.

\textbf{D. Evidence Against Suggested Models}

As pointed out by several authors, the occurrence of small numbers of very resistant individuals could be regarded as a normal feature of a population of microorganisms. To verify the assumption that CDDR curves and tailing result from the type of the distribution of resistance in the population, at least two types of experiments must be done: (1) particles surviving more drastic treatments must be assayed in order to show if their resistance is greater than that of the majority of individuals in the population and (2) the resistance of decreasingly smaller fractions of the population must be
assayed to determine whether the CDDR curves become progressively exponential as cell counts decrease.
The first type of experiment has been performed by few authors, and all failed to show survivors of greater resistance (to heat, radiation, or disinfectant) than in the parent population. The second type of experiment has been performed by more authors. Bigelow and Esty reported heat resistance data obtained using thermophilic bacterial spores treated at temperatures ranging from 100 to 140°C. They used mother suspensions containing more than $10^5$ spores per sample and diluted these down to 3 spores per sample. Surprisingly, since it was unexpected both on the basis of a hypothetical distribution of the resistance among individuals in the spore population and on the basis of expected exponential inactivation, it was found that the time required to destroy 90% of the spores increased as particle concentrations ($N_0$) decreased. More than 80% of the assays carried out by Bigelow and Esty showed such an effect. The decreasing death rate found as spore concentrations decreased was increasingly evident (and obviously statistically more reliable) as treatment temperatures were lowered. This phenomenon escaped the attention of many researchers. Many authors followed the suggestion of Stumbo et al. and averaged the DE values they obtained, disregarding the actual meaning of the decrease in death rate with increasing treatment time. Reed et al. found a similar phenomenon in heat destruction rate studies on PA 3679 spores. Pflug and Esselen found increased resistance of PA 3679 spores at all 14 temperatures tested (ranging from 112.8 to 148.9°C), as treatment time increased. Kempe et al. found a linear relationship between the logarithm of Clostridium botulinum spores counts and a dose of $\gamma$-radiation. At spore concentrations ranging from $4 \times 10^4$ to $4 \times 10^2$ the decimal reduction dose ranged from 0.6 to 0.8 Mrad; the $D_{90}$ increased to 1.5 Mrad at lower concentrations and reached as high as 12.0 Mrad at a spores concentration of four in ten samples. Amaha found a linear relationship between $\log_{10}(time)$ and $\log_{10}(N_0)$ using spores of C. sporogenes, Bacillus megaterium, and B. natto treated at temperatures ranging from 105 to 120°C. Using PA 3679 spores Casolari found an increase in $D_T$ value as spore concentrations decreased from $9 \times 10^6$ to $1.2 \times 10^0$, employing ten different media for the recovery of treated spores. Using five C. botulinum strains (types A, B, and E) and six PA 3679 strains, the extent of inhibition of growth of the vegetative cells brought about by nitrite-dependent-compounds (inhibitory substances present in heat treated solutions containing nitrite) was found to be linearly correlated with the logarithm of the initial cell concentration, ranging from 4 to $10^6$ per sample. Analogous results, although unrecognized or disregarded, were obtained by Greenberg and Roberts and Ingram among others, using heat treated substrates containing nitrite. Greater heat resistance by low concentrations of yeasts was shown by Williams (see Morris and Casolari and Castelvetri. Campanini et al. found the same phenomenon using S. faecalis. Spores of B. polymyxa ($4 \leq N_0 \leq 10^5$ per experimental unit) and vegetative cells of Staphylococcus aureus ($4 \leq N_0 \leq 200$/ml) and E. coli ($0.1 \leq N_0 \leq 10^2$ cells per 10 ml) were treated with $\gamma$-radiation from a $^{60}$Co source using three dose rates (from 1 to 22 krad / min). The results showed clearly that the initial concentration of particles affected survival probability at all the dose rates tested. When S. aureus was irradiated in solutions containing cysteine at $10^{-3}$ M, the $D_{90}$ doubled at No = 4.6 cells per 10 ml [$D_{90}(200$ cells) = 31 krad, $D_{90}(4.6$ cells) = 73 krad] and was three times higher at a cell concentration of 0.93/10 ml ($D_{90} = 214$ krad). According to the above observations, CDDR curves neither result from heterogeneity in the resistance of individuals in a population, nor from the acquisition of resistance during treatment. Therefore, a different hypothesis must be formulated.
V. APPROACH TO A GENERAL MODEL
Some years ago a model was devised\textsuperscript{155} which related in some way concepts suggested by Ball and Olson\textsuperscript{7} about the likely mechanism of microbial heat inactivation. The model was based originally on what might be envisioned to occur in the process of heat inactivation, although, as will be shown later, it applies to radiation and chemical inactivation processes as well.

A. Heat Inactivation
The basic reasoning was that as shown experimentally, a single factor is of paramount importance in the heat inactivation process. This is the water content of the environment. A suspension of microbial cells in aqueous medium can be regarded as a biphasic system consisting of about $3 \times 10^{22}$ water molecules and less than $10^9$ microbial particles per milliliter. Energy supplied to a system will be taken up by the more concentrated components of the system and then transferred by collision to less concentrated ones. Accordingly, kinetic energy supplied to a microbial suspension is expected to be taken up by water molecules and then transferred to microbial cells. Brownian movement of particles results from this collision process. If the energy transferred between the particles is sufficiently high, the physicochemical structure of the microbial particle is damaged. If the damage is great enough the particles lose their ability to functionally relate with their environment and become unable to multiply, viz., they die. A more detailed hypothesis about the death mechanisms has been reported elsewhere.\textsuperscript{155}

Let the survival probability $P(S) = C_t / C_0$ where $C_0$ and $C_t$ represent the concentration of living organisms initially and after a treatment time $t$, respectively. Based on the experimental evidence outlined above, the probability with which particles elude collisions ($q$) with water molecules carrying lethal energy $E_d$ can be regarded as inversely related to the living particle concentration in the suspension at the time $t$:

$$q = 1/C_t$$

(92)

The probability $P(T)$ with which particles elude lethal collisions at temperature $T$ is expected to depend on the frequency $P_c$ of collision at the temperature $T$, so that:

$$P_0(T) = q^{P_c}$$

(93)

and during $t$ min at the temperature $T$:

$$P_0(T) = q^{t \cdot P_c}$$

(94)

The collision frequency $P_c$ depends on both the probability of there being a given number of water molecules with more than $E_d$ energy, that is $P(n_E)$, and on the probability $P(h)$ that available $n$, molecules strike microbial particles, so that:

$$P_c = P(n_E) \cdot P(h)$$

(95)

Taking into account the relative size of microbial particles (about $10^{11}$ times greater than a water molecule), the probability $P(h)$ almost equals the probability of having $n$, molecules per unit volume, so that Equation 95 can be rewritten:

$$P_c = (P(n_E))^2$$

(96)
The $P(n_E)$ value comes from the Maxwellian distribution of energy from which, in the simplified form (Equation 60), the number of molecules carrying more than $E_d$ energy present in 1 ml of water will be

$$P(n_E) = (6.02295 \times 10^{23} / 18) \times \exp(- E_d / R*T)$$

so that:

$$Pc = M = (6.02295 \times 10^{23} / 18)^2 \times \exp(- 2*E_d / R*T)$$

that is

$$M = \exp(103.7293 – 2*E_d / R*T)$$

It follows that the survival probability after time $t$ at temperature $T$ is described by the equation:

$$P(S) = C_t / Co = q^{M*t} = (1/C_t)^{M*t} = C_t^{M*t}$$

Dividing by $C_t$:

$$Co = C_t^{(1 + M*t)}$$

or

$$C_t = Co^{1/(1 + M*t)}$$

that is:

$$C_t = Co^{(1+t) \times \exp[103.7293 – 2*E_d / (R*T)]}$$

To fit experimental data using Equation 103 we must know values for $Co$, $C_t$ and ‘$t$’. With these values for a single temperature, the value of $M$ can be obtained from Equation 102:

$$M = ((\log C_o / \log C_t) - 1) / t$$

and hence the $Ed$ value:

$$Ed = 0.5*R*T*(103.7293 - \log M)$$

The $Ed$ value is the most important single parameter characterizing the heat resistance of microbial cells in defined environmental conditions. It can be expected that for a given microorganism, $Ed$ will depend on the environmental conditions pertaining after heat treatment. Knowing the $Ed$ for a given microorganism, the inactivation curves at any temperature and at any environmental water content can be obtained by simple computation.

1. Expected Shape of Survivor Curves
   Survivor curves obtained by plotting $\log_{10} C_t$ against time $t$ (min) are expected to be, according to the model, fundamentally concave. Nevertheless:
   1. At a given temperature $T$, survivor curves of microorganisms having high $Ed$ are expected to be nearly exponential (i.e., statistically indistinguishable from an exponential decay curve); those of organisms having intermediate values of $Ed$ are expected to be concave (i.e., of the CDDR type); and survivor curves of microorganisms having low $Ed$ are expected to be nearly exponential initially, followed by a phase of CDDR type and finally tailing (Figure 5).
2. A population of organisms having a given $E_d$ is expected to yield nearly exponential inactivation curves at low temperature, concave (CDDR type) survivor curves at intermediate temperatures.
temperatures, and curves nearly exponential at first (short treatment times), followed by a CDDR phase and then tailing (Figure 6).

![Figure 6](image.png)

FIGURE 6. Predicted shape of survivor curves of a heat sensitive microorganism ($E_a = 35$ kcal/mol) as a function of the concentration of untreated organisms ($C_0$) and of the temperature, according to the general model. The correlation coefficients of linear regression from ten pairs of data obtained at 55°C are all greater than -0.99.

Depending on the concentration of cells, survival curves at a given temperature $T$ are more concave (high concentration) or less concave (low particle concentration) (Figure 7). Equation 103 agrees quite well with experimental data, as already shown.\(^{155}\)

Pflug and Esselen\(^{148}\) reported a total of 95 experimentally determined decimal reduction times obtained by treating PA 3679 spores at 14 temperatures; the relationship obtained by plotting $\log_{10} D_T$ against temperature yields $\log_{10} D_T = 12.986 - 0.107^*T$ ($r = -0.9992$).

Figure 8 shows the ratio between experimental $D_T$ values and the $D_T$ expected by interpolation, using the above equation, together with those expected from the model. The computation of $D_T$ values as performed by authors was possible only if a fraction of heat treated samples was sterile, since in order to obtain the number of survivors, they used the first term of the Poisson distribution, known as the Halvorson and Ziegler formula:

$$\bar{N}_t = \log (A / H)$$  \hspace{1cm} (106)

where $N_t$ is the average number of survivors per sample, $A$ is the total number of treated samples, and $H$ is the fraction of sterile samples. To use the model, the first term of the Poisson distribution was used in order to obtain a $C_t$ value and to compute an $M$ value from Equation 104. A parameter analogous to the decimal reduction time, subsequently called $P(10,T)$, was obtained from Equation 100:

$$P(10,T) = 1 / M^* \log_{10} C_t$$  \hspace{1cm} (107)
To use the above equation, a $C_t$ value corresponding to 37% sterility of treated samples was chosen for all temperatures; Pflug and Esselen\textsuperscript{148} used $N_t$ values derived from sterility fractions ranging from 4 to 96%. As can be seen from Figure 8, the experimental data are quite scattered around the interpolated values. Nevertheless, both experimental $D_T$ values and those predicted by the model show a defined trend; that is, they are not equally distributed about values expected from the regression obtained using all data, although they show a defined concavity around interpolated values obtained by assuming that $z$ is constant. Such behavior is pertinent to the controversy regarding the linear dependence of inactivation rate against temperature.\textsuperscript{156, 157} The question arises as to whether the Arrhenius activation energy or the $z$ value can be regarded as being constant. The moderate concavity obtained by plotting microbial heat destruction rates against $T^{-1}$ (that is $\log_{10} D_T$ vs. $1/T$) is statistically indistinguishable from the plot of $\log_{10} D_T$ against $T$, taking into account the low level of experimental accuracy attainable in practice. Hence, the controversy is fed.\textsuperscript{156, 157}

![Figure 8](image)

**FIGURE 8.** Relationship between temperature and (1) the ratio of single experimental $D_i$ values ($D_i(e)$) reported by Pflug and Esselen\textsuperscript{148} to those obtainable from the regression equation ($D_i(i)$) calculated using the 95 experimental data ($r = -0.9999$), (2) the ratio of $P(10,T)$ (the parameter analogous to $D_T$ from the general model) to $D_T(i)$. Full circles = $D_i(e) / D_T(i)$; empty circles = $P(10,T) / D_T(i)$. In using $P(10,T) = 1/M \log_{10} C_i$ five experimental survival fractions were chosen at random and $M$ values calculated using Equation 104.

The fact that $D_T$-equivalent data arising from the model (i.e., $P(10,T)$) do show concave behavior is not surprising, since the model requires that $E_D$ must be constant; the unexpected evidence coming from the experimental data reported by Pflug and Esselen,\textsuperscript{148} on the contrary, suggests that $\log D_T$ is linearly correlated with $1/T$ and not with $T$ (i.e., it is lethal energy which is constant, not $z$). It follows that a plot of $\log_{10} D_T$ against $T$ is not appropriate.
2. Tailing-Off

According to Equation 107, the inactivation rate decreases as \( C_t \) decreases. At a low \( C_t \) level following the M level in the environment (i.e., temperature, free water, etc.), the inactivation rate is expected to be very low and it approaches zero as \( C_t \) approaches unity.

Tailing can be regarded as a phenomenon produced by the increasingly low probability of collision between water molecules having more than Ed energy and microbial particles. If microbial particles are about 10 µm apart on the average (as in suspensions containing \( 10^9 \) particles per milliliter), water molecules carrying lethal energy can be expected to have a greater probability of striking the particles than when the particles are more than 1000 µm apart (as in suspensions containing less than \( 10^3 \) particles per milliliter). Actually, a water molecule having high energy behaves like a long-lived bullet, able to overcome a large number of collisions with other water and/or solute molecules, while traveling through the medium, keeping enough energy to kill living particles it meets along its path. Nevertheless, in environmental conditions which result in more tailing-off, the concentration of M molecules is very low. In heat inactivation curves, the tail appears after shorter treatments as temperature increases. The probability of finding the tailing-off of survivor curves decreases as temperature increases, since microbial particles are inevitably struck with increasing frequency and also by molecules having less than Ed energy while having a value high enough to damage the particles. The probability of occurrence of molecules having enough energy to damage the particles is expected to increase with temperature, according to the Maxwell-Boltzman distribution of energy. It follows that a fraction of particles could become incapable of growing, although surrounded by suitable environmental conditions, being extensively damaged not by molecules carrying more than Ed energy, but by those carrying less than Ed energy. Such an event is not accounted for by the model developed, while it can be expected to occur following the premise of random collisions. However, these considerations concern almost exclusively the last living particle, since the model predicts that inactivation curves tend to become exponential as temperature increases owing to the ensuing increase of M value. According to the model, the death of the last particle cannot be expected to occur.

Tailing occurs at decreasingly low particle concentrations, as temperature increases. There are intermediate temperatures at which tailing occurs with a greater probability, as well as extreme ones (low or high temperatures) at which inactivation curves resemble a straight line.

Very low survivor values are often disregarded on the basis that they are not statistically reliable. On the other hand, they are accepted for exponential inactivation curves at values as low as \( 10^{-12} \).

3. Inactivation Rate, Temperature, and Energy

As noted previously, a relevant parameter employed to define heat resistance in microorganisms is \( z \) (Equation 15), related to the temperature coefficient, \( Q_{10} \), by the relationship expressed in Equation 16.

According to Equations 99 and 107, the inactivation rate increases as the M value increases, and M increases with temperature. The model suggested is able to supply an exact definition of \( z \) and \( Q_{10} \) in terms of M: the inactivation rate changes \( Q_{10} \) times for each \( 10^\circ \)C, since the M value changes \( Q_{10} \) times for each \( 10^\circ \)C; the inactivation rate changes ten times for each change of z degrees, since the M value changes ten times for each change of z degrees.

According to the model (Equation 107):

\[
(P(10, T))^{-1} = M \times \log_{10} C_t
\]

then:

\[
(108)
\]
\[ Q_{10} = M_{(T+10)} \times \log_{10} C_t / [M_T \times \log_{10} C_t] \]
\[ = M_{(T+10)}/M_T \]  \hspace{1cm} (109)

It follows that:

\[ Q_{10} = \exp((2*Ed/R)(10/(T+10)T)) \]  \hspace{1cm} (110)

and from Equation 99,

\[ \log Q_{10} = (2*Ed/R)(10/(T+10)T) \]  \hspace{1cm} (111)

Therefore, Ed can be obtained as a function of Q_{10}:

\[ Ed = (\log Q_{10}/10)(R/2)(T+10)T \]  \hspace{1cm} (112)

and from Equation 16,

\[ Q_{10} = \exp(23.03/z) \]  \hspace{1cm} (113)

so that,

\[ Ed = (\log 10)*R*T*(T+10)/(2*z) \]  \hspace{1cm} (114)

and

\[ Ed = R*T(T+10) \log Q_{10} / 20 \]  \hspace{1cm} (115)

since, according to Equation 113,

\[ z = (10*\log 10)/\log Q_{10} \]  \hspace{1cm} (116)

Accordingly, given \( Q_{10} = z = 10 \) in the temperature range 110 to 120°C and following Equations 112 and 114, E, will equal 34459.6348 cal/mol. Therefore, using Equation 99, the value of M will be \( 5.4208 \times 10^5 \) at 110°C, a value which is \( z = Q_{10} = 10 \) times lower than the value of M at 120°C where it is \( 5.4208 \times 10^6 \). Similarly, given a \( Q_{10}(a) = 22 \) (i.e., \( z = 7.4449 \)) at 60°C ≤ T ≤ 70°C, and a \( Q_{10}(b) = 16 \) (i.e., \( z = 8.305 \)) at 110°C ≤ T ≤ 120°C, from Equations 112 and 114, Ed (a) = 5107.2379 and Ed (b) = 41493.5348. In the former case \( M_{70°C} = 2.1196 \), which is \( Q_{10} \) (a) = 22 times greater than \( M_{60°C} \), which is equal to 0.096. In the latter case, \( M_{120°C} = 0.082 \) which is \( Q_{10} \) (b) = 16 times greater than \( M_{110°C} \) which has a value of \( 5.1178 \times 10^{-3} \).

At the same time, at the temperature of 60°C+ \( z = 67.449°C \), M equals 0.963 which is 10 times higher than \( M_{60°C} \) and at 120°C - z = 111.695°C, M equals 0.0082 which is a value 10 times lower than that of \( M_{120°C} \).

Equation 110 shows the rate of change of \( Q_{10} \), as a function of Ed and temperature. The rate of change of z as a function of Ed and temperature is described by:

\[ z = (\log 10)(T+10) \times T \times R/(2 \times Ed) \]  \hspace{1cm} (117)
Taking into account the inactivation rate of heat-sensitive microbial particles destroyed in measurable times at 60°C it can be computed that according to the model, the expected $Q_{10}$ values must range between about 18 and 26 at $60^\circ C \leq T \leq 70^\circ C$, so that $8^\circ C \leq z \leq 7^\circ C$ is expected in the same temperature range, as shown in Table 4. For more resistant bacterial spores, destroyed in measurable times at 110 to 120°C, the expected values of $Q_{10}$ range between about 13 and 20, so that $7.5^\circ C \leq z \leq 9^\circ C$ is expected in the same range of temperature. As can be seen in Table 4, the expected value of $z$ increases as temperature increases; in the same temperature range, it decreases as $E_d$ value increases (i.e., increasing the resistance of the organism). The opposite is true with $Q_{10}$. It follows that the energy, $E_d$, required to inactivate less resistant organisms ranges between about 33 and 37 kcal/mol, while $E_d$ values required to inactivate more resistant organisms range between about 38 and 44 kcal/mol. Thus, less energy is required to kill less resistant organisms, and vice versa, a result quite reasonable but in opposition to what is predicted by the classical thermodynamic treatment of exponential kinetics [see: 1. Thermodynamic Inconsistencies].

<table>
<thead>
<tr>
<th>$E_d$</th>
<th>$z$</th>
<th>$Q_{10}$</th>
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<td>33</td>
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<td>21.71</td>
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<td>7.28</td>
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<td>$85-95$</td>
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</table>

For more resistant bacterial spores, destroyed in measurable times at 110 to 120°C, the expected values of $Q_{10}$ range between about 13 and 20, so that $7.5^\circ C \leq z \leq 9^\circ C$ is expected in the same range of temperature. As can be seen in Table 4, the expected value of $z$ increases as temperature increases; in the same temperature range, it decreases as $E_d$ value increases (i.e., increasing the resistance of the organism). The opposite is true with $Q_{10}$. It follows that the energy, $E_d$, required to inactivate less resistant organisms ranges between about 33 and 37 kcal/mol, while $E_d$ values required to inactivate more resistant organisms range between about 38 and 44 kcal/mol. Thus, less energy is required to kill less resistant organisms, and vice versa, a result quite reasonable but in opposition to what is predicted by the classical thermodynamic treatment of exponential kinetics [see: 1. Thermodynamic Inconsistencies].

The number of water molecules having $E_d$ values predicted by the model is quite reasonable, in opposition to that predicted from thermodynamic treatments. At an intermediate $E_d$ value of 35 kcal/mol, for instance, for less resistant organisms, the M values range between about 0.1 and 3/ml at 60 and 70°C, respectively; given $E_d = 40$ kcal/mol for more resistant organisms, the M value equals 0.25 at 110°C and 3.8/ml at 120°C.

### 4. Inactivation Rate and Water Content of the Environment
The single most relevant environmental condition affecting heat resistance of microbial particles is the amount of water contained in the medium in which microorganisms are treated. This phenomenon can be explained simply by the model proposed. The M value is linked to the number of water molecules per milliliter or gram of substrate, according to Equation 98 which can
FIGURE 9. Relationship between M value in a fully hydrated environment at 120°C (for $E_a = 40$ kcal/mol) and (1) the temperature ET at which an equivalent value of M is expected to occur, according to the general model, as water content of the environment decreases ($ET = f(\log_{10} W)$, where $W = g$ of water/100 g of medium), (2) the temperature $T'$ that must be reached, according to the model, to obtain a value of M equal to that occurring in a fully hydrated environment ($T' = f(\log_{10} W)$). Relationships of the type $\log_{10} P(10,T) = f(\log_{10} W)$ represent the expected decrease of $P(10,T)$ as water content of the environment also decreases, and were computed using three $C^1$ values for illustrative purposes.

rewritten as:

\[ M = \exp(A - 2*Ed / (R*T)) \]  
\[ A = 94.5190 + 2*\log W \]

and 94.519 is the natural logarithm of the squared number of water molecules per gram of medium containing 1 g of water and $W = g$ of water in 100 g of medium. Using Equations 118 and 119 we may obtain the value of M at any given water content ($W \leq 100$). Obviously, as the water content decreases, so does the M value. From Equations 118 and 119:

\[ \log M_w = 94.519 + 2*\log W - 2*Ed / (R*T) \]

where $M_{w}$ is the expected reduced M value following a decrease in the $W$ value.

A decrease in water content of the environment is expected to be equivalent to a decrease in temperature.

The temperature $T_e$ (equivalent temperature) corresponding to an M value lower than the one expected in a fully hydrated environment can be obtained by substituting M obtained from Equation 120 into Equation 99, i.e.:

\[ T_e = 2 * Ed / R*(103.7293 - \log M_{w}) \]

where $T_e$ is lower than $T$ (the true temperature in a fully hydrated environment), it follows that decreasing the water content in the medium results in an increase in microbial resistance, because a decrease in water content is equivalent to a decrease in temperature. As shown in Figure 9, a temperature of 120°C in a fully hydrated environment is equivalent to about 87.6°C in a medium containing 1 g of water in 100 g of medium.
Taking into account the $z$ (M) and $Q_{10}$ (M) expected values for both sensitive and resistant particles, a change of water content from 100 to 1 g in 100 g of medium at a given temperature $T$ (°K), the inactivation rate decreases about 1000 times (Figure 9). The following equation:

$$T' = \frac{2 \cdot Ed}{(R(94.519 + 2\cdot \text{Log } W - \text{Log } M_T))}$$ (122)

can be used to compute the temperature $T'$ that must be reached to obtain a $P(10,T)$ equal to that expected at the temperature $T$ in a fully hydrated environment. As shown in Figure 9, a 100 times change in $W$ requires that $T$ reaches about $T' = T + 40°C$ (letting $Ed = 40$ kcal/mol).

The agreement between resistance data at different water contents in the medium, and those predicted by the model, has already been shown.

<table>
<thead>
<tr>
<th>Table 5</th>
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<tbody>
<tr>
<td>LOSS OF WATER PREDICTED BY THE GENERAL MODEL, TO OBTAIN HIGH $z$ (LOW $Q_{10}$) VALUES, AS A FUNCTION OF THE TEMPERATURE RANGE AND OF THE ENERGY $E_a$ (kcal/mol), IN MICROBIAL INACTIVATION KINETICS</td>
</tr>
</tbody>
</table>

- For $E_a = 35,800$ (Heat-Sensitive Organisms):
  - Temperature range (°C):
    - $55–65$:
      - $z = 10.00$: $35.34$,
      - $z = 15.64$: $55.55$,
      - $z = 26.16$: $63.44$,
      - $z = 30.25$: $69.99$,
      - $z = 40.17$: $72.73$,
      - $z = 50.15$: $74.26$
    - $65–75$:
      - $z = 10.00$: $39.17$,
      - $z = 15.64$: $51.74$,
      - $z = 26.16$: $60.17$,
      - $z = 30.25$: $57.17$,
      - $z = 40.17$: $70.13$,
      - $z = 50.15$: $71.80$
    - $75–85$:
      - $z = 10.00$: $23.00$,
      - $z = 15.64$: $47.54$,
      - $z = 26.16$: $56.69$,
      - $z = 30.25$: $64.24$,
      - $z = 40.17$: $67.52$,
      - $z = 50.15$: $69.34$
    - $85–95$:
      - $z = 10.00$: $16.45$,
      - $z = 15.64$: $43.15$,
      - $z = 26.16$: $53.24$,
      - $z = 30.25$: $61.40$,
      - $z = 40.17$: $64.93$,
      - $z = 50.15$: $66.90$

- For $E_a = 40,000$ (Heat-Resistant Bacterial Spores):
  - Temperature range (°C):
    - $120–130$:
      - $z = 10.00$: $11.20$,
      - $z = 15.64$: $39.39$,
      - $z = 26.16$: $35.67$,
      - $z = 30.25$: $50.96$,
      - $z = 40.17$: $58.78$,
      - $z = 50.15$: $64.45$
    - $130–140$:
      - $z = 10.00$: $5.57$,
      - $z = 15.64$: $31.88$,
      - $z = 26.16$: $46.90$,
      - $z = 30.25$: $55.59$,
      - $z = 40.17$: $62.55$,
      - $z = 50.15$: $64.32$
    - $140–150$:
      - $z = 10.00$: $28.16$,
      - $z = 15.64$: $46.78$,
      - $z = 26.16$: $53.19$,
      - $z = 30.25$: $57.84$,
      - $z = 40.17$: $63.84$,
      - $z = 50.15$: $63.94$
    - $150–160$:
      - $z = 10.00$: $40.71$,
      - $z = 15.64$: $51.46$,
      - $z = 26.16$: $55.14$,
      - $z = 30.25$: $58.02$

Several workers have pointed out that heat inactivation rates in dry environments have greater $z$ values than those obtained in fully hydrated ones. According to Fox and Pflug, it is reasonable to suppose that cells subjected to high temperatures in dry environments lose some water. The increase of $z$ in dry environments can be explained, according to the model, accepting the suggestion of these authors. Otherwise, as Ed is constant for any given microorganism, $z$ must change thereby changing the water content of the environment. Let $Q'_{10}$, and $z'$ be values of $Q_{10}$ and $z$ expected when the water content at the temperature $T$ is $W_T$ and that at the temperature $T + 10°C$ is $W_{T+10°C}$. Then:
\[
\log Q_{10} = 2(\log W_{T + 10^\circ C} - \log W_T) + \frac{(2*Ed / R)(10 / T (T + 10^\circ C))}{10}
\]  
(123)

so that:

\[
z' = 10 \log 10 \left/ \left(2(\log W_{T + 10^\circ C} - \log W_T) + \frac{(2*Ed / R)(10 / T (T + 10))}{10} \right) \right.
\]  
(124)

Table 5 shows the fraction of water that must be lost in dry conditions by a 10°C increase in treatment temperature in order to obtain z values higher than those expected in moist conditions (W = 100 g water/100 g of medium). As can be seen, a loss of only about 50% of the water content, coming from a lower to a higher temperature, is required to yield very high z values (very low Q_{10} values), somewhat independently of the temperature range and/or the Ed value.

**B. Radiation Inactivation**

Survivor curves of microorganisms treated with radiation can be convex, sigmoid, or concave. A function like that suggested above for heat inactivation (Equation 102) can describe these types of survivor curves (Figure 10):

\[
Cd = Co^{1 / (1 + S*D)}
\]  
(125)

where Cd is the concentration of microorganisms surviving the dose d (Mrad) of radiation, D is the squared dose of radiation, and S is a sensitivity parameter, specific to the microorganisms employed.

![Image](image-url)

**FIGURE 10.** Shape of survivor curves of microorganisms treated with ionizing radiations, predicted by the general model, as a function of the value of the ratio \( S = 10^8 / \left[ -SH \right] \), where \([ -SH \] is the number of surface -SH groups /cell. Values of 0.2 \( \leq S \leq 100 \) were selected since \( [ -SH \] content of most bacteria lies in this range.

Experimental radiation survival curves can be fitted, according to Equation 125. \(^{155}\)

Following the model, lethal effects of radiations are expected to be mainly "indirect". Radicals produced by radiation in aqueous media are expected to damage microbial structure, in particular surface structures, as occurs with several lethal agents. \(^{53, 55, 163-167}\) Microbial resistance to radiation is higher in microorganisms with a higher content of [ - SH] groups. \(^{168}\) Let the maximum level of [-
SH]-/cell be about $10^8$. If $S$ in Equation 125 equals $10^8/G$, where $G$ is the expected [-SH] content of a single microorganism, or more precisely the [-SH] fraction present at the surface of a microbial cell, experimental survival curves can be predicted quite accurately. Figure 11 shows the relationship between total [-SH] content/colony-forming-unit for different microorganisms and their decimal reduction doses\(^{168}\) as compared with the analogous parameter $P(10,R)'$, i.e., the first decimal reduction dose, obtained from the model:

$$P(10,R)' = ((\log_{10} C_0 / \log_{10} (C_0 /10)) - 1) / S$$  \hspace{1cm} (126)

As can be seen, the agreement is quite satisfactory, taking into account the uncertainties associated with the evaluation of the [-SH] content per cell\(^{168}\) and mostly the approximation made for computation purposes (i.e., 1/4 of the [-SH] content per cell was assumed to be at the surface of the cells, on the basis of data reported by Bruce et al.\(^{168}\)). The high resistance of Micrococcus radiodurans could result from the exceptionally active repair system of this organism.\(^{169}\)

![Graph showing the relationship between the -SH level per cell and the first decimal reduction dose](image)

**FIGURE 11.** Relationship between the -SH level per cell and the first decimal reduction dose (comprehensive of the shoulder) of some bacteria, as reported by Bruce et al.,\(^{168}\) together with the first decimal reduction dose $P(10,R)'$ as predicted by the general model using $S' = 0.25*S$ = surface -SH content /cell. ($\circ$) = Micrococcus radiodurans; (●) = other microorganisms.

C. Chemical Inactivation

Microbial inactivation kinetics by chemical compounds can be expected to be described by a function of the form:

$$C_t = C_0^{1/(1+Q^t)}$$  \hspace{1cm} (127)

and

$$C_q = C_0^{1/(1+Q^S)}$$  \hspace{1cm} (128)

where $C_t$ is the concentration of organisms surviving $t$ (min) of treatment with a concentration, $q$, of lethal chemical compounds, $C_q$ is the concentration of organisms surviving a fixed time of
treatment with a concentration, q, of lethal compounds, \( Q = q^2 \), and S is a sensitivity parameter specific to the microorganism being treated. Survivor curves from Equation 127 are inevitably concave (Figure 6); those from Equation 128 are convex, sigmoid, or concave, according to the lethal concentration of the chemical compound and specific microbial sensitivity (Figure 10).

A general model for disinfection has not been developed, but several observations seem to suggest that a more accurate description of survivor curves could be obtained by an equation of the following type:

\[
C_{t,q} = C_0 \frac{1}{(1+Q^t)^t}
\]  

(129)

where:

\[
Q = \frac{(N*m/1000)^2 \times \exp(-2*Ed/(R*T))}{1000}
\]  

(130)

‘m’ being the molarity of the lethal chemical compound used, N is Avogadro's number, and Ed is a measure of the specific sensitivity of the microorganism tested to the chemical employed.

D. Observations

The general model developed seems to meet the fundamental requirements of a theory: it supplies a general mechanism of cell-environment interaction, based on random collisions between lighter molecules with sufficiently high energy and microorganisms; it describes most known experimental observations; it possesses a quite high predictive power; it suggests further improvements. In addition it can be used to describe microbial inactivation kinetics by heat, radiations, and chemical compounds, whatever the shape of the survivor curve; a single parameter (Ed), obtained simply from a single triplet of experimental data, can allow the description of inactivation kinetics, whatever the temperature and the water content of the medium; it supplies an exact explanation of the meaning of \( Q_{10} \) and \( z \); changes of \( Q_{10} \) and \( z \) values following changes of water content of the medium can be easily computed; it explains radiation inactivation kinetics on the basis of microbial content of a defined chemical group, viz., [-SH]; it supplies a provisional theory of chemical inactivation kinetics, suggesting (Equations 129 and 130) a promising approach to a comprehensive theory of the disinfection process. Further, microbial growth kinetics can be described on the same basis. More recently, the model has been used to fit human embryo and human tumor growth kinetics, linking the specific rate of growth to quantitatively defined molecules in the surface structure of metazoan cells.

E. Practical Consequences of the Model

As far as the process of thermal sterilization is concerned, high temperatures must be preferred, since by increasing treatment temperature the probability of tailing decreases and microbial inactivation kinetics approaches exponential type behavior. A high water content in the substrate to be sterilized must be preferred, since it implies eventually higher temperature of treatment. Sterilization by ionizing radiations cannot be expected to be straightforward, even at very high doses, because of the high content of [-SH] groups in microbial surface structures, which probably operate by scavenging radiation-induced radicals (indirect effect) whose targets are expected to be enzymatic complexes with the ability to repair intracellular (mostly nucleic acids) radiation damage. A great deal of work must be done on application of the model to the disinfection process.
VI. CONCLUDING REMARKS

Lazzaro Spallanzani (1729 - 1799) proved in 1765 that microorganisms were killed by heat. Louis Pasteur (1822 - 1895) improved our knowledge of this phenomenon and promoted its practical application. Afterwards, experimental observations of microbial inactivation by physical and chemical agents increased in quality and number. Perhaps unfortunately, a first-order kinetics theory of microbial death was developed, having the attractive features that it was linked to chemical kinetics, it was seemingly valid for all lethal agents, and it was easy to treat mathematically. Later, other theories were developed, including the target theory.

At present, in an attempt to recognize in the cobweb of experimental observations and associated theories, only the events really relevant to understanding facts and mechanisms, I am inclined to believe that:

1 - The theory of microbial exponential inactivation kinetics can be regarded as fundamentally wrong since it does not explain a large quantity of experimental data and it does not supply any insight into the mechanisms of microbial death.

2 - The most relevant contribution to the understanding of the mechanisms of microbial death was made by Ball and Olson, who suggested that "the cause of death must be outside the cell. It must be in the medium", and that at the microbial level statistical concepts break down and must be replaced "by energy considerations involving molecules in the discrete sense".

3 - The general theory based on a random process of collision between "discrete" units and microorganisms as a fundamental mechanism of cell-environment interaction is able to accommodate all relevant experimental observations, is amenable to modifications, and can be regarded as a useful approach to the understanding of the mechanisms of death.

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