

A CLASS OF STOCHASTIC MODELS OF RESPONSE AFTER INFECTION IN THE ABSENCE OF DEFENSE MECHANISM

PREM S. PURI

UNIVERSITY OF CALIFORNIA, BERKELEY

1. Introduction

In experimental studies of quantal response to infection three factors of essential interest are (i) the microorganisms, henceforth called particles, (ii) the host, and (iii) the type of response. The particles are *self-reproducing* entities, usually bacteria or viruses, which are inoculated with differing intensities into groups of hosts, such as animals, egg membranes, or tissue cultures. The response which the particles elicit from the host during the course of time may be death, the development of a tumor or a local lesion, or some other detectable symptom. The phenomenon of particular interest here is observed in the following.

At time $t = 0$ a certain dose of a suspension of specified virulent particles is injected into each of n experimental hosts. If $n(t)$ denotes the number of hosts not responding by time t , the plot against t of either $n(t)$ itself or of the proportion $q(t) = n(t)/n$ is known as the time dependent response curve. If the response is the death of the host, the curve is also called the survival curve. As is well known, the response curves differ with the dose and with the type of particles injected. Generally, the larger the injected dose, the sooner the host responds, that is, the steeper is the decrease in $q(t)$. The purpose of the present paper is to examine a class of stochastic models for the time dependent response curves with the hope that some of these will be useful in certain situations to be discussed later. It is to be emphasized, however, that we will not concern ourselves here with those situations where the response causing agent is not a self-reproducing entity. The reader may find discussion of these elsewhere [7].

Most of the earlier mathematical models related to the time dependent response curves treat the case where the response is the death of the host, although this is by no means a restriction of their applicability to other cases. A brief reference to these is desirable here.

Wiggins [20] has studied a stochastic model of survival of an animal injected with a certain dose of virulent bacteria. He assumes that the body of the host is divided into three regions R_1 , R_2 , and R_3 , with the following properties. If a bacterium enters R_3 , it is rendered noninfectious, and nothing happens to the

This research has been prepared with partial support from USPHS Grant GM-10525.

host. If, on the other hand, it enters R_1 , the bacterium remains therein for a time so short that practically no cell division occurs. Thus, the birth rate in R_1 is assumed to be zero. However, the bacteria are allowed to migrate from R_1 to R_2 . Finally, the region R_2 is considered to be a sensitive region which acts as a "trap" in the sense that, once a bacterium reaches R_2 from R_1 , it cannot return. The bacteria in R_2 undergo a simple birth and death process; the fundamental assumption is that when the population in R_2 reaches a certain fixed number N_0 for the first time, the animal dies. The number N_0 may be called the lethal threshold. The same hypothesis of a fixed lethal threshold seems to underlie the experimental work of Meynell and Meynell [12]. In fact, the latter authors produced experimental results tending to support this hypothesis. The theory of Wiggins, while interesting, did not produce formulas that could be compared with the observations.

In a more recent paper, Gart [8] has considered two stochastic models, which he calls (i) the individual action model and (ii) the collective action model. Let m denote the number of particles inoculated into the host at time $t = 0$, the growth of each particle being governed independently by some growth process. Then the total number of particles present in the host at time t is given by $X(t) = \sum_{j=1}^m X_j(t)$, where $X_j(t)$ is the contribution from the j th inoculated particle. The key assumption of model (i) is that a positive response is observable at time t whenever, for at least some $j = 1, 2, \dots, m$, and for some $t' \leq t$, $X_j(t') > N_0$, where N_0 is the lethal threshold. On the other hand, the assumption underlying model (ii) is that a response is observable at any time t , whenever for some $t' \leq t$, $X(t') > N_0$. The hypothesis involved in model (i) is similar to the hypothesis of independent action proposed by Meynell and Stocker [11], as noted by Gart himself. Furthermore, an extended model used by Meynell and Meynell [12], although completely deterministic in character, has some resemblance to Gart's model (ii). In another recent paper, Williams [21] considers the same problem with only partial success, again under the hypothesis of existence of a lethal threshold.

We note that the hypothesis of existence of a fixed lethal threshold is a common feature of all the models considered so far. In the present paper, however, this hypothesis is abandoned, for two reasons. First, it is not likely to be strictly correct; and second, it is mathematically intractable because of the involvement of the first passage time problem. We adopt an alternative hypothesis suggested by LeCam [9], namely, that the connection between the number $X(t)$ of particles in a host at time t and the host's response is indeterministic in character. In other words, it is assumed that the value of $X(t)$, or of a random variable whose distribution is dependent on the process $\{X(t)\}$, determines not the presence or absence of response, but only the probability of response of the host.

In the next two sections, we shall discuss in detail two stochastic models, A and B (model B being a special case of model A), based on this indeterministic hypothesis. In section 4, we shall introduce a class \mathcal{C} of stochastic models, which will include A and B as elements. In section 5, the theoretical distribution

under model B of the number of virulent bacteria at the time of death of the host is obtained and is compared with actual observations. Later, in section 6, we attempt to fit a typical model of class C, with certain appropriate modifications, to response curves based upon data on guinea pigs injected with varying doses of tubercle bacilli, the response in this case being death. Finally, in section 7, consideration of the biological mechanisms underlying the response to infection leads to suggestions for further research to improve models.

2. Model A

2.1. *Underlying assumptions.* We assume the origin on the time scale to be the moment when we start the experiment by giving the host a certain dose of particles. Let $X(t)$ be the number of live particles in the body of the host at time t , with $X(0)$ equal to m , the number of live particles injected at the start of the experiment. We can treat m as a fixed number or as a random variable, usually assumed to be Poisson distributed. To begin with, m will be treated as a constant.

We assume that the particle growth is governed by a simple homogeneous birth and death process with birth and death rates λ and μ , respectively, both assumed constant over time. The basic assumptions underlying the following developments are that all the events that might occur during $(t, t + \tau)$ to a particle alive at t are independent (i) of the events occurring to the other particles, (ii) of the events that occurred to this particle in the past, and (iii) of the state (response or nonresponse state) of the host. With these assumptions $\{X(t)\}$ is a Markov process. Another of the key assumptions we make here is that all the hosts are uniform in their susceptibility to the response causing mechanism. Let $a + f(X(t), Y(t)|t)$ be the risk function for the response of the host, so that

$$(2.1) \quad P\{\text{host responds during } (t, t + \tau) | X(t) = x, Y(t) = y, Z(t) = 1\} \\ = [a + f(x, y)]\tau + o(\tau),$$

where $a \geq 0$, $x = 0, 1, 2, \dots$, and

$$(2.2) \quad Y(t) = \int_0^t X(\tau) d\tau, \\ Z(t) = \begin{cases} 1 & \text{if there is no response by time } t, \\ 0 & \text{otherwise.} \end{cases}$$

A practical realization of the above assumption concerning the risk function for host response might be found in the case where the survival of an animal inoculated with virulent bacteria is in question. Here, the function $f(X(t), Y(t)|t)$ is nonnegative, nondecreasing in both of its arguments, with $f(0, 0|t) \equiv 0$, and represents the risk of death solely due to the bacterial invasion. The component a , on the other hand, represents the constant risk of death due to other causes and may be taken as zero in cases where the response is other than

death and/or where no other causes are operating. The function $Y(t)$ is contemplated as a measure of the amount of toxin produced by the live bacteria during the interval $(0, t)$, assuming, of course, that the rate of toxin excretion is constant per bacterium per unit time. The toxin excreted by certain types of bacteria is well known to be a factor contributing to the disease process and thereby to the ultimate death of the host. It therefore seems natural to believe that the risk function for the death of the host should depend not only on $X(t)$ but also on the total amount of toxin that has been produced by time t .

As a first attempt, we shall assume in model A, for the sake of mathematical simplicity, that

$$(2.3) \quad f(X(t), Y(t)|t) = bX(t) + cY(t),$$

with $b \geq 0$, $c \geq 0$. Then the natural course is to study the joint distribution of the process $\{X(t), Y(t), Z(t)\}$ which is of the Markovian type. The distribution of the process $\{X(t), Y(t)\}$ has already been studied extensively, along with the limiting behavior of some of the related processes [18], [19]. Let $\varphi(u, v, w; t)$, or φ for short, denote the joint characteristic function (ch. f. for short) of the process $\{X(t), Y(t), Z(t)\}$ defined as

$$(2.4) \quad \varphi(u, v, w; t) = E\{e^{iuX(t)+ivY(t)+iwZ(t)}|X(0) = m\},$$

where $Y(0) = 0$ and $Z(0) = 1$. We derive in the next section a differential equation satisfied by the ch. f. φ .

2.2. *Differential equation for the ch. f. $\varphi(u, v, w; t)$.* Let $(\delta X, \delta Y, \delta Z)$ be the element of change in $(X(t), Y(t), Z(t))$ during an infinitesimal interval of time $(t, t + \tau)$, in which at most a single event may occur. Then, given $X(0) = m$, $Y(0) = 0$ and $Z(0) = 1$,

$$(2.5) \quad \varphi(u, v, w; t + \tau) = E\{\exp[iuX(t) + ivY(t) + iwZ(t)] \\ E[\exp(iu\delta X + iv\delta Y + iw\delta Z)|X(t), Y(t), Z(t)]\}.$$

We proceed first to derive the expression for

$$(2.6) \quad E[\exp(iu\delta X + iv\delta Y + iw\delta Z)|X(t) = n, Y(t) = y, Z(t) = z]$$

separately for $z = 1$ and 0 , under the assumptions of the previous section. First of all, we note that, given $X(t) = n$, $Y(t) = y$ and $Z(t) = 1$ and that no event affecting the n particles occurs during the interval $(t, t + s)$, the probability that the host does not respond during $(t, t + s)$ is given by

$$(2.7) \quad \exp\left\{-\left(a + bn + cy\right)s - \frac{1}{2}cns^2\right\}.$$

Let the event, if it occurs, occur at time $t + s$, where $0 < s < \tau$, and let the conditional probability density function (p.d.f., for short) of s , given that $Z(t) = 1$ and that a birth occurs during $(t, t + \tau)$, be $f_{B,1}(s)$. The probability density function $f_{D,1}(s)$ is correspondingly defined given that $Z(t) = 1$ and a death occurs during $(t, t + \tau)$. Similarly, let $f_{B,0}(s)$ and $f_{D,0}(s)$ be the corresponding p.d.f.'s given $Z(t) = 0$. Then it is easy to show that

$$\begin{aligned}
 f_{B,1}(s) &= \frac{\exp\left\{(\mu + \lambda + b + c\tau)s - \frac{1}{2}cs^2\right\}}{\int_0^\tau \exp\left\{(\mu + \lambda + b + c\tau)s - \frac{1}{2}cs^2\right\} ds}, \\
 f_{D,1}(s) &= \frac{\exp\left\{-(\mu + \lambda + b + c\tau)s + \frac{1}{2}cs^2\right\}}{\int_0^\tau \exp\left\{-(\lambda + \mu + b + c\tau)s + \frac{1}{2}cs^2\right\} ds}, \\
 f_{B,0}(s) &= \frac{(\lambda + \mu) \exp\{(\lambda + \mu)s\}}{\exp\{(\lambda + \mu)\tau\} - 1}, \\
 f_{D,0}(s) &= \frac{(\lambda + \mu) \exp\{-(\lambda + \mu)s\}}{1 - \exp\{-(\lambda + \mu)\tau\}},
 \end{aligned}
 \tag{2.8}$$

where $0 \leq s \leq \tau$.

Given $Z(t) = 1$, let the probability that no event occurs in $(t, t + \tau)$, given $X(t) = n, Y(t) = y$, be denoted by $P_{0,n,1}$, and let $P_{\lambda,n,1}, P_{\mu,n,1}$ and $P_{r,n,1}$ denote the probabilities under the same given conditions, of a particle birth, of a particle death, and of the response of the host, respectively; then

$$\begin{aligned}
 P_{0,n,1} &= \exp\left\{-(\lambda + \mu)n\tau - (a + bn + cy)\tau - \frac{1}{2}nc\tau^2\right\}, \\
 P_{\lambda,n,1} &= n\lambda \exp\left\{-(\lambda + \mu)(n + 1)\tau - (a + bn + b + cy)\tau \right. \\
 &\quad \left. - \frac{1}{2}(n + 1)c\tau^2\right\} \int_0^\tau \exp\left\{(\lambda + \mu + b + c\tau)s - \frac{1}{2}cs^2\right\} ds, \\
 P_{\mu,n,1} &= n\mu \exp\left\{-(\lambda + \mu)(n - 1)\tau - (a + bn - b + cy)\tau \right. \\
 &\quad \left. - \frac{1}{2}(n - 1)c\tau^2\right\} \int_0^\tau \exp\left\{-(\lambda + \mu + b + c\tau)s + \frac{1}{2}cs^2\right\} ds, \\
 P_{r,n,1} &= \exp\{-(\lambda + \mu)n\tau\} \left\{1 - \exp\left[-(a + bn + cy)\tau - \frac{1}{2}nc\tau^2\right]\right\}.
 \end{aligned}
 \tag{2.9}$$

Similarly, given $Z(t) = 0$, the corresponding expressions for $P_{0,n,0}, P_{\lambda,n,0}$, and $P_{\mu,n,0}$ are

$$\begin{aligned}
 P_{0,n,0} &= \exp\{-(\lambda + \mu)n\tau\}, \\
 P_{\lambda,n,0} &= \frac{n\lambda}{\lambda + \mu} \exp\{-(\lambda + \mu)(n + 1)\tau\} \{\exp[(\lambda + \mu)\tau] - 1\}, \\
 P_{\mu,n,0} &= \frac{n\mu}{\lambda + \mu} \exp\{-(\lambda + \mu)(n - 1)\tau\} \{1 - \exp[-(\lambda + \mu)\tau]\}.
 \end{aligned}
 \tag{2.10}$$

Thus we have

$$\begin{aligned}
 (2.11) \quad & E\{\exp(iu\delta X + iv\delta Y + iw\delta Z)|X(t) = n, Y(t) = y, Z(t) = 1\} \\
 &= P_{0,n,1} e^{ivn\tau} + P_{\lambda,n,1} \int_0^\tau \exp\{iu + iv[ns + (n+1)(\tau-s)]\} f_{B,1}(s) ds \\
 &\quad + P_{\mu,n,1} \int_0^\tau \exp\{-iu + iv[ns + (n-1)(\tau-s)]\} f_{D,1}(s) ds \\
 &\quad + P_{r,n,1} \exp\{ivn\tau - iw\} + o(\tau),
 \end{aligned}$$

and

$$\begin{aligned}
 (2.12) \quad & E\{\exp(iu\delta X + iv\delta Y + iw\delta Z)|X(t) = n, Y(t) = y, Z(t) = 0\} \\
 &= E\{\exp(iu\delta X + iv\delta Y)|X(t) = n\} \\
 &= P_{0,n,0} e^{ivn\tau} + P_{\lambda,n,0} \int_0^\tau \exp\{iu + iv[ns + (n+1)(\tau-s)]\} f_{B,0}(s) ds \\
 &\quad + P_{\mu,n,0} \int_0^\tau \exp\{-iu + iv[ns + (n-1)(\tau-s)]\} f_{D,0}(s) ds + o(\tau),
 \end{aligned}$$

where $o(\tau)$ corresponds to the contribution due to the occurrence of more than one event.

Combining (2.11) and (2.12), we may write the general expression for (2.6), for $Z(t) = z$, as

$$\begin{aligned}
 (2.13) \quad & zE\{\exp(iu\delta X + iv\delta Y + iw\delta Z)|X(t) = n, Y(t) = y, Z(t) = 1\} \\
 &+ (1-z)E\{\exp(iu\delta X + iv\delta Y + iw\delta Z)|X(t) = n, Y(t) = y, Z(t) = 0\}.
 \end{aligned}$$

Let the first partial derivatives of $\varphi(u, v, w; t)$ with respect to u, v, w and t be denoted by $\varphi_u, \varphi_v, \varphi_w$ and φ_t , respectively, and likewise the second partial derivatives by $\varphi_{uw}, \varphi_{vw}, \dots$, and so on. These derivatives exist, since $X(t)$ and $Y(t)$ are nonnegative and all the moments of $X(t)$, and consequently those of $Y(t)$, exist. Substituting the expression for (2.13) into (2.5), subtracting $\varphi(u, v, w; t)$ from both sides, dividing both sides by τ , and finally letting $\tau \rightarrow 0$, we have

$$\begin{aligned}
 (2.14) \quad \varphi_t &= E(\exp[iuX(t) + ivY(t) + iwZ(t)] \cdot \{ivX(t) - (\lambda + \mu)X(t) \\
 &\quad - Z(t)[a + bX(t) + cY(t)] + \mu X(t) e^{-iu} \\
 &\quad + \lambda X(t) e^{-iu} + e^{-iw} [a + bX(t) + cY(t)]Z(t)\}),
 \end{aligned}$$

which may be written as the differential equation

$$\begin{aligned}
 (2.15) \quad \varphi_t &= \{v + i[(\lambda + \mu) - \lambda e^{iu} - \mu e^{-iu}]\} \varphi_u \\
 &\quad + (1 - e^{-iw})(ia\varphi_w + b\varphi_{uw} + c\varphi_{vw}),
 \end{aligned}$$

with the initial condition

$$(2.16) \quad \varphi(u, v, w; 0) = \exp\{ium + iw\}.$$

2.3. *Some remarks on the solution of the differential equation for φ .* Unfortunately, equation (2.15) lends itself to solution only in special cases, for instance when either $\mu = 0$ or $\lambda = 0$. However, we are not interested in the complete solution for φ . What we would like to obtain is the expression for $Q(t|m)$, the

probability of no response until time t , which can be expressed in a variety of ways, such as

$$(2.17) \quad Q(t|m) = P\{Z(t) = 1\} = E[Z(t)] = \frac{1}{i} \varphi_w(0, 0, 0; t).$$

Differentiating (2.15) with respect to w , and putting $u = v = w = 0$, we find that

$$(2.18) \quad \frac{dQ(t|m)}{dt} = -aE[Z(t)] - bE[Z(t)X(t)] - cE[Z(t)Y(t)].$$

This equation does not help us in obtaining the solution for $Q(t|m)$, but it does demonstrate how the rate of decrease of $Q(t|m)$ depends upon the various elements given on the right side of (2.18).

Taking another approach we write the identity

$$(2.19) \quad \varphi(u, v, w; t) \equiv e^{iw} g_1(u, v; t) + g_0(u, v; t),$$

where for $j = 0, 1$

$$(2.20) \quad g_j(u, v; t) = \int_0^\infty \sum_{k=0}^\infty e^{iuk+ivv} d_y P\{X(t) = k, Y(t) \leq y, Z(t) = j\}.$$

Since $Q(t|m) = g_1(0, 0; t)$, we need only to solve for the function $g_1(u, v; t)$. Substituting the expression on the right side of (2.19) in equation (2.15) and equating the coefficients of e^{iw} and terms independent of w on both sides, we find the differential equations for $g_1(u, v; t)$ and $g_0(u, v; t)$ to be

$$(2.21) \quad g_{1t} = \{v + i[(\lambda + \mu + b) - \lambda e^{iu} - \mu e^{-iu}]\} g_{1u} + icg_{1v} - ag_1,$$

$$(2.22) \quad g_{0t} = \{v + i[(\lambda + \mu) - \lambda e^{iu} - \mu e^{-iu}]\} g_{0u} + (ag_1 - ibg_{1u} - icg_{1v}),$$

with the side conditions $g_1(u, v; 0) = e^{imu}$ and $g_0(u, v; 0) = 0$.

Although equation (2.21) appears much simpler than (2.15), unfortunately, this also lends itself to solution only in special cases, such as with either $\mu = 0$ or $\lambda = 0$. Without going into the details of solving for these special cases, we give here, for the sake of completeness, the corresponding expressions for $Q(t|m)$ as

$$(2.23) \quad Q(t|m, \mu = 0) = e^{-at} \left\{ \exp \left[(b + \lambda)t + \frac{ct^2}{2} \right] - \lambda \int_0^t \exp \left[(b + \lambda + c\tau)\tau - \frac{c\tau^2}{2} \right] d\tau \right\}^{-m},$$

and

$$(2.24) \quad Q(t|m, \lambda = 0) = e^{-at} \left\{ \exp \left[-(\mu + b)t - \frac{ct^2}{2} \right] + \mu \int_0^t \exp \left[-(b + \mu + c\tau)\tau + \frac{c\tau^2}{2} \right] d\tau \right\}^m.$$

When $\lambda, \mu > 0$, one may derive an approximate expression for $Q(t|m)$, valid only for sufficiently small values of the parameter c (see Puri [17]).

Finally, equation (2.21) can be completely solved if we assume that $c = 0$, or

equivalently that $Y(t)$ plays no role in bringing about the response. This modified model (model A with $c = 0$, so that $f[X(t)|t] = bX(t)$), henceforth called model B, will be discussed in the next section.

3. Model B: theoretical time dependent response curves

Under model B, let

$$(3.1) \quad G_{x,1}(s; t) = \sum_{x=0}^{\infty} s^x P_{x,1}(t), \quad |s| \leq 1,$$

where for $x = 0, 1, 2, \dots$,

$$(3.2) \quad P_{x,1}(t) = P\{X(t) = x, Z(t) = 1 | Z(0) = 1, X(0) = m\}.$$

The partial differential equation for the probability generating function (p.g.f., for short) $G_{x,1}(s; t)$ under model B can be derived by the standard procedure. It is easier, however, to obtain it by taking $c = 0$, $v = 0$ and $e^{iu} = s$ in equation (2.20) with $j = 1$, and again in equation (2.21), wherefrom we find that $G_{x,1}(s; t) = g_1(-i \log s, 0; t)$ and that this satisfies the equation

$$(3.3) \quad G_t = [\lambda s^2 - (\lambda + \mu + b)s + \mu]G_s - aG,$$

subject to the initial condition

$$(3.4) \quad G_{x,1}(s; 0) = s^m,$$

where G_t and G_s denote, as before, the corresponding first order partial derivatives of G .

From the auxiliary equations associated with (3.3) we have

$$(3.5) \quad \frac{ds}{dt} = -\lambda s^2 + (\lambda + \mu + b)s - \mu,$$

whence

$$(3.6) \quad C = \left[\frac{(s - r_1)}{(s - r_2)} \right] \exp[\lambda(r_1 - r_2)t],$$

where C is the constant of integration and r_1 and r_2 are the roots of

$$(3.7) \quad \lambda y^2 - (\lambda + \mu + b)y + \mu = 0;$$

that is, they are, with positive and negative signs, respectively,

$$(3.8) \quad \frac{1}{2\lambda} \{(\lambda + \mu + b) \pm [(\lambda + \mu + b)^2 - 4\mu\lambda]^{1/2}\}.$$

Again from the auxiliary equations,

$$(3.9) \quad G_{x,1}(s; t) = -at + \text{constant} = -at + h(C),$$

where h is an arbitrary function. Using the initial condition (3.4), we obtain after some manipulation

$$(3.10) \quad G_{x,1}(s; t|m) = e^{-at} \left[\frac{(r_1 - r_2 C)}{(1 - C)} \right]^m$$

or, on substituting for C ,

$$(3.11) \quad G_{X,1}(s; t|m) = e^{-at} \left\{ \frac{r_1(s - r_2) - r_2(s - r_1) \exp[\lambda(r_1 - r_2)t]}{(s - r_2) - (s - r_1) \exp[\lambda(r_1 - r_2)t]} \right\}^m.$$

Since $(1 - r_1)(1 - r_2) = -b/\lambda < 0$, it is easy to see that

$$(3.12) \quad 0 < r_2 < 1 < r_1.$$

If $m = 1$, the p.g.f. (3.11) can be written as

$$(3.13) \quad G_{X,1}(s; t|m = 1) = e^{-at} \left[r_1 r_2 \alpha + (1 - r_1 \alpha)(1 - r_2 \alpha) s \sum_{x=0}^{\infty} \alpha^x s^x \right],$$

where

$$(3.14) \quad \alpha = \{1 - \exp[-\lambda(r_1 - r_2)t]\} \{r_1 - r_2 \exp[-\lambda(r_1 - r_2)t]\}^{-1}.$$

From (3.13), it follows that for $m = 1$,

$$(3.15) \quad P_{x,1}(t) = \begin{cases} (1 - r_1 \alpha)(1 - r_2 \alpha) \alpha^{x-1} \exp(-at) & \text{for } x \geq 1, \\ r_1 r_2 \alpha \exp(-at) & \text{for } x = 0. \end{cases}$$

The probability of no response until time t is given by

$$(3.16) \quad Q(t|m) = G_{X,1}(1; t|m).$$

Also, the median effective dose, which is just enough to get response, during a fixed time interval $(0, t)$, from 50 per cent (on the average) of the hosts, is given by the solution for m of the equation

$$(3.17) \quad G_{X,1}(1; t|m) = \frac{1}{2}.$$

Let us now consider m , the number of particles which initiate the biological process at $t = 0$, to be a random variable having a Poisson distribution with parameter β . Then, taking the expectation of (3.11) over m and substituting $s = 1$, we have the probability of no response as

$$(3.18) \quad Q(t|\beta) = \exp \left\{ -at - \beta(1 - r_2)(r_1 - 1) \left[\frac{1 - \exp\{-\lambda(r_1 - r_2)t\}}{(1 - r_2) \exp\{-\lambda(r_1 - r_2)t\} + (r_1 - 1)} \right] \right\}.$$

Now if the random variable T denotes the waiting time for response, usually known as the incubation period, then its density function $p_\beta(t)$ is given by

$$(3.19) \quad p_\beta(t) = -\frac{d}{dt} Q(t|\beta).$$

In figure 1, this density function is graphed for $a = 0.01$, $\lambda = 6$, $\mu = 5$, $b = 10^{-6}$ and for various typical values of β . It is to be noticed that as β increases, the distribution contracts and moves leftwards, as one would expect. Furthermore, there is a striking initial rise and subsequent falling off of the distributions, as also observed by Williams [21] through investigations based on the threshold hypothesis. These observations suggest that in general as β increases both the expectation and variance of T tend to decrease.

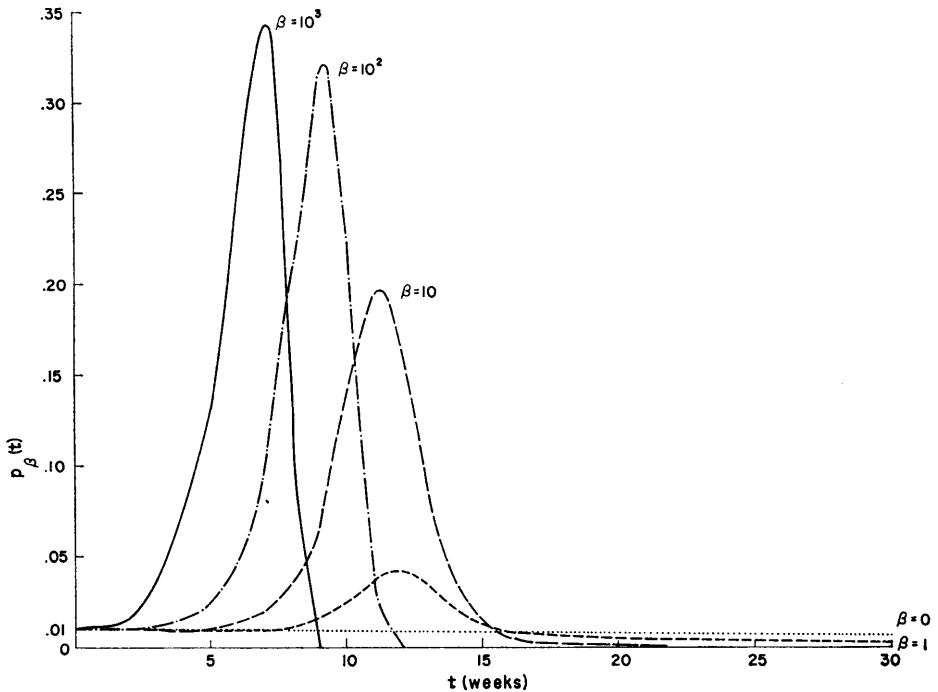


FIGURE 1

Theoretical curves for the density function $p_\beta(t)$ for the incubation period, for various values of β , with $a = 0.01$, $\lambda = 6$, $\mu = 5$, $b = 10^{-6}$.

The mean and variance of T , if they are finite, are given by

$$(3.20) \quad E(T) = \int_0^\infty Q(t|\beta) dt,$$

$$\sigma^2 = 2 \int_0^\infty tQ(t|\beta) dt - \left[\int_0^\infty Q(t|\beta) dt \right]^2.$$

Unfortunately, it is not possible to express these in closed form. Instead we consider the median incubation period M_T , which is given by the solution for t of the equation $Q(t|\beta) = 1/2$, or equivalently, of

$$(3.21) \quad \frac{r_1 - r_2}{(1 - r_2) \exp[-\lambda(r_1 - r_2)t] + (r_1 - 1)} = \frac{\log 2 - at + \beta(r_1 - 1)}{\beta(r_1 - 1)}.$$

With $a = 0$, as will be the case if the response can be initiated only by the injected particles, we note that $P\{T < \infty\} = 1 - \exp[-\beta(1 - r_2)]$. However, neglecting $\exp[-\beta(1 - r_2)]$ for large β , we have from (3.21) with $a = 0$,

$$(3.22) \quad M_T = -\frac{1}{\lambda(r_1 - r_2)} \log \left[1 - \frac{\lambda(r_1 - r_2) \log 2}{b\beta + \lambda(1 - r_2) \log 2} \right],$$

which for large $b\beta$ can be approximated to yield

$$(3.23) \quad \frac{1}{M_T} \approx \lambda(1 - r_2) + \frac{b\beta}{\log 2}.$$

Thus, for model B, for large $b\beta$, the inverse of the median incubation period is approximately linearly related to β , the average number of particles injected at $t = 0$. Note, however, that since b is usually small this approximation requires extremely large β .

REMARK. In model B, the risk function for response, namely, $a + bX(t)$, suggests that, while the constant a corresponds to the risk due to causes unrelated to the particles, the risk of response due to particles is b for a single particle and is additive over the particles present at any moment. While this additivity brings about independence of action among the m particles starting at $t = 0$ (this is similar to the hypothesis of independent action proposed by Meynell and Stocker [11]), the additivity between a and $bX(t)$ essentially implies independence of action between the causes due to the particles' invasion and the ones unrelated to this one.

4. A class \mathcal{C} of stochastic models

We propose here a class \mathcal{C} which consists of stochastic models that are essentially based on the hypothesis suggested by Meynell and Stocker [11], of independent action of the particles. In the present case this independence is introduced into the models through the risk function $a + f(\cdot|t)$; here a is, in general, a constant, but may depend upon β , the initial average dose injected, and/or may possibly be taken as a function of time; the function $f(\cdot|t)$ is a non-negative and nondecreasing function of the random variable $X(t)$ and/or some other related random variables. For instance, we had in model B, $f(\cdot|t) = bX(t)$, while in model A, $f(\cdot|t) = bX(t) + cY(t)$, with $Y(t) = \int_0^t X(\tau) d\tau$. Later, we shall give a few more examples of the function f .

Let $Q(t|m)$ be, as before, the probability of no response until time t , given that m particles were injected at $t = 0$. For a given risk function $a + f(\cdot|t)$ let $\psi_r(t)$ denote the probability $Q(t|m)$ evaluated at $m = 1$ and $a = 0$, so that we have

$$(4.1) \quad Q(t|m = 1) = e^{-at} \psi_r(t).$$

We may now define the class \mathcal{C} as consisting of those models satisfying the assumptions of section 2.1 except that $\lambda(t)$ and $\mu(t)$ may be functions of time and that the risk function $f(\cdot|t)$ may be any function such that

$$(4.2) \quad Q(t|m) = e^{-at} [\psi_r(t)]^m; \quad m = 1, 2, \dots$$

It is evident from (3.11) that the model B, with $f(\cdot|t) = bX(t)$, belongs to \mathcal{C} . The expression for $\psi_r(t)$ in this case is in the square brackets on the right side of (3.11) with s replaced by one. The relation (4.2) clearly implies independence

among the m initial particles in their response causing action. This in turn requires, generally speaking, that the function $f(\cdot|t)$ be such that the contributions of the m particles to the components of $f(\cdot|t)$ are additive. This and the fact that even model A with $f(\cdot|t) = bX(t) + cY(t)$ belongs to \mathfrak{C} , can be seen heuristically as follows.

For a given realization of $X(\tau)$ for all τ with $0 \leq \tau \leq t$, it is clear that the probability of no response during $(0, t)$ is given by

$$(4.3) \quad \exp \left\{ - \int_0^t [a + f(\cdot|\tau)] d\tau \right\}.$$

Taking the expectation of this over all realizations of $\{X(\tau); 0 \leq \tau \leq t\}$, we have

$$(4.4) \quad Q(t|m) = e^{-at} E \left[\exp \left\{ - \int_0^t f(\cdot|\tau) d\tau \right\} \mid X(0) = m \right],$$

where $f(\cdot|\tau)$ is a function of the random variables $X(\tau)$ and so on. Comparing (4.2) and (4.4), we have for every model of class \mathfrak{C} , for $m = 1, 2, \dots$,

$$(4.5) \quad E \left[\exp \left\{ - \int_0^t f(\cdot|\tau) d\tau \right\} \mid X(0) = m \right] = [\psi_f(t)]^m,$$

so that

$$(4.6) \quad \psi_f(t) = E \left[\exp \left\{ - \int_0^t f(\cdot|\tau) d\tau \right\} \mid X(0) = 1 \right].$$

Now, since $X(t) = \sum_{j=1}^m X_j(t)$, where $X_j(t)$ for $j = 1, 2, \dots, m$, is the contribution of the j th inoculated particle, we have

$$(4.7) \quad Y(t) = \sum_{j=1}^m \int_0^t X_j(\tau) d\tau = \sum_{j=1}^m Y_j(t),$$

so that, for instance, for model A

$$(4.8) \quad f[X(t), Y(t)] = bX(t) + cY(t) = \sum_{j=1}^m [bX_j(t) + cY_j(t)].$$

Because of the hypothesis of independent growth of the particles (section 2.1), it is thus clear that for model A, (4.5) and hence (4.2) are satisfied, so that model A belongs to \mathfrak{C} . Furthermore, the relations (4.4), (4.5), and (4.6) pave another equivalent way of deriving the expression for $Q(t|m)$, namely, from the differential equation of $\psi_f(t)$. For instance, for model A,

$$(4.9) \quad Q(t|m) = e^{-at} E \left[\exp \left\{ -bY(t) - c \int_0^t Y(\tau) d\tau \right\} \mid X(0) = m \right].$$

Now let, for $X(0) = m$,

$$(4.10) \quad g(u, v, w; t) = E \left[\exp \left\{ iuX(t) + ivY(t) + iw \int_0^t Y(\tau) d\tau \right\} \right].$$

It is obvious that knowledge of $g(u, v, w; t)$ is sufficient for obtaining $Q(t|m)$. Following the procedure of section 2.2, it is not difficult to show that the ch.f. $g(u, v, w; t)$ satisfies the partial differential equation

$$(4.11) \quad g_t = \{v + i[(\lambda + \mu) - \lambda e^{iu} - \mu e^{-iu}]\} g_u + w g_v,$$

with the initial condition

$$(4.12) \quad g(u, v, w; 0) = e^{imu}.$$

Interestingly enough, equation (4.11) resembles (2.21), the equation for the function $g_1(u, v; t)$; in fact (2.21) can be obtained from (4.11).

Again in (4.2), assuming m to be Poisson distributed with parameter β , the probability $Q(t|\beta)$ for a typical model of class \mathcal{C} is given by

$$(4.13) \quad Q(t|\beta) = \exp\{-at - \beta[1 - \psi_f(t)]\}.$$

Other forms of the function $f(\cdot|t)$ which could be considered are, for example,

$$(4.14) \quad f(\cdot|t) = bX(t) + cN(t)$$

and

$$(4.15) \quad f(\cdot|t) = bX(t) + cX^2(t),$$

where the random variable $N(t)$ denotes the number of particle deaths occurring during $(0, t)$, and has been studied elsewhere by the author [19]. A model with $f(\cdot|t)$ given by (4.14) is applicable when the bacteria are known to yield a certain type of toxin only at the time of their death. Clearly, this model belongs to class \mathcal{C} . However, a model with $f(\cdot|t)$ expressed by (4.15) does not belong to \mathcal{C} , especially since $X^2(t) \neq \sum_{j=1}^m X_j^2(t)$.

Finally, we remark that, although the above models are, in general, applicable to several experimental situations, nevertheless, for certain situations they may require modification. In section 6 we shall attempt to fit the models of class \mathcal{C} , with certain modifications, to survival data obtained from a dilution series experiment on guinea pigs injected with tuberculosis bacilli. First, in the next section, we obtain the distribution under model B (section 3) of the number of particles at the time of death of the host, which is relevant to the hypothesis of existence of a fixed lethal threshold.

5. Distribution of the number of particles at time of death of the host under LeCam's model

Let X_T be the number of particles at the time T of death (or other response) of the host. We shall now derive the distribution of X_T under the model (B) suggested by LeCam, under the restriction $a = 0$, that is, that the response can be caused only by the particles. However, in the case of $a = 0$, there is a positive probability for $T = \infty$; in fact from (3.11) and (3.16) it follows easily that

$$(5.1) \quad P\{T = \infty\} = Q(\infty|m) = r_2^m.$$

The appropriate distribution for us to consider is, therefore, $P\{X_T = x|T < \infty\}$, for $x = 0, 1, 2, \dots$. Let

$$(5.2) \quad H(u|m) = \sum_{x=0}^{\infty} u^x P\{X_T = x|T < \infty\}, \quad |u| \leq 1,$$

be the corresponding p.g.f. To find $H(u|m)$ we may proceed as follows.

For $t < t_1$, consider the p.g.f.

$$(5.3) \quad G_{X,1}(u, v; t, t_1 | m) \\ = \sum_{j=0}^{\infty} \sum_{k=0}^{\infty} u^k v^j P\{X(t) = k, X(t_1) = j, Z(t_1) = 1 | X(0) = m, Z(0) = 1\},$$

where $|u| \leq 1$ and $|v| \leq 1$. Using (3.11), the expression for the p.g.f. (5.3) can easily be derived, but will not be reproduced here.

It is clear that for $x = 0, 1, 2, \dots$,

$$(5.4) \quad P\{X(t) = x, T = t_1\} = -\frac{d}{dt_1} P\{X(t) = x, T > t_1\} \\ = -\frac{d}{dt_1} P\{X(t) = x, Z(t_1) = 1\},$$

and that the density function for T is given by

$$(5.5) \quad p_T(t_1) = \frac{d}{dt_1} P\{T > t_1\} = -\frac{d}{dt_1} P\{Z(t_1) = 1\},$$

with

$$(5.6) \quad p_T(t_1 | T < \infty) = -\frac{1}{1 - r_2^m} \frac{d}{dt_1} P\{T > t_1\}.$$

Thus, we have

$$(5.7) \quad P\{X(t) = x | T = t_1\} = \frac{\frac{d}{dt_1} P\{X(t) = x, T > t_1\}}{\frac{d}{dt_1} P\{T > t_1\}},$$

which yields

$$(5.8) \quad P\{X(t) = x | T = t\} = \frac{\frac{d}{dt_1} P\{X(t) = x, T > t_1\} |_{t_1=t}}{\frac{d}{dt} P\{T > t\}}.$$

Using (5.6) to take the expectation of (5.8) over T , we have the unconditional distribution of X_T , given by

$$(5.9) \quad P\{X_T = x | T < \infty\} = \frac{-1}{1 - r_2^m} \int_0^{\infty} \frac{d}{dt_1} P\{X(t) = x, T > t_1\} |_{t_1=t} dt,$$

from which it follows easily that

$$(5.10) \quad H(u|m) = \frac{-1}{1 - r_2^m} \int_0^{\infty} \frac{d}{dt_1} G_{X,1}(u, 1; t, t_1) |_{t_1=t} dt.$$

Substituting the expression for $G_{X,1}(u, 1; t, t_1)$ in (5.10), we obtain after some simplification

$$(5.11) \quad H(u|m) = \frac{(1 - r_1)(1 - r_2) u(u^m - r_2^m)}{(1 - r_2^m)(u - r_1)(u - r_2)},$$

and this yields easily

$$(5.12) \quad P\{X_T = x|T < \infty\} = \begin{cases} 0 & \text{for } x = 0, \\ \frac{b}{\lambda r_1(1 - r_2^m)} \left[r_2^{m-x} + r_2^{m-x+1} \left(\frac{1}{r_1}\right) + \dots + r_2^{m-1} \left(\frac{1}{r_1}\right)^{x-1} \right] & \text{for } 1 \leq x \leq m, \\ \left(\frac{1}{r_1}\right)^{x-m} P\{X_T = m|T < \infty\} & \text{for } x > m. \end{cases}$$

Also, we have

$$(5.13) \quad E(X_T|T < \infty; m) = \frac{m}{1 - r_2^m} + \frac{\lambda - \mu}{b},$$

and

$$(5.14) \quad \text{Var}(X_T|T < \infty; m) = \frac{(\lambda - \mu)^2 + b(\lambda + \mu)}{b^2} - \frac{m^2 r_2^m}{(1 - r_2^m)^2}.$$

At this stage, we refer to the experimental work of Meynell and Meynell [12], where several groups of mice were injected with varying doses of virulent bacteria (*Salmonella typhimurium*). For each animal, a bacterial count was made immediately after death (within half an hour). In figure 2, which is taken

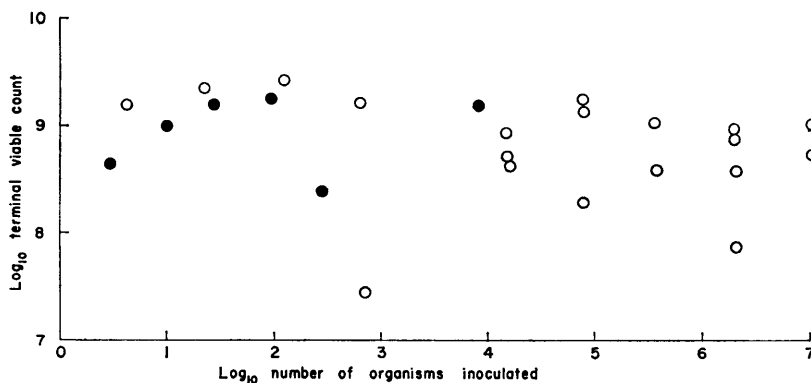


FIGURE 2

Logarithm of observed terminal viable count plotted against logarithm of dose, showing that the former is approximately constant with mean of $10^{8.75}$, for all doses given to two lines of mice of differing resistance. Counts from experiment 1.

($LD_{50} = 320$ organisms; open points) and experiment 4 ($LD_{50} = 3$ organisms; solid points) [12].

from [12], a plot is shown of the logarithm of the number of bacteria at death against the logarithm of the injected dose. One observes in this plot an absence of any trend in the number of bacteria at death with changing size of injected dose and also that this number appears to remain constant on the average. It is this observation which seems to have led Meynell and Meynell to support the

hypothesis of the existence of a threshold. The average number of bacteria observed at death in their experimental work was about $10^{8.75}$. This high estimate of the left side of (5.13) suggests that $\lambda > \mu$ and that b must be extremely small. Thus, it may be relevant to approximate the distribution of X_T for small values of b . To this end, then, our next step is to find the limiting distribution of bX_T as $b \rightarrow 0$. The ch.f. of the limiting distribution of bX_T as $b \rightarrow 0$ is given by

$$(5.15) \quad \lim_{b \rightarrow 0} H(e^{iwb}|m) = \frac{1}{1 - (\lambda - \mu)iw},$$

which is the ch.f. of $(\lambda - \mu)\chi_2^2/2$, where χ_2^2 is the central chi square with two degrees of freedom. Thus, for small b , we have the approximation

$$(5.16) \quad X_T \approx \frac{\lambda - \mu}{2b} \chi_2^2.$$

It is interesting to note that this approximation is independent of m , the number of bacteria injected. Thus, the observations made by Meynell and Meynell could be explained easily under LeCam's model without relying on the threshold hypothesis. It may be remarked here that under the threshold hypothesis X_T is constant and the variability exhibited in figure 2 is to be attributed only to measurement errors; by contrast, under the models considered here, X_T is a random variable and the variability is thus intrinsic.

Using (5.16), it is easy to obtain an approximation for the distribution of $\log X_T$. The numbers at death observed by Meynell and Meynell, were read from the plot of figure 2 and the distribution of $\log X_T$ was fitted to these by the

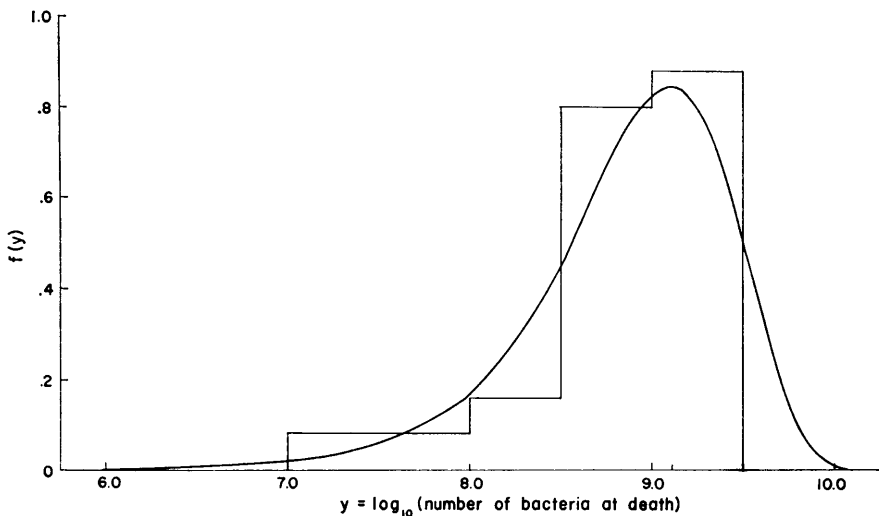


FIGURE 3

Histogram for the \log_{10} (observed number of bacteria at death) and the theoretical curve under LeCam's model.

minimum chi square method. The result is presented in figure 3. The fit is found to be excellent, with the calculated value of chi square (2 d.f.) slightly less than two.

Another paper based on the experimental work of Berry, DeRopp, Fair, and Schur [4], has recently come to the author's attention. In this work it was found that, for small and moderately large m , the number of bacteria at death showed no trend with changing dose m ; but if the injected dose m was very large, the number at death was observed to be larger than for the smaller values of m . In this case, then, the limiting procedure used in approximating the distribution of X_T should be slightly different. In the approximation (5.16), m is assumed to be only moderately large, so that for very small values of b we could neglect terms of the order bm . However, for very large doses it seems appropriate to avoid this assumption and consider instead, the limiting distribution of X_T/EX_T as $b \rightarrow 0$. Using (5.11), it is easy to establish that

$$(5.17) \quad \lim_{b \rightarrow 0} E \left\{ \exp \left[\frac{i\omega X_T}{EX_T} \right] \middle| m \right\} = \frac{1}{1 - i\omega},$$

wherefrom we have for small b

$$(5.18) \quad X_T \approx \left[\frac{\lambda - \mu}{2b} + \frac{m}{2(1 - r_2^m)} \right] \chi^2.$$

6. An application of models of class **C** to survival data

6.1. *Effective number of organisms.* Meynell and Meynell point out [12] that their results can be given the interpretation that host response to inoculation is caused by only a fraction of the administered organisms. They hypothesize that the fates of individual inoculated organisms are randomly and independently determined, so that each inoculated organism has a certain chance of experiencing an event or succession of events *in vivo* which will permit it to multiply sufficiently to cross the threshold and thereby cause a response. According to them, this chance, denoted by p , depends upon the virulence of the organism. If the organisms are of maximum virulence, the inoculation of a single organism will invariably lead to a response so that $p = 1$. But if the organism is of intermediate virulence, so that $p < 1$, then in most cases more than one organism will have to be inoculated before a response will follow. Thus, on the average, only a proportion p of the total inoculated organisms will multiply sufficiently to initiate a response. Meynell and Meynell call such organisms "effectives." In their model, which is purely deterministic in nature and is based on the threshold hypothesis, p is assumed to be constant for a given system and is unaffected by the number of injected organisms. They do, however, agree (see [12], p. 325) that an alternative and perhaps more realistic hypothesis could be stated on the assumption that p varies with the size of the dose. Following this suggestion, we modify our models to include (i) the existence of p , the probability that a particular organism among the m inoculated will be

effective and (ii) the assumption that this p depends on β , the average number of organisms per unit volume of the dose injected. One could assume the dependence of p on the random variable m , rather than on its expectation β , but this is probably an unnecessary complication. Finally, it is assumed that the effective organisms initiate the disease process at time zero, and are solely responsible for the eventual response of the host, whereas the noneffective ones are considered dead right from the start, possibly killed by the defense mechanism of the host. Under these assumptions, we proceed to derive a modified expression for the probability of survival.

Let η be the total number of effective organisms among the m injected, then for given $\eta = n$, we have, from (4.2),

$$(6.1) \quad Q(t|\eta = n) = e^{-at}[\Psi_f(t)]^n.$$

It is clear that for given m , η has a binomial distribution with probability $p(\beta)$ of a single organism being effective, where $\beta = E(m)$. Taking the expectation of (6.1) over η yields, for given m and $p(\beta)$,

$$(6.2) \quad Q[t|m, p(\beta)] = e^{-at}\{p(\beta)[\Psi_f(t) - 1] + 1\}^m.$$

Finally, taking the expectation of this expression over m , we have

$$(6.3) \quad Q[t|\beta, p(\beta)] = \exp\{-at - \beta p(\beta)[1 - \Psi_f(t)]\}.$$

6.2. *A dilution series experiment.* We now turn to an actual realization of a dilution series experiment, the response in this case being death. What is usually done is that a standard volume of suspension with a certain concentration of organisms is injected into each of a group of animals. This suspension is then diluted, say d times, and a standard volume of this diluted suspension is given to each of another group of animals. This new suspension is further diluted d times and standard volumes administered to a third group of animals. This process of dilution and inoculation is carried out, say, ℓ times, and all the groups of animals are then followed for their survival. Assuming that the process of dilution was carried out uniformly and that the laboratory conditions (temperature, and so on) are so adjusted that changes in the population of organisms during the time it takes for dilution and inoculation are negligible, it is clear that if β is the Poisson parameter of the distribution of m for the last group (inoculated with the lowest concentration), then the Poisson parameters for the various groups are given, from last to first, by βd^k , with $k = 0, 1, 2, \dots, \ell$, respectively. Then for the group associated with Poisson parameter βd^k , the probability $Q_k(t)$ of a particular animal being alive at time t is, from (6.3),

$$(6.4) \quad Q_k(t) = \exp\{-at - \beta d^k p(\beta d^k)[1 - \Psi_f(t)]\},$$

for $k = 0, 1, 2, \dots, \ell$. Unfortunately, the exact dependence of p on βd^k is unknown. Therefore, it appears more appropriate to consider several p_k for $k = 0, 1, 2, \dots, \ell$, instead of taking an arbitrary function $p(\beta)$. In fact, in this way we might be able to get some idea of the form of the function $p(\beta)$ by estimating the p_k . With this modification, the $Q_k(t)$ of (6.4) become

$$(6.5) \quad Q_k(t) = \exp\{-at - \beta d^k p_k [1 - \Psi_f(t)]\},$$

for $k = 0, 1, 2, \dots, \ell$.

We shall denote by $Q(t)$, without subscript, the probability that an animal belonging to the control group (which received no inoculation, except possibly an equal volume of saline for the sake of comparison) is alive at time t . Assuming that the saline has no effect on the normal survival of the animal, it is clear that

$$(6.6) \quad Q(t) = \exp\{-at\}.$$

Now we attempt to fix the expressions (6.5) and (6.6) to some actual data on the survival of guinea pigs injected with tubercle bacilli. These data were obtained by Bjerkedal and Palmer (see [5] and [6], study "P") in a dilution series experiment, conducted along the lines explained above with $k = 0, 1, 2, 3$ and 5 (for some reason the data for the series with $k = 4$ are not presented) and $d = 10$. Each of these five groups was composed of about 158 guinea pigs. The animals of a sixth group received no bacilli and served as controls. All six groups were observed for their survival for 52 weeks after inoculation. While the reader may refer to [5] and [6] for complete observed survival curves, these are shown in figure 4 only for the first 30 weeks of observation, and have been labeled by the corresponding Poisson parameters, namely βd^k , with $k = 0, 1, 2, 3$ and 5 .

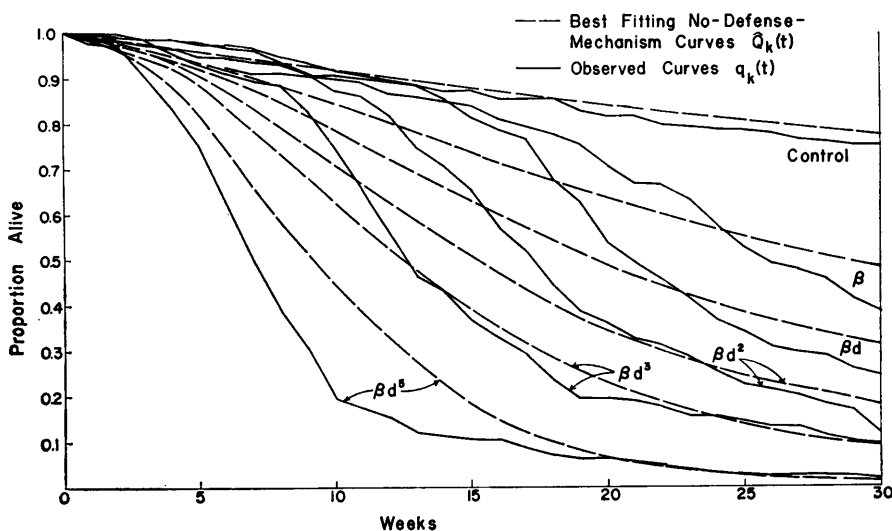


FIGURE 4

Observed and fitted survival curves of guinea pigs
after injection of tubercle bacilli.
(Data of Bjerkedal and Palmer [6].)

For a given $f(\cdot|t)$, if $\Psi_f(t)$ is known (as in model B), one may proceed to fit the formulas (6.5) and (6.6) to the above data. The usual approach would be to

break the time axis into intervals, consider the numbers of deaths occurring during the various intervals as multinomial variables, and estimate the parameters of interest by some standard method such as that of minimum chi square. However, because of the complexity of the formula (6.5) (in view of the expression for $\Psi_f(t)$) and the multiplicity of the parameters involved (for instance for model B, the parameters involved are $a, b, \beta, \lambda, \mu$ and $p_k, k = 0, 1, \dots, \ell$), this approach was dropped. Instead we adopted an empirical approach, which is not only simple but also useful for fitting (6.5) without prior knowledge of the function $\Psi_f(t)$. The function $\Psi_f(t)$ instead is estimated from the data. If this estimate is satisfactory, one could, as a next step compare it with the corresponding theoretical expression for $\Psi_f(t)$ for various particular models of the class C.

We shall restrict ourselves, for all except the control group, to the data observed up to 30 weeks after inoculation, in order to avoid the effect on our analysis of the large fluctuations which occur during the latter part of the study period when relatively few animals remain alive for observation.

Let $q_k(t)$ represent the observed proportion of animals alive at t in the k th series. From formula (6.6) for $Q(t)$, we estimate the parameter a from the observed survival curve for the control series by using the standard method of least squares on $\log q(t)$. The estimate \hat{a} turns out to be 0.00858. From now on, we shall consider a known to be equal to \hat{a} . Taking the logarithms of (6.5) and (6.6), we have

$$(6.7) \quad \log Q_k(t) + at = -\beta d^k p_k [1 - \Psi_f(t)],$$

for $k = 0, 1, 2, \dots, \ell$; and

$$(6.8) \quad \log Q(t) + at = 0.$$

Taking now a combined average over both sides of (6.8) and (6.7), for $k = 0, 1, 2, \dots, \ell$, we have for each fixed t

$$(6.9) \quad \overline{\log} Q(t) + at = -\bar{d}_p \beta [1 - \Psi_f(t)],$$

where

$$(6.10) \quad \overline{\log} Q(t) = \frac{1}{\ell + 2} [\log Q(t) + \sum_{k=0}^{\ell} \log Q_k(t)],$$

$$\bar{d}_p = \frac{1}{\ell + 2} \sum_{k=0}^{\ell} d^k p_k.$$

Thus, with $a, Q_k(t)$, and $Q(t)$ replaced, respectively, by $\hat{a}, q_k(t)$, and $q(t)$, we can estimate empirically, for each t , the left side of (6.9) by $\overline{\log} q(t) + \hat{a}t$, where

$$(6.11) \quad \overline{\log} q(t) = \frac{1}{\ell + 2} \left[\log q(t) + \sum_{k=0}^{\ell} \log q_k(t) \right].$$

In order to smooth the plot of $\overline{\log} q(t) + \hat{a}t$ for t , a cubic

$$(6.12) \quad \chi(t) = \alpha_1 t + \alpha_2 t^2 + \alpha_3 t^3$$

was fitted by the ordinary least squares method, the term independent of t in $\chi(t)$ being zero, since both functions given by (6.9) and $\overline{\log} q(t) + \hat{a}t$ pass through the origin. Thus, we have an empirical estimate of $\overline{\log} Q(t) + \hat{a}$ given by

$$(6.13) \quad \bar{\chi}(t) = \hat{\alpha}_1 t + \hat{\alpha}_2 t^2 + \hat{\alpha}_3 t^3.$$

Using (6.9), (6.7) can be rewritten as

$$(6.14) \quad \log Q_k(t) + at = \left(d^k \frac{p_k}{\bar{d}_p} \right) [\overline{\log} Q(t) + at].$$

However, the left side of this can be estimated empirically by $\log q_k(t) + at$, which suggests that if we fit for $k = 0, 1, 2, \dots, \ell$, a function $\hat{\delta}_k \bar{\chi}(t)$ to $\log q_k(t) + at$, where $\bar{\chi}(t)$ is given by (6.13), then $\hat{\delta}_k$ will give us an estimate of $(d^k p_k / \bar{d}_p)$, for $k = 0, 1, 2, \dots, \ell$. Thus, we may write the estimate of p_k / \bar{d}_p as

$$(6.15) \quad \text{est}(p_k / \bar{d}_p) = \hat{\delta}_k / d^k.$$

Also, we have an estimate for $Q_k(t)$, given by

$$(6.16) \quad \hat{Q}_k(t) = \exp\{-at + \hat{\delta}_k \bar{\chi}(t)\}.$$

The plot against k of values of $\text{est}(p_k / \bar{d}_p)$ obtained from the data (figure 5)

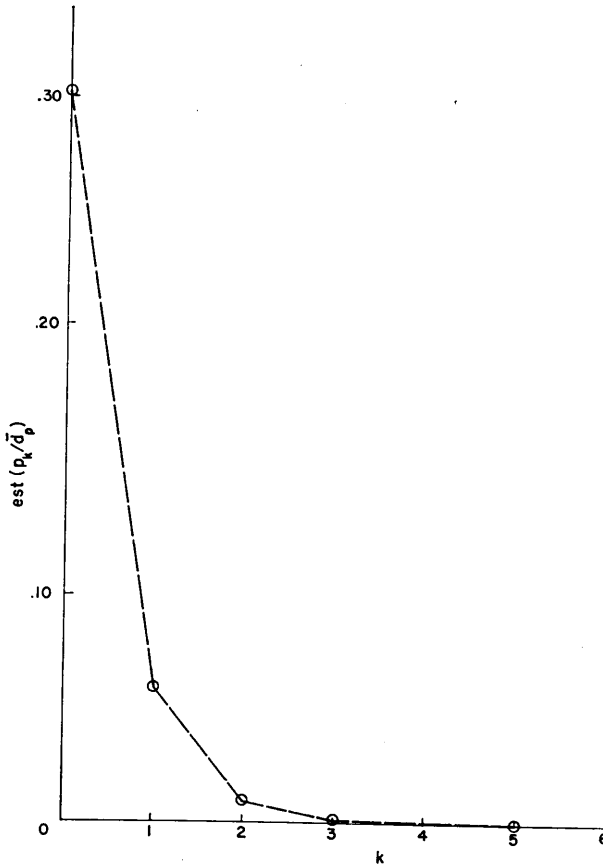


FIGURE 5

Plot of $\text{est}(p_k / \bar{d}_p)$ against k .

shows the variation of p_k with k (except for a multiplicative constant). It seems clear that, in general, the larger the dose inoculated, the smaller is the probability $p(\beta)$. Again, in order to see how well the models of class © fit the observations, the estimates $\hat{Q}_k(t)$ given by (6.16) were plotted along with the observed $q_k(t)$ (figure 4), for $k = 0, 1, 2, 3$, and 5. Although the general form of the fitted curves is similar to that observed, the fit is, nevertheless, quite poor because of the presence of systematic bumps in all the observed curves which are absent in the corresponding curves based on $\hat{Q}_k(t)$. In actual fact, the animals die more slowly in the beginning than indicated by the fitted curves, and the opposite behavior is exhibited during the later weeks. Several improvements might be made in the present models to take into account other relevant factors, such as the initiation of some defense mechanism within the host, interaction between the various causes of death and so on. In order not to overload a single paper, these considerations are discussed only briefly in the next section.

7. Discussion

A great many biological factors influence the response phenomenon; for example, (i) the virulence of the particles, (ii) the number of particles administered to the host or more generally, the amount of exposure, (iii) variation in the susceptibility of the host, (iv) the defense mechanism, if any, induced by the particles in the host and developed during the course of the experiment, for instance the generation of antibodies, and (v) interaction among various causes of response, particularly when the response is death; the list can be greatly extended. Some of these factors were incorporated in a simple manner in the above models, whereas others were left to future investigations. The stochastic models suggested here appear to explain only the broad features of the time dependent response curves, but not the finer details relating to the mechanism itself. In this sense, using the terminology of Neyman, Park, and Scott [16], we may regard these as "models in the large." Still deeper models incorporating the finer points such as (iv) and (v) are very much needed. Improvements of the above models based on some of these points are suggested below.

7.1. *Variation in susceptibility of the host population.* In the above models, it is assumed that the hosts are uniform in their susceptibility to the response causing particles. However, as has been pointed out by Armitage and Spicer [3], Armitage [2], Moran [13], [14], and several others, this assumption may not be valid. One effect of this might be that the probability p_k of a particle being effective will vary not only with the dose (that is, with k) but also from host to host. Assuming that in the k th series this probability varies over the host population with a distribution function $F_k(p)$, the new expression for the probability $Q_k(t)$, using (6.5), is given by

$$(7.1) \quad Q_k(t) = e^{-at} \int_0^1 \exp\{-\beta d^k p [1 - \Psi_f(t)]\} dF_k(p).$$

However, the problem faced by the above authors still remains since we need to know the exact form of $F_k(p)$. As a first step one might wish to test the hypothesis of uniformity of the probabilities p_k within each series, by using large sample tests following Neyman [15] and Armitage [1].

7.2. *Defense mechanism.* Meynell and Meynell [12] observed some indications in their experimental studies that the hypothesis of independent action might not apply to attenuated *Salmonella* deposited within the host tissues. In the same context, they suggest the possibility of a fall in resistance of the host because the initial effective particles might enable initially ineffective particles to multiply and thus contribute to the ultimate response. At the same time, a force in the opposite direction might be exerted by the production of defense entities, such as antibodies, which cause a rise in the resistance of the host, thus contributing to its struggle against the invading particles. One or the other of these forces may predominate in a given biological situation, or both may be present, counteracting each other. Models taking these forces into account are greatly needed, even though they may be mathematically complicated.

7.3. *Interaction among different causes of death.* In the above models, it was assumed that both causes of death, namely, the bacterial and the nonbacterial ones, operate independently. This was reflected through the additivity of a and $f(\cdot|t)$ in the risk function. In the actual fact, this assumption is hardly tenable. On the other hand, the question remains as to what kind of interdependence one should introduce among the various causes. A simple improvement that comes to mind, which retains the independence between the two causes of death in our models, is to allow the risk a to vary from series to series, a kind of dose dependence, giving from (6.5) for $k = 1, 2, \dots, \ell$,

$$(7.2) \quad Q_k(t) = \exp\{-a_k t - \beta d^k p_k [1 - \Psi_f(t)]\}.$$

7.4. *Other forms of risk functions.* In the above class of models, the hypothesis of independent action of the particles starting at time zero was incorporated by taking an appropriate risk function $f(\cdot|t)$. In reality, this hypothesis may not quite hold, in which case one needs to try some other forms of the function $f(\cdot|t)$, for instance

$$(7.3) \quad f[X(t)|t] = bX(t) + cX^2(t).$$

Unfortunately, with such modifications the algebra becomes somewhat complicated.

7.5. *Other types of particle growth processes.* In the above class of models the particles were assumed to undergo a linear birth and death process with birth and death rates $\lambda(t)$ and $\mu(t)$, respectively. One needs, however, to consider growth processes such as quadratic birth and death processes. In actual fact, the growth process might become even more complicated in the presence of a defense mechanism within the host. Again, the Markovian assumption made

for the growth process may also be questionable. The simplest form of non-Markovian behavior that one could introduce in the above models would be to let the rates λ and μ depend upon m , the number of particles injected at $t = 0$.

8. Summary

In mathematical models of the phenomena resulting from the infection of an animal (host) by a population of bacteria (or, more generally, self-reproducing particles) and culminating in the animal's "response" (for example, death), it has been customary to assume that the response occurs when the number of the infecting particles reaches a fixed threshold. In the present paper, the hypothesis of a fixed threshold is abandoned in favor of a more realistic assumption, suggested by Professor L. LeCam. Given that at time t the host is alive, the occurrence of its death between t and $t + \tau$ is treated as a random event, the probability of which can be written as $[a + f(\cdot|t)]\tau + o(\tau)$, where $a \geq 0$ and f is a nonnegative and nondecreasing function of the number of particles present in the body of the host at time t and, possibly, of certain other relevant characteristics of the process of infection. One possibility is to assume $f = bX(t) + cY(t)$, where $X(t)$ stands for the number of particles in the body of the host at time t and $Y(t) = \int_0^t X(u)du$, with $b, c \geq 0$. With this treatment, the number of particles in the body of the host at the moment of its death becomes a random variable, say X_T ; the deduction of the distribution of X_T is one of the problems treated in this paper.

The author is greatly indebted to Professor J. Neyman for his inspiration and guidance, and to Professor L. LeCam for his invaluable suggestions and discussions, during the course of these investigations.

REFERENCES

- [1] P. ARMITAGE, "Host variability in dilution experiments," *Biometrics*, Vol. 15 (1959), pp. 1-9.
- [2] ———, "An examination of some experimental cancer data in the light of one-hit theory of infectivity titrations," *J. Natn. Cancer Inst.*, Vol. 23 (1959), pp. 1313-1330.
- [3] P. ARMITAGE and C. C. SPICER, "The detection of variation in host susceptibility in dilution counting experiments," *J. Hyg., Camb.*, Vol. 54 (1956), pp. 401-414.
- [4] L. J. BERRY, M. K. DEROPP, M. K. FAIR, and E. M. SCHUR, "Dynamics of bacterial infections in mice under conditions known to alter survival time," *J. Infect. Dis.*, Vol. 98 (1956), pp. 198-207.
- [5] T. BJERKEDAL, "Degree of tuberculin sensitivity in guinea pigs infected with different doses of virulent tubercle bacilli," *Amer. J. Hyg.*, Vol. 71 (1960), pp. 299-313.
- [6] T. BJERKEDAL and C. E. PALMER, "Competing forces of mortality in long-term animal experiments," *Amer. J. Hyg.*, Vol. 70 (1959), pp. 246-253.
- [7] D. J. FINNEY, *Statistical Method in Biological Assay*, London, Griffin, 1952.
- [8] J. J. GART, "Some stochastic models relating time and dosage in response curves," *Biometrics*, Vol. 21 (1965), pp. 583-599.
- [9] L. M. LECAM, Personal communication.

- [10] G. G. MEYNELL, "The applicability of the hypothesis of independent action to fatal infections in mice given *Salmonella typhimurium* by mouth," *J. Gen. Microbiol.*, Vol. 16 (1957), pp. 396-404.
- [11] G. G. MEYNELL and B. A. D. STOCKER, "Some hypotheses on the aetiology of fatal infections in partially resistant hosts and their application to mice challenged with *Salmonella paratyphi-B* or *Salmonella typhimurium* by intraperitoneal injection," *J. Gen. Microbiol.*, Vol. 16 (1957), pp. 38-58.
- [12] G. G. MEYNELL and E. W. MEYNELL, "The growth of microorganisms *in vivo* with particular reference to the relation between dose and latent period," *J. Hyg., Camb.*, Vol. 56 (1958), pp. 323-346.
- [13] P. A. P. MORAN, "The dilution assay of viruses I," *J. Hyg., Camb.*, Vol. 52 (1954), pp. 189-193.
- [14] ———, "The dilution assay of viruses II," *J. Hyg., Camb.*, Vol. 52 (1954), pp. 444-446.
- [15] J. NEYMAN, "Optimal asymptotic tests of composite statistical hypotheses," *Probability and Statistics* (The Harald Cramér Volume), Uppsala, Almqvist and Wiksells, 1959, pp. 416-444.
- [16] J. NEYMAN, T. PARK, and E. L. SCOTT, "Struggle for existence. The *Tribolium* model: biological and statistical aspects," *Proceedings of the Third Berkeley Symposium on Mathematical Statistics and Probability*, Berkeley and Los Angeles, University of California Press, 1956, Vol. 4, pp. 41-79.
- [17] P. S. PURI, "Two stochastic models of survival after infection in the absence of defense mechanism," Ph.D. thesis, University of California, Berkeley, 1964. (Abstract, *Biometrics*, Vol. 20 (1964), p. 906.)
- [18] ———, "On the homogeneous birth-and-death process and its integral," *Biometrika*, Vol. 53 (1966), pp. 61-71.
- [19] ———, "Some further results on the homogeneous birth-and-death process and its integral," to appear *Proc. Camb. Phil. Soc.*, 1967.
- [20] A. D. WIGGINS, "A statistical study of the mechanism of bacterial toxicity," unpublished Ph.D. thesis, University of California, Berkeley, 1957.
- [21] T. WILLIAMS, "The basic birth-and-death model for microbial infections," *J. Roy. Statist. Soc. Ser. B*, Vol. 27 (1965), pp. 338-360.