A KINETIC MODEL FOR BACTERIAL SPORE GERMINATION*

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Germination is the process by which the bacterial spore is rendered metabolically active. Because germination involves a significant loss of mass,[1] it is easily measured and so has been given an accurate kinetic description.[2,3] Germination can be triggered by a single compound, L-alanine. The properties of this reaction are in large part responsible for a prevalent notion that a special enzyme(s) causes germination.[4,5] Very recently, evidence for an L-alanine-dependent "germination enzyme" has been found.[6]

The purpose of the present communication is to show that the kinetics of spore germination are readily explained on the basis of a simple enzyme model. Although such a model does not elucidate biochemical details of the process, it is useful in explaining how the spore can confine germination to a physiologically acceptable range of conditions. It also makes a number of predictions that can be used to test further its validity and/or refine our understanding of the process.

The Model: Simple Formulation.—Let us assume that germination occurs when the level of some substance, P, in the spore reaches a critical value, P_c, and that substance P is produced by a "germination enzyme" contained in the spore. Let us further assume that, to a first approximation, germination enzymes are distributed among the individual spores in a spore population according to a simple Poisson distribution. (Thus the spore population can be divided theoretically into subpopulations each characterized by having a particular, characteristic number, n, of enzymes per spore in every individual of the subpopulation.) This distribution will be called the *enzyme distribution*, to be distinguished from the *germination distribution*, and encountered shortly.

The rate of production of P can be written:

$$\frac{dP}{dt} = Kn, \qquad (1)$$

where n is the number of germination enzymes in each spore of any given subpopulation and K is a constant (which may be a function of other parameters in the system). The time it takes a spore to accumulate the critical amount of germination product, P_c, is then

$$t_c = a/n, \qquad \text{where } a \equiv P_c/K, \qquad (2)$$

it being assumed that n remains constant and that $P = 0$ at $t = 0$. Thus a *time* distribution can be derived from the (Poisson) *enzyme distribution* simply by plotting the latter as a function of the quantity a/n, rather than against n—as is customarily done. This time distribution is then the theoretical *germination distribution.* Figure 1 shows several theoretical *germination distributions*—in

which the fraction of the whole spore population germinated by time t is plotted as a function of t. (The curves in Fig. 1 have been plotted—by adjusting the parameter a—so that the time value corresponding to the average value, $\bar{n}$, of germination enzymes per spore has the same position on the abscissa in each case.)

The theoretical *germination distributions* derived on the simple assumptions above are seen to be step functions. This is because we have implicitly assumed

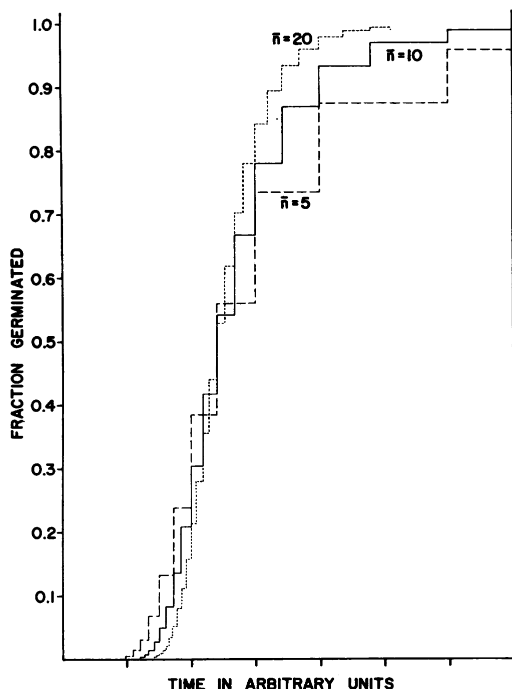

FIG. 1.—Theoretical germination distributions for spore populations with average numbers of germination units as shown. *Abscissa*, time in arbitrary units; *ordinate*, fraction of total population germinated by time t. See text for details.

that in any given spore subpopulation (characterized by every spore's having exactly n germination enzymes) all spores germinate precisely at the same time, a time determined by the value of n. In actuality this is an unreal assumption. It is far more likely that within each spore subpopulation there exists a distribution of germination times about some mean value. However, it is not possible to make an *a priori* theoretical assessment of this sort of spread. The effect of such a spread in germination times for the individual spore subpopulations would be twofold: (1) a tendency to obliterate the step function character to the over-all *germination distribution*, and (2) some broadening at the over-all *germination distribution.*

Theoretical versus Experimental Germination Distributions.—Figure 2 presents the experimental *germination distribution*, determined by the method of Vary and Halvorson for spores of *Bacillus cereus*,[6] and the theoretical distribution with $\bar{n} = 9$, which gives the best fit to experimental results. It can be seen that the theoretical curve is an excellent approximation of the experimental one, except

for a small fraction of spores that germinate somewhat earlier than would be expected. This exception may be the result of the above-mentioned broadening of the *germination distribution* caused by a spread in the germination time for any given spore subpopulation. However, there are alternative explanations for these "early germinators."

Two features of the experimental *germination distribution* have been used in fitting the theoretical curve to it. One is the general shape of the experimental

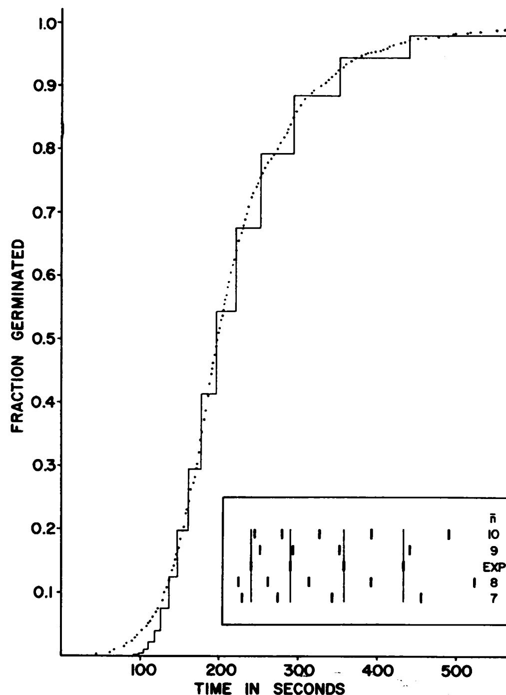

FIG. 2.—Experimental germination distribution (*solid circles*) and theoretical distribution with $\bar{n} = 9$. Abscissa and ordinate as in Fig. 2. The inset shows the positions where steps occur in theoretical germination for which $\bar{n} = 7$, 8, 9, and 10 when these are optimally fitted to the experimental distribution. Also shown are the approximate positions of the discernible "steps" in the experimental distribution.

distribution. A comparison of Figures 1 and 2 shows that theoretical distributions with $\bar{n}$ as high as 20 or as low as 5 are either too sharp or too broad, respectively, to accommodate the experimental data. The other is that the experimental distribution does show a slight residual "step-function" character, as predicted. (This can best be seen by viewing Fig. 2 diagonally, edge-on.) This feature then permits a "Vernier" sort of analysis of the data. The inset in Figure 2 shows the positions where steps occur in the theoretical curves with $\bar{n} = 7$, 8, 9, and 10 when these curves are each fitted optimally to the data. Also shown are the approximate positions of the discernible "steps" in the experimental distribution. It is clear that the curve with $\bar{n} = 9$ gives the best fit in a "Vernier" analysis. Thus we tentatively conclude that the average spore in the population contains about nine germination enzymes. (This average number may have to be raised slightly in view of a consideration to be discussed below.)

The Ungerminating Fraction: Expanded Model.—The above model predicts that a certain fraction of spores, those which according to the Poisson distribu-

tion must contain zero germination enzymes, will be incapable of germination, no matter how long germination time extends. When $\bar{n} = 9$, this "ungerminating fraction" is predicted to comprise approximately 0.01 per cent of the total population. Measurement shows the actual ungerminating fraction to be far greater than this—i.e., of the order of 1 per cent.[6] An explanation for this discrepancy is suggested by the characteristics of the ungerminating fraction—a fraction that *varies* as a function of germination conditions. For example, when spores are germinated in suboptimal concentrations of L-alanine, or at temperatures above optimal, or when certain strains of spores receive suboptimal heat activation, the ungerminating fraction increases dramatically; it can approach 100 per cent of the spore population.[3, 7, 8]

Let us now investigate the consequences of considering the substance P to be labile in such a way that we can rewrite equation (1) as

$$\frac{dP}{dt} = Kn - k_2P, \tag{3}$$

where k_2 is the rate constant for "breakdown" of P. In this situation, P approaches a steady-state level, P_s, with increasing time, this level being a linear function of n, the number of germination enzymes in the spore:

$$P = \frac{Kn}{k_2}(1 - e^{-k_2t}), \tag{4}$$

$$P_s = Kn/k_2. \tag{5}$$

If the germination level of P, P_c, is high enough, those subpopulations of the total spore population for which $P_c > P_s$ will be incapable of germinating. Also, the ungerminating fraction has now become *variable*, a function of various germination parameters. Accepting the above-mentioned experimental figure that approximately 1 per cent of spores do not germinate under optimal conditions, and assuming the value $\bar{n} = 9$ from the theoretical analysis of the germination distribution, we can calculate that possession of two to three germination enzymes or less places a spore in the ungerminating fraction.

Equation (3) modifies slightly the theoretical *germination distributions*. There is little point in going into the details of an expanded theory at this time. Suffice it to say that the greatest effect of assuming "breakdown" of P is seen for the small values of n—i.e, for large values of t_c—and that this modification of the *germination distribution* has to a first approximation the same effect as decreasing the value of $\bar{n}$. Thus, while the distribution with $\bar{n} = 9$ provides the best fit to the data on the simple theory, the expanded theory would require a distribution with $\bar{n} = 10$–11 for best fit.[9]

Let us now see how the expanded model can account for some of the characteristics of spore germination.

Heat activation: Many spore strains require heating (50–80°C) before they will germinate. These "activation" termperatures are themselves too high to cause actual germination. On the above model it is reasonable to assume that it is the germination enzymes that are being activated at the high tempera-

ture. The simplest assumption is that the activation of any one germination enzyme is an all-or-none process and is independent of the activation of any other enzyme in the same spore. This model then makes certain definite predictions about the phenomenon of heat activation, distinguishing it from the following reasonable alternative explanations: (1) The heat activation of any given spore is an all-or-none, one-step process. (2) The activation of a spore is a continuous process (so that one encounters a continuum, ranging from the inactive, dormant spore to the fully activated one).

Were heat activation of a spore all-or-none, suboptimal heating would divide the whole spore population into two subpopulations, one which did not germinate at all, and one which germinated with "optimal" kinetics. On the present model, suboptimal heat activation would affect the whole spore population, causing it to behave as a (slightly broadened) population with lowered value on $\bar{n}$. Thus, all spores would germinate more slowly, and the ungerminating fraction would rise in accordance with the fraction of the population that contained two to three or less *active* germination enzymes. The present model cannot be distinguished from the continuum model on this basis, but these two can be distinguished in one or both of the following ways: The present model predicts the existence of a *"maximum germination time"*; that is, a maximum time after which essentially no more spores can germinate. This time is determined by the average time it takes spores with three to four (active) germination enzymes to germinate—since, as mentioned above, those with two to three germination enzymes or less are probably incapable of germinating at all. Certain forms of a "continuum hypothesis" predict no upper limit to germination times. The present model also predicts that suboptimally heat-activated spores should show the typical "steps" in their germination distributions (Fig. 2), which is impossible on a continuum model.

Although this last prediction has not yet been tested, all the other predictions of the present model with regard to heat activation have been verified to a first approximation.[10]

Effect of germination temperature: At suboptimal temperatures the germination distribution resembles that obtained at optimal temperatures, except that the times involved are longer. However, at above-optimal temperatures the germination distribution is rather different. For one thing, the over-all distribution becomes broadened; yet the time taken for some of these spores to germinate continues to shorten as temperature increases. For another, the ungerminating fraction increases with increasing temperature, approaching 100 per cent of the spore population.[4, 7, 8] Although detailed studies of this phenomenon have yet to be done, the present model can account for the existing crude data in the following simple way: Assume that the rate constants controlling the production and "breakdown" of P are different functions of temperature, i.e., k_2 increases with temperature faster than K does. The effect of increased germination temperature on the *initial* rate of formation of P will be to increase it (since this quantity depends only upon K and n), but the steady-state level of P, P_s (which depends upon the ratio K/k_2—equation (5)), will drop as temperature increases. Those spores that contain a relatively large number

of germination enzymes will germinate more and more rapidly with increasing temperature, but fewer spores will find the number of germination enzymes they contain sufficient to generate the critical level P_c. Therefore, an increase in the ungerminating fraction will occur, as will some (relative) increase in time taken to reach that level for many of those spores that can do so—i.e., those whose number of germination enzymes is close to the minimum now necessary to generate P_c—thus producing the broadened germination distribution.

Germination as a function of L-alanine concentration: As the concentration of L-alanine in which the spores germinate is decreased from some optimal value (above which no change occurs in the kinetics), two things are seen: The overall germination distribution shifts toward larger time values and lower $\bar{n}$, and the ungerminating fraction simultaneously increases.[4, 7, 8] This behavior is very like that seen when heat-activation time is decreased. Thus, L-alanine may be controlling the number of "active" germination enzymes in the cell. Alternatively, L-alanine may be the substrate for the germination enzyme and so limits the rate of P production when it is present at levels below "saturation." The fit of theory to data here is at present semiquantitative,[9] but we are now attempting a more precise test of the theory's predictions, which should distinguish between the above two alternative roles of L-alanine.

Discussion and Summary.—The above model provides a good kinetic description of bacterial spore germination. The basic features of the model are that germination occurs when the level of a certain "germination substance," P, attains some critical value. The "germination substance" in turn is somehow produced by a "germination enzyme," E. The model provides a close fit to the experimental *germination distribution* if we assume there are an average of nine "germination enzymes" per spore. By making the assumption that the "germination substance" is labile, it is possible to account for a previously puzzling characteristic of germination—i.e., that under various suboptimal conditions the spore population divides into two subpopulations (that vary in relative amount according to germination conditions), one that germinates, and one that does not no matter how long germination time extends. In this way it has been possible to rationalize the characteristics of germination at high temperatures or low L-alanine concentrations, as well as the kinetics of germination of spores given suboptimal heat activation. The model in addition predicts a so-called "maximum germination time," a time after which essentially no more spores will germinate. This time limit should be independent of degree of heat activation, and of concentration of L-alanine, but is, of course, a function of germination temperature.

Since we have no *a priori* certainty that this particular scheme is a unique explanation, it is important to assess how much evidence can be accumulated for the existence and nature of the postulated components and their relationships.

The strongest evidence regarding the mechanisms involved in germination is the residual "step-function" character to the experimental *germination distribution.* This is proof that the total spore population is divided into functional distinguishable subpopulations. Although one would need more evidence to

state the point with certainty, it does seem as though these subpopulations germinate at (mean) times that vary as the ratios of the reciprocals of small numbers. We can see no reasonable explanation for such a phenomenon other than that a spore contains a small number of "units" that are responsible for germination, and that some rate-limiting reaction in germination proceeds at a rate directly proportional to the number of such "units" in a spore. However, it is not necessary that such a "unit" be actually an enzyme.

We do not know the nature of the hypothetical P substance. It is possible that it is produced from L-alanine, but it is equally possible that it is not—L-alanine could be acting merely as an activator, an allosteric effector, of the enzyme producing P. These alternative roles for L-alanine are probably distinguishable: if L-alanine were to control the number of *active* germination "enzymes"—i.e., if it were an allosteric effector—then at suboptimal L-alanine concentrations nonintegral values of active enzymes per spore would be possible, and so the *germination distribution* under such conditions would lose its "step-function" character. If L-alanine were a substrate, it could not effect the number of active germination enzymes per spore, and so the "step-function" character of the *germination distribution* would be retained at suboptimal L-alanine concentrations. Also, it seems possible to test the prediction of the model that the spore can accumulate a subcritical level of P and not germinate, as well as the prediction that P is labile.

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