

On A Mathematical Theory of Quantal Response Assays[†]

by

Prem S. Puri^{*} and Jerome Senturia^{**}

Department of Statistics

Division of Mathematical Sciences

Mimeograph Series No. 268

October, 1971

[†] To appear in the Proceedings of the Sixth Berkeley Symposium on Mathematical Statistics and Probability (Biology - Health Section) held in June, 1971.

^{*} This investigation was supported in part by the U.S. Public Health Service, N.I.H. Grant GM-10525, at the Statistical Laboratory, University of California, Berkeley.

^{**} This investigation was supported by the National Science Foundation Traineeship Grant 3392-80-1399 at Purdue University.

On A Mathematical Theory of Quantal Response Assays

by

Prem S. Puri* and Jerome Senturia**

Purdue University, Lafayette

1. INTRODUCTION. About seven years ago, one of the authors (See Puri [16]) was confronted with the following biological phenomenon. At time $t = 0$, each member of a group of hosts such as animals is injected with a dose of a specified virulent organism such as viruses or bacteria, which elicit a characteristic response from the host during the course of time. This response may be death, development of a tumor or some other detectable symptom. 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 $n(t)/n(0)$ is known as the time dependent response curve. These response curves differ with the dose and with the type of the organism. However, generally speaking, the larger the injected dose, the sooner the host responds. The question was raised as to how one could explain these observed response curves through a suitable stochastic model. Upon a search of the existing literature at the time, it was found that most of the models considered until then, were based on the hypothesis of existence of a fixed threshold, namely while the organisms are undergoing a certain growth process within the host, as soon as their number touches a fixed threshold N , the host responds. In [16], this hypothesis was

* This investigation was supported in part by the U.S. Public Health Service, N.I.H. Grant GM-10525, at the Statistical Laboratory, University of California, Berkeley.

** This investigation was supported by the National Science Foundation Traineeship Grant 3392-80-1399 at Purdue University

abandoned; first, because this hypothesis is not strictly correct; second, it is not clear as to what value one ought to choose for N in a given situation, and third, because this hypothesis makes the algebra unnecessarily intractable due to the involvement of the first passage time problem. Instead an alternative hypothesis originally suggested by Professor LeCam was adopted. Here, unlike in the threshold hypothesis, the connection between the number $Z(t)$ of organisms in a host at time t and the host's response is indeterministic in character. More exactly, it is assumed that the value of $Z(t)$ (or possibly of a random variable whose distribution is dependent on the process $\{Z(t)\}$) determines not the presence or the absence of response, but only the probability of response of the host. Mathematically, this amounts to postulating the existence of a nonnegative risk function $f(x,t)$ such that

$$(1) \quad P(\text{host responds during } (t, t+\tau) \mid \text{not responded until } t \text{ and } Z(t) = x) \\ = \delta f(x,t)\tau + o(\tau) \quad ,$$

where $\delta \geq 0$ and f satisfies certain mild regularity conditions. Stochastic models based on this more appropriate alternative hypothesis have been explored with a reasonable amount of success in a paper which appeared in the last Berkeley Symposium [16] and again in a later paper connected with bacteriophage reproduction (see Puri [17]). In fact in [16], it is assumed that the risk function f depends not only on $Z(t)$ but also on the integral $\int_0^t Z(\tau)d\tau$, which 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 toxin excretion rate is constant per bacterium per unit time.

The above models (see [16], [17]) apply to the situations where the response causing agents are self-reproducing such as viruses, bacteria etc. A

natural question arises as to how a similar model based on the alternative hypothesis would behave in situations where the agent is not self-reproducing. Such would be the case where, for instance, the agent is a chemical poison, insecticide or a drug. This then brings up the typical phenomenon that one faces in what is commonly described as Quantal Response Assays. The classical theory of quantal response assays can be found in books such as by Finney ([7], [8]), Bliss [6], and others. One of the purposes of this theory has been to help in arriving at an estimate of the relative potency of one drug against another by using measures such as E.D.50, the dose which is just about enough to cause response among on the average about 50 per cent of the subjects. Here, typically the experimenter chooses a set of doses of each drug and tests each dose on a batch of subjects. At the end of the test, the experimenter records how many of the subjects responded. In order to analyse the data so obtained, it has been customary to make the following assumptions:

- (i) For each subject there exists a tolerance limit or a threshold level T . This limit for a subject is the dose which will be just sufficient to produce the response, so that the subject will respond if $z \geq T$ and will not if $z < T$, where z is the dose injected.
- (ii) The threshold level T is assumed to be a random variable varying over the population of subjects, with a common distribution. Thus the probability that a randomly chosen subject responds after receiving a dose z , is given by

$$(2) \quad P(z) = P(T \leq z) \quad .$$

It is a common practice with the experimenters to use log dose or $x = \log z$, known as the dose metameter of z . Now if $g(y)$ is a probability density function so that $\int_{-\infty}^{\infty} g(y) dy = 1$, the form of the distribution of

$\log T$ typically can be represented by the density

$$(3) \quad dQ(x) = g(\gamma + \eta x) dx ,$$

where γ and η are the usual location and scale parameters respectively.

With this, one easily obtains

$$(4) \quad P(z) = \int_{-\infty}^{\gamma + \eta \log z} g(y) dy .$$

In practice, the choice of $g(y)$ and hence of the distribution of the tolerance limit T is rather arbitrary. Some of the typical choices of $g(y)$ that have been used in the literature are given below.

$$(5) \quad \begin{aligned} (a) \quad g(y) &= (2\pi)^{-1/2} \exp(-\frac{1}{2} y^2); & -\infty < y < \infty \\ (b) \quad g(y) &= \begin{cases} \sin 2y & 0 \leq y \leq \pi/2 \\ 0 & \text{otherwise} \end{cases} , \\ (c) \quad g(y) &= \frac{1}{2} \operatorname{sech}^2 y, & -\infty < y < \infty \end{aligned}$$

The last one has been used by Berkson in his well known work in this area (see [5]).

While the above classical theory has been found useful and is still being used, there are however certain unattractive features in it that make one feel like giving it another look. Some of these are as follows: First is the same objection of assuming the existence of a tolerance limit or a threshold level for each subject even though the random element is introduced only through allowing this limit to vary randomly from subject to subject. Second, the model as it stands does not lend itself to the consideration of any biological mechanism going on within the host leading to its response. And third, it does not

allow the consideration of the time when the response actually occurs if it does; all it considers is whether the response does or does not occur within a fixed length of time. These same features also underlie the more recent work of Ashford [1], Ashford and Smith [2] and Plackett and Hewlett [13], [14], in the case of mixture of drugs.

It is the purpose of this paper to give the classical theory of quantal response assays a fresh look and to construct new stochastic models which attempt to eliminate the above objections. This has been achieved by adopting the alternative approach of the nonthreshold type as discussed above. For the biological phenomenon under consideration, a typical stochastic model of the present type would involve the consideration of the following three main components.

(A) THE INPUT PROCESS. This describes the manner in which the drug is introduced into the subject. We call it the 'Input Process'. One could visualise, depending upon the situation in question, several possibilities of inputs such as a continuous time deterministic input, discrete time deterministic input or a random input according to some random mechanism.

(B) THE RELEASE PROCESS. This describes the manner in which the subject attempts to reduce the level of the drug within its body. This may be carried out either through the process of direct elimination of the drug through natural means or by changing the composition of the drug itself through biochemical processes. We shall call this as the 'Release Process'. In principle, this would involve the mechanism going on within the body of the subject which takes into account the manner in which the subject copes with the drug. In experimental situations, the input process is generally controlled by the experimenter. The release process on the other hand is much more involved. It

requires a great deal of experience and knowledge of the biological system on the part of the experimenter. This in turn involves, in general, a considerable amount of experimentation while probing into the nature of the release mechanism. There has been, in fact, much work done in the past in an attempt to describe this mechanism for certain situations. For instance, the compartment models of, among others, Sapirstein et al [22], Bellman [3], [4], are attempts towards a better understanding of functioning of specific organs and of various biological systems. Unfortunately not too many of these models are stochastic in nature. Again the models in dam theory (see Moran [11], Gani [9], and Prabhu [15], to cite only three references from this vast literature) could be found suitable for combining the aspects of both the input and the release processes.

(C) THE RISK FUNCTION. The most important aspect which appears not to have been considered before in the context of the classical quantal response assays is the consideration of a risk function which ties up the input and the release processes of the drug to the causation of the subject's response. Whether, in any given situation, the risk function depends only on the level $Z(t)$ of the drug at time t , or on some other factors characterising the biological mechanism going on within the body of the subject, would entail a considerable knowledge of the biological system.

In the next few sections, we shall attempt to incorporate the three aspects listed above into a stochastic model. Although, this has been done here under rather simplified assumptions, the results do indicate that there is something to be gained by approaching this problem from a structural point of view. In this context the reader may also find, among others, the work of Neyman and Scott [12] of great interest. Here the response causing agent is Urethane, while the response is the appearance of a tumor in mice.

2. A STOCHASTIC MODEL BASED ON A QUANTAL RESPONSE PROCESS.

2.1. ASSUMPTIONS AND NOTATION. As a first attempt, we consider here a simple stochastic model along the lines discussed above. More comprehensive models incorporating detailed mechanisms suitable for certain situations shall be reported elsewhere. Following the lines of classical quantal response assays, we assume that for each subject the experiment starts with the administration (input) of a single dose $Z(0) = z$ at time $t = 0$, with no other inputs thereafter. Thus if $Z(t)$ denotes the amount of drug present at time t in the body of the subject, it is evident that with probability one $Z(t)$ is nonincreasing with t . The release process is assumed to have two components. The first one determines how often and at what times the releases occur, while the second one associates with each such occurrence a nonnegative random variable Y denoting the amount of the drug to be released if available. More specifically, if $N(t)$ denotes the number of releases occurring during $(0, t]$, we assume, for simplicity, that $N(t)$ is a Poisson process with parameter $\mu > 0$. Also, given $N(t)$, let $Y_1, Y_2, \dots, Y_{N(t)}$ denote the random amounts to be released if available, at the release time points as determined by the Poisson process. In particular, it is assumed that conditionally given $N(t)$, the random variables $Y_1, Y_2, \dots, Y_{N(t)}$ are independently distributed with a common distribution having the probability density function

$$(6) \quad h(y) = \begin{cases} \beta \exp(-\beta y), & y > 0, \\ 0 & \text{elsewhere,} \end{cases}$$

where $\beta > 0$. Of course, if at any time, the random amount Y_i is greater than the amount actually available, all the available amount is then released. From the above construction, it follows that

$$(7) \quad Z(t) \equiv \max \left(0, Z(0) - \sum_{j=0}^{N(t)} Y_j \right); \quad t \geq 0$$

where, by convention, $Y_0 = 0$. Under the Poisson process assumption, it is clear that how often and at what times the releases occur is not influenced by the changes over time in the amount of the drug actually present. This however may not be realistic in certain situations. In Section 5 we shall briefly consider a more general model incorporating this dependence in an appropriate manner. Finally we introduce what we shall call a 'Quantal Response Process' $\chi(t)$ defined as

$$(8) \quad \chi(t) = \begin{cases} 1, & \text{if the subject does not respond until } t \\ 0, & \text{otherwise,} \end{cases}$$

where $\chi(0) = 1$. Also it is assumed that

$$(9) \quad P(\chi(t+\tau) = 0 | \chi(t) = 1, Z(t) = x) = \delta f(x, t) \tau + o(\tau) ,$$

where $\delta > 0$, and $f(\cdot, \cdot)$ defined for $x \geq 0$ and $t \geq 0$, is a nonnegative bounded function, assumed to be continuous almost everywhere with respect to both of its arguments. Using a standard argument it is easy to show that

$$(10) \quad P(\chi(t) = 1 | \omega) = \exp\{-\delta \int_0^t f(Z(\tau, \omega), \tau) d\tau\} ,$$

where $Z(\tau, \omega)$ denotes the state of the process $\{Z(t)\}$ at time τ for a given realisation ω of this process. From (10), we easily obtain the transform

$$(11) \quad E(\chi(t) \cdot \exp(-s Z(t))) = E[\exp\{-s Z(t) - \delta \int_0^t f(Z(\tau), \tau) d\tau\}] ,$$

where $\text{Re}(s) \geq 0$. In particular, this yields

$$(12) \quad P(L > t) = P(\chi(t) = 1) = E[\chi(t)] = E[\exp\{-\delta \int_0^t f(Z(\tau), \tau) d\tau\}] ,$$

where L is the length of time the subject takes to respond. Taking δ in

(12) as a dummy variable, it follows that the response time distribution can equivalently be studied by obtaining the distribution of the integral

$\int_0^t f(Z(\tau), \tau) d\tau$. Reader may find this particular connection explored in detail elsewhere (see Puri [18], [19], [20], [21]). Again, the random variable L , in general, may not be a proper random variable, so that

$$(13) \quad P(L = \infty) = P(\text{no response}) = \lim_{t \rightarrow \infty} E(\chi(t)) = E(\exp\{-\delta \int_0^{\infty} f(Z(\tau), \tau) d\tau\}).$$

At this point, we introduce the following notation:

$$W_1(t, z, x) = P(Z(t) \leq x, \chi(t) = 1 | Z(0) = z, \chi(0) = 1),$$

$$W_1(t, z) = P(\chi(t) = 1 | Z(0) = z, \chi(0) = 1),$$

$$W(t, z, x) = P(Z(t) \leq x | Z(0) = z) ,$$

$$\phi_1(\theta, z, x) = \int_0^{\infty} \exp(-\theta t) W_1(t, z, x) dt ,$$

$$\phi_1(\theta, z) = \int_0^{\infty} \exp(-\theta t) W_1(t, z) dt ,$$

$$\phi(\theta, z, x) = \int_0^{\infty} \exp(-\theta t) W(t, z, x) dt ,$$

where $\text{Re}(\theta) > 0$, $0 \leq x \leq z$, and

$$W(t, 0, x) = W(t, z, z) = 1, \quad \text{for } x \geq 0 ,$$

$$W(t, z, x) = W_1(t, z, x) = 0, \quad \text{for } x < 0 .$$

Here the last line follows from the fact that zero is an absorption state for the process $Z(t)$.

In the next subsection, we shall attempt to obtain expressions for the quantities defined above, through setting up the usual Kolmogorov backward integral equations involving these quantities.

2.2. CERTAIN INTEGRAL EQUATIONS AND THEIR SOLUTIONS.

Unless mentioned to the contrary, we assume henceforth that the risk function f does not explicitly depend on time t and depends only on the level $Z(t)$. Moreover, it is assumed that $f(x)$ is differentiable for all $x \geq 0$.

By considering the moment of the first release during $(0, t)$ and the amount to be released, it is easy to establish the following Kolmogorov backward integral equation for the probability $W_1(t, z, x)$ for $x < z$.

$$(14) \quad W_1(t, z, x) = \mu\beta \int_0^t \exp\{-(\mu + \delta f(z))u\} \left[\int_0^{z-x} W_1(t-u, z-y, x) \exp(-\beta y) dy \right. \\ \left. + \int_{z-x}^z W_1(t-u, z-y) \exp(-\beta y) dy + \exp(-\delta f(0)(t-u)) \int_z^{\infty} \exp(-\beta y) dy \right] du.$$

Taking Laplace transform of both sides of (14) we have for $\text{Re } \theta > 0$,

$$(15) \quad (\mu + \theta + \delta f(z)) \phi_1(\theta, z, x) = \mu\beta \int_0^{z-x} \phi_1(\theta, z-y, x) \exp(-\beta y) dy \\ + \mu\beta \int_{z-x}^z \phi_1(\theta, z-y) \exp(-\beta y) dy \\ + \exp(-\beta z) [\mu / (\theta + \delta f(0))]$$

Similarly, we have the corresponding equation for $W_1(t, z)$ given by

$$(16) \quad W_1(t, z) = \exp\{-(\mu + \delta f(z))t\} + \mu\beta \int_0^t \exp\{-(\mu + \delta f(z))u\} \int_0^z W_1(t-u, z-y) \exp(-\beta y) dy du \\ + \mu \exp(-\beta z) \int_0^t \exp\{-(\mu + \delta f(z))u - \delta f(0)(t-u)\} du,$$

or equivalently in terms of its Laplace transform, by

$$(17) \quad (\mu + \theta + \delta f(z)) \phi_1(\theta, z) = 1 + \exp(-\beta z) [\mu / (\theta + \delta f(0))] \\ + \mu \beta \exp(-\beta z) \int_0^z \exp(\beta v) \phi_1(\theta, v) dv .$$

Equation (17) can easily be converted into the differential equation

$$(18) \quad \phi_1' + \phi_1 [\beta + (\delta f' - \mu \beta) (\mu + \theta + \delta f)^{-1}] = \beta (\mu + \theta + \delta f)^{-1} ,$$

where ϕ_1' and f' are the corresponding derivatives with respect to z .

Solving (18) subject to the initial condition

$$(19) \quad \phi_1(\theta, 0) = (\theta + \delta f(0))^{-1} ,$$

we obtain

$$(20) \quad \phi_1(\theta, z) = (\theta + \mu + \delta f(z))^{-1} \exp\{-\beta \int_0^z A(u) du\} \cdot \\ \cdot [(A(0))^{-1} + \beta \int_0^z \exp\{\beta \int_0^v A(u) du\} dv] ,$$

where

$$(21) \quad A(u) = [\theta + \delta f(u)] [\theta + \mu + \delta f(u)]^{-1} , \quad u \geq 0 .$$

Substituting (20) in (15) and solving (15) in an analogous manner we have the solution for (15) given by

$$(22) \quad \phi_1(\theta, z, x) = (\theta + \mu + \delta f(z))^{-1} \exp\{-\beta \int_0^z A(u) du\} \cdot \\ \cdot [(A(0))^{-1} - \exp\{\beta \int_0^x A(u) du\} + \beta \int_0^x \exp\{\beta \int_0^v A(u) du\} dv] ,$$

where $x < z$. As a check, letting $x \rightarrow z$ in (22) and subtracting the result from (20) we obtain, as expected,

$$(23) \quad \int_0^{\infty} P(Z(t) = z, \chi(t) = 1 | Z(0) = z, \chi(0) = 1) \exp(-\theta t) dt \\ = \phi_1(\theta, z) - \phi_1(\theta, z, z^-) = (\theta + \mu + \delta f(z))^{-1} .$$

The expressions for the transforms as given by (20) and (22), in principle, are sufficient for determining the joint distribution of $\chi(t)$ and $Z(t)$. Unfortunately, to carry out the inversion of these transforms in this generality is rather cumbersome. Later on, we shall carry out their inversion for a special case. Again, if $f(0) > 0$, using a Tauberian argument it follows from (20) that

$$(24) \quad \psi(z) \equiv P(L = \infty | Z(0) = z) = \lim_{\theta \rightarrow 0} \theta \phi_1(\theta, z) = 0 ,$$

so that L is a proper random variable. In fact, using the relation

$$(25) \quad \phi_1(\theta, z) = \int_0^{\infty} \exp(-\theta t) P(L > t) dt = \frac{1}{\theta} (1 - E[\exp(-\theta L)])$$

and (20) we have

$$(26) \quad E[\exp(-\theta L)] = 1 - (\theta + \mu + \delta f(z))^{-1} \exp\{-\beta \int_0^z A(u) du\} \\ \cdot [\theta(A(0))^{-1} + \beta \theta \int_0^z \exp\{\beta \int_0^v A(u) du\} dv] .$$

One could now easily obtain moments of L from (26). In particular, it follows from (25) that

$$(27) \quad E(L) = \lim_{\theta \rightarrow 0} \theta \phi_1(\theta, z) = \phi_1(0, z) .$$

2.3. PROBABILITY OF NO RESPONSE FOR THE CASE WITH $f(0) = 0$.

If $f(0) > 0$, this would mean that the response could be caused even without the presence of the drug. However, in most of the practical situations this appears unrealistic, except when the response is the death of the subject.

Even in the latter case, one could define response as the death caused by the drug and not by other causes; or as an approximation to the actual situation one could ignore the other causes, in which case $f(0) = 0$ would be a reasonable requirement. A more realistic model of this latter situation would be the one which incorporates other causes besides the one due to the drug, since, in principle, all these causes simultaneously compete against each other for the life of the subject. However, at present we shall not venture into this refinement and instead assume $f(0) = 0$ in what follows. With this assumption the random variable L is no longer a proper random variable, since the probability that the subject never responds will be positive. Again, in quantal response assays, where the actual response times are often not reported, one is typically interested only in the probability that the subject never responds. This is valid only as an approximation assuming, of course, that the subject has been under observation for a sufficient length of time. Using (20) with $f(0) = 0$, this probability, denoted by $\psi(z)$, is now given by

$$(28) \quad \psi(z) = P(\text{subject never responds} \mid Z(0) = z) \\ = \lim_{\theta \rightarrow 0} \theta \phi_1(\theta, z) = \mu(\mu + \delta f(z))^{-1} \exp\left\{-\beta \delta \int_0^z f(u) [\mu + \delta f(u)]^{-1} du\right\}.$$

Alternatively, one could easily justify either from (24) and (17) with $f(0) = 0$ or by a direct probabilistic argument that $\psi(z)$ must satisfy the integral equation

$$(29) \quad \psi(z) \exp(\beta z) [\mu + \delta f(z)] = \mu + \mu \beta \int_0^z \exp(\beta v) \psi(v) dv.$$

This, when solved subject to the boundary condition $\psi(0) = 1$, yields again the expression (28). In the next section, we exhibit a comparison of the classical

quantal response model with the present one through the use of the expression (28).

3. A COMPARISON OF THE PRESENT MODEL WITH THE CLASSICAL ONE.

It appears rather natural at this stage to look for some kind of direct comparison between the present theory and the classical one. Unfortunately there does not appear to be any simple way of making such a comparison, mainly because the two theories are based on entirely different points of view. However, if we insist on making one for the sake of amusement, the only way which appears reasonable is to equate the end result common to both the theories. More specifically, by equating the probability of no response under the classical theory, namely

$$(30) \quad 1 - P(z) = P(T > z) = \int_{\gamma + \eta \log z}^{\infty} g(y) dy \quad ,$$

to the probability of no response under the present model, namely $\psi(z)$, we ask what risk function $f(\cdot)$ of the present model would correspond to a given density function $g(y)$ used in the classical theory. To this end, one can easily solve (28) for $f(z)$ in terms of ψ yielding

$$(31) \quad \delta f(z) = [\psi(z)]^{-1} \exp(-\beta z) [\mu \{1 - \psi(z) \exp(\beta z)\} + \mu \beta \int_0^z \exp(\beta v) \psi(v) dv] \quad .$$

Now by replacing $\psi(z)$ with $1 - P(z)$, one obtains the desired risk function f corresponding to a given density g of the classical model. For instance, the risk function corresponding to the normal density (5a) is given by

$$(32) \quad \delta f(z) = \mu \exp(-\beta z) [1 - H(\gamma + \eta \log z)]^{-1} \quad .$$

$$[1 - \exp(\beta z) (1 - H(\gamma + \eta \log z)) + \beta \int_0^z \exp(\beta v) (1 - H(\gamma + \eta \log v)) dv] \quad ,$$

where $H(x) = (2\pi)^{-1/2} \int_{-\infty}^x \exp(-\frac{1}{2} \tau^2) d\tau$. Similarly for the density function of (5c), we have

$$(33) \quad \delta f(z) = \mu \exp(-\beta z) [1 + \exp\{2(\gamma + \eta \log z)\}] \cdot \{1 - \exp(\beta z) \cdot [1 + \exp\{2(\gamma + \eta \log z)\}]^{-1} + \beta \int_0^z \exp(\beta v) [1 + \exp\{2(\gamma + \eta \log v)\}]^{-1} dv\} .$$

As expected, since $P(0) = 0$, we have $f(0) = 0$ in the above formulas. Similar expressions can be obtained for f that correspond to other densities often used in the classical theory. Unfortunately, as is evident, all such expressions will usually be complicated, so that there appears to be no rationale for choosing one or the other form of the risk function in practice. In the next section, we consider the simplest form of the risk function, namely the linear function $\delta f(x) = \delta x$, which appears reasonable at least as a first approximation. The results obtained by using this simple risk function are then applied to some observed data.

4. AN APPLICATION OF THE MODEL TO OBSERVED DATA.

We shall now restrict ourselves to the case of a linear risk function with $\delta f(x) = \delta x$. For this, we have from (22),

$$(34) \quad \phi_1(\theta, z, x) = (\theta + \mu + \delta z)^{-1} [C(z)]^{-1} \exp(-\beta z) \cdot [(1 + \mu/\theta) - C(x) \exp(\beta x) + \beta \int_0^x C(v) \exp(\beta v) dv] ,$$

where $x < z$ and

$$(35) \quad C(v) = [(\theta + \mu)/(\theta + \mu + \delta v)]^{\beta\mu/\delta} , \quad v \geq 0 .$$

Also the expression (20) now takes the form

$$(36) \quad \phi_1(\theta, z) = [(\theta + \mu)/(\theta + \mu + \delta z)] [C(z)]^{-1} \exp(-\beta z) \cdot \\ \cdot [1/\theta + \{\beta/(\mu + \theta)\} \int_0^z C(v) \exp(\beta v) dv] .$$

The transforms (34) and (36) can be easily inverted to produce the expressions for $W_1(t, z, x)$ and $W_1(t, z)$ respectively; for instance, when $\beta\mu/\delta$ is not an integer,

$$(37) \quad W_1(t, z) = \sum_{k=0}^{\infty} \frac{1}{k!} [A_k(\frac{\beta\mu}{\delta} - 1) \cdot \{\delta z/(\mu + \delta z)\}^k \exp(-\beta z) I_{k, \mu + \delta z}(t) \\ + A_k(\beta\mu/\delta) \cdot (\delta/\beta)^k I_{k+1, \beta}(z) \cdot t^k \exp\{-(\mu + \delta z)t\}] ,$$

where

$$A_k(x) = x(x+1)(x+2) \dots (x+k-1), \quad k \geq 1; \quad A_0(x) \equiv 1,$$

and for $\alpha > 0$,

$$I_{k, \alpha}(x) = \int_0^x \frac{\alpha y^{k-1}}{\Gamma(k)} \exp(-\alpha y) dy; \quad k \geq 1; \quad I_{0, \alpha}(x) \equiv 1.$$

It is easy to verify that by letting δ tend to zero in (34) one obtains

$$(38) \quad \lim_{\delta \rightarrow 0} \phi_1(\theta, z, x) = \phi(\theta, z, x) = \mu [\theta(\theta + \mu)]^{-1} \exp\{-\beta\theta(\theta + \mu)^{-1}(z-x)\}; \quad x < z,$$

a result for the process $Z(t)$ alone without the consideration of the quantal response process $\chi(t)$. Again, for the present case with $f(x) = x$, we have from (28) the expression for the probability of no response, as given by

$$(39) \quad \psi(z) = \exp(-\beta z) [1 + \frac{\delta}{\mu} z]^{-1 + \beta\mu/\delta} .$$

It is this probability which is relevant to the fitting of our model to appropriate data on quantal response assays. The expression (39) contains

essentially two parameters, since μ and δ always appear as μ/δ . However, for fitting the above formula to suitable data, it was found convenient to introduce the reparameterization

$$(40) \quad \rho = \delta/\mu, \quad \lambda = \rho(\beta - \rho),$$

so that

$$(41) \quad \psi(z) = (1 + \rho z)^{\lambda/\rho^2} \exp\{-(\rho + \lambda/\rho)z\}.$$

Let Z_0 (E.D.50) denote the dose which will produce a response with probability one-half. From (41), it follows that Z_0 satisfies the relation

$$(42) \quad \ln \frac{1}{2} = -\left(\rho + \frac{\lambda}{\rho}\right) Z_0 + \frac{\lambda}{\rho^2} \ln(1 + \rho Z_0).$$

The formula (41) was fitted to the data based on a study of the toxicity of an insecticide known as Deguelin. The data are due to Martin [10] and have also been used by Berkson[5] in an attempt to fit the classical model of the quantal response assays. In the study proper, concentrations at different dose levels z_i of Deguelin were prepared in an alcohol medium. These were then sprayed on groups of respective sizes n_i of the test insects (Adult Apterous Female) *Aphis Rumicis*. These sprayings were performed in a carefully controlled way using a special atomizer. After spraying, the insects without further handling were placed in tubes with a small amount of bean foliage. They were checked after about 20 hours for the number r_i of deaths in the i th group. These data are given in Table 1.

Concentration mg/litre (z_i)	10.1	20.2	30.3	40.4	50.5
Total number (n_i)	48	48	49	50	48
Number of Deaths (r_i)	18	34	47	47	48

TABLE 1: MARTIN'S DATA ON TOXIC EFFECT OF DEGUELIN

The formula (41) was fitted to the above data by using the standard method of minimum chi-square. The fit appears quite satisfactory, since the observed value of the chi-square is 3.69 (3 degrees of freedom), which is not significant at the 5 percent level where the table value is 7.81. Also the method of maximum likelihood lead to the estimates for the parameters ρ and μ , along with their standard errors, as given below. Using these and the relation (42), an estimate \hat{Z}_0 of E.D.50 was obtained by using a computer search procedure for finding the appropriate root \hat{Z}_0 of (42). The various estimates of the standard errors, as given here, are based on the standard large sample formulas valid for the maximum likelihood estimates.

$$\begin{aligned} \hat{\lambda} &= 0.00526, & \hat{\rho} &= 0.02428, & \hat{Z}_0 &= 13.117 \\ \text{S.E}(\hat{\lambda}) &= 0.00102, & \text{S.E}(\hat{\rho}) &= 0.0143, & \text{S.E}(\hat{Z}_0) &= 0.011 \end{aligned}$$

As a passing remark, it may be appropriate to mention here that we also fitted the formula (41) to data reported in [5] which pertain to responses to certain bacteria. Here, as expected, the fit was considerably worse. For the four degrees of freedom available in that case, the observed chi-square was 12.9. This being significant indicates the sensitivity of the present model to situations where the response causing agent is self-reproducing. The

models appropriate for such situations have already been dealt with elsewhere (see Puri [16],[17]). The present model is, of course, not designed for such situations.

5. A MODEL WITH A GENERALISATION OF THE RELEASE PROCESS.

In the release process as adopted in the above model, how often and at what times the releases occur is not influenced by the changes over time in the amount of the drug actually present in the subject. When the above work was presented at this symposium a question was raised from the audience inquiring whether it was possible to modify the release process of the model in order to take into account the possible effect of the changes over time in the level of the drug on the frequency of the releases. We attempt here to accomplish this through a generalisation of the Poisson process of Section 2. Let $\mu(z)$ be a nonnegative bounded function, which may be called as the risk function for the release, such that

$$(43) \quad \begin{aligned} P(\text{a release occurs during } (t, t+\tau) | Z(t) = z) &= \mu(z)\tau + o(\tau) \quad , \\ P(\text{more than one release occur during } (t, t+\tau) | Z(t) = z) &= o(\tau) \quad . \end{aligned}$$

The random variables Y_1, Y_2, \dots , denoting the amounts to be released, if available, at the release points governed by (43), are as before independently distributed with the common distribution given by (6). Clearly, when $\mu(z)$ is a positive constant, we are back to the case of the Poisson release process. All the other assumptions of the model as outlined in Section 2 remain the same with the only exception of (43) and that we assume that $f(0) = 0$. It may be remarked here that there is no loss in generality so far as the distribution of the quantal response process $\chi(t)$ is concerned, if we allow $\mu(0)$ to be positive. In the latter case we can still fictitiously talk of the releases, even though the level of the drug may be zero. Thus we assume that $\mu(0) > 0$, for convenience. Let $N(t)$ denote the number of releases occurring during $(0, t]$. Also we introduce the following notation.

$$(44) \quad \begin{cases} V_1(k, t, z) = P(\chi(t) = 1, N(t) = k | Z(0) = z, \chi(0) = 1), & k \geq 0, \\ V_1(t, z) = P(\chi(t) = 1 | Z(0) = z, \chi(0) = 1), \\ V(k, t, z) = P(N(t) = k | Z(0) = z), & k \geq 0. \end{cases}$$

It is not too difficult to show that the random variable $N(t)$ is a proper random variable for every $t \geq 0$, so that the probability of an infinite number of releases occurring during a finite time interval is zero. As such

$$V_1(t, z) = \sum_{k=0}^{\infty} V_1(k, t, z) .$$

Again taking into account the first release, if it occurs, it is easy to establish the following system of recurrence relations for the V 's.

$$(45) \quad V_1(0, t, z) = \exp\{-(\mu(z) + \delta f(z))t\} .$$

$$(46) \quad V_1(k, t, z) = \mu(z) \int_0^t \exp\{-(\mu(z) + \delta f(z))u\} \left\{ \int_0^z \beta \exp(-\beta y) V_1(k-1, t-u, z-y) dy \right. \\ \left. + \exp(-\beta z) V(k-1, t-u, 0) \right\}, \quad k \geq 1 .$$

Let

$$(47) \quad V_1^*(k, \theta, z) = \int_0^{\infty} \exp(-\theta t) V_1(k, t, z) dt ,$$

$$V^*(k, \theta, z) = \int_0^{\infty} \exp(-\theta t) V(k, t, z) dt ,$$

where $\text{Re } \theta > 0$. Then from (45) and (46) we have

$$(48) \quad V_1^*(0, \theta, z) = [\theta + \mu(z) + \delta f(z)]^{-1}$$

$$(49) \quad V_1^*(k, \theta, z) = \mu(z) [\mu(z) + \delta f(z) + \theta]^{-1} \cdot \{ \exp(-\beta z) V^*(k-1, \theta, 0) \\ + \beta \int_0^z \exp(-\beta y) V_1^*(k-1, \theta, z-y) dy \}, \quad k \geq 1 .$$

Clearly

$$(50) \quad V(k,t,0) = \frac{[\mu(0)t]^k}{k!} \exp(-\mu(0)t) ,$$

so that

$$(51) \quad V^*(k,\theta,0) = [\mu(0)]^k [\mu(0)+\theta]^{-k-1} .$$

Using this, one can easily solve the system (48)-(49) recursively. However, our aim is to obtain $\psi(z)$, the probability of no response. To this end, adding (48)-(49) over the possible values of k , we obtain

$$(52) \quad V_1^*(\theta,z) = [\theta+\mu(z)+\delta f(z)]^{-1} \cdot [1+\mu(z) \exp(-\beta z) \{ \theta^{-1} + \beta \int_0^z \exp(\beta v) V_1^*(\theta,v) dv \}] .$$

We assume now, for simplicity, that besides $f(z)$, the risk function $\mu(z)$ is also differentiable for $z \geq 0$. With this (52) can be easily transformed into the differential equation,

$$(53) \quad \partial V_1^* / \partial z + [\mu(\delta f' + \beta \theta + \beta \delta f) - \mu'(\theta + \delta f)] [\mu(\theta + \mu + \delta f)]^{-1} V_1^* = [\beta - (\mu'/\mu)] ,$$

where f' and μ' denote, respectively, the derivatives of f and μ . Here we have suppressed, for convenience, the arguments of all the functions such as f, μ , etc. Equation (53) can be easily solved subject to the initial condition $V_1^*(\theta,0) = 1/\theta$, yielding

$$(54) \quad V_1^*(\theta,z) = \frac{\mu(z)(\theta+\mu_0)}{\mu_0(\theta+\mu(z)+\delta f(z))} \exp(-\beta B(z)) \left[\frac{1}{\theta} + \frac{\mu_0}{\theta+\mu_0} \cdot \int_0^z \{ \beta \mu(s) - \mu'(s) \} \{ \theta + \mu(s) + \delta f(s) \} \{ \mu(s) \}^{-2} \exp(\beta B(s)) ds \right] ,$$

where $\mu_0 = \mu(0)$ and

$$(55) \quad B(s) = \int_0^s \frac{\delta f(v)}{\mu(v) + \delta f(v)} dv .$$

Finally, since $\psi(z) = \lim_{\theta \rightarrow 0} \theta V_1^*(\theta, z)$, it follows from (54), that

$$(56) \quad \psi(z) = \mu(z) [\mu(z) + \delta f(z)] \exp\{-\beta B(z)\} .$$

This then is the generalisation of the formula (28) where $\mu(z)$ was assumed to be a positive constant. Finally for the special case with $f(x) = x$ and $\mu(x) = \mu_0 + vx$ such that $\mu_0 + vx > 0$ for $0 \leq x \leq z$, we have

$$(57) \quad \psi(z) = \exp\left\{-\frac{\beta\delta}{v+\delta} z\right\} \left[1 + \frac{vz}{\mu_0}\right] \left[1 + \frac{\delta+v}{\mu_0} z\right]^{-1+\beta\delta\mu_0(\delta+v)^{-2}}, \quad z \geq 0 .$$

6. DISCUSSION. The present work is inspired by an earlier work of one of the authors (see Puri [16]) and by the need of giving a fresh look to the classical theory of quantal response assays (see Finney [7]), which, in the opinion of the authors, appears to have certain unappealing features. Although most of the mathematical models of random phenomena incorporate assumptions, which tend to simplify the real situation, yet, by now, it is evident that there are certain fundamental differences in the approach adopted here from the one classically used. For instance, the present approach permits the consideration of the response time, while the classical one does not. Unlike the classical approach, the present one is based on a nonthreshold hypothesis which appears more appealing. Most importantly, however, the present model allows ample room for the consideration of the mechanism of the causation of the response, while the classical theory does not. The mechanism incorporated in the model studied here may be oversimplified for certain situations. However, this, in general, can easily be rectified by incorporating more complicated yet realistic mechanisms into the present theory, usually, of course, at the cost of making the algebra more involved.

In the present model the only input allowed is at the start of the experiment. However, this can easily be extended to cover the general case, where the input pattern over time is controlled and determined ahead of time by the experimenter (see Neyman and Scott [1]). Also situations such as exposure to natural radiation, or to specific chemicals as part of certain occupational hazards, involve perhaps a random mechanism for the input process. Such models involving more elaborate input and release processes will be reported elsewhere (see also Senturia [23]).

The classical theory of quantal response process has been extended to the case of multiple responses to one or several drugs (see Ashford [1]) or to the case of a single response to mixture of drugs (see Ashford and Smith [2]). It appears worthwhile to examine and extend the present approach to cover these cases. Also, deeper models along the present lines, while incorporating the role of the defense mechanism utilized by the subject in order to cope with the drug, are very much needed. This mechanism, of course, may vary considerably from one situation to another. In several situations, to gain knowledge of this mechanism itself would need a considerable amount of further experimentation.

Again, in many situations it may appear realistic to consider the risk function f not only dependent on the level $Z(t)$ of the drug but also on some other relevant functionals of the process $Z(t)$. (See for instance, Puri [16], [17], and the work done at the Statistical Laboratory, University of California, Berkeley, to appear in the present volume).

In the present model, a special form (6) of the common distribution of Y_1, Y_2, \dots , the amounts released, was assumed. This can be generalised to the case with an arbitrary distribution function, say $H(y)$, for the random variables Y 's. One can easily set the integral equations analogous to (15) and (17) for this case. For instance, the equation (17) now takes the form

$$(58) \quad (\mu + \theta + \delta f(z)) \phi_1(\theta, z) = 1 + \mu(1 - H(z)) (\theta + \delta f(0))^{-1} + \mu \int_0^z \phi_1(\theta, z-y) dH(y) \quad .$$

Unfortunately, however, the solution of these equations becomes relatively cumbersome.

Finally, it is hoped that, in due course, the approach adopted here will find its proper place in its usefulness in comparison to the classical approach. This will emerge even more when the experimenter wishes to use the data on response times of the subjects for an appropriate analysis, rather than only on whether the subject does or does not respond in a given period of time.

7. SUMMARY. The classical theory of Quantal Response Assays (see Finney [7]) is based on the hypothesis of existence of a threshold level T (tolerance limit), such that if the injected dose z of the drug is smaller than T , the subject does not respond, and it does respond if $z \geq T$. The threshold level T is assumed to vary randomly over the population of subjects with a common distribution, usually with an arbitrarily chosen form. As it stands, the classical theory has several unattractive features. First, the hypothesis of existence of a threshold level may not be strictly correct. Second, the classical model does not lend itself to the consideration of any biological mechanism leading to the subject's response. And third, it does not allow for consideration of the time when the response actually occurs, if it does. In view of these objections, the present paper gives a fresh look to the problem. As a result, a new stochastic model is constructed along the more realistic lines (see Puri [16], [17]) adopted elsewhere for a similar situation. Here it is assumed that

$$P(\chi(t+\tau) = 0 | \chi(t) = 1, Z(t) = x) = \delta f(x)\tau + o(\tau) \quad ,$$

where $\chi(t)$ is one, if the subject has not responded until time t , and is

zero otherwise; the random variable $Z(t)$ denotes the level of the drug at time t ; $\delta > 0$ and f is a nonnegative risk function defined for all $x \geq 0$, and is assumed to satisfy certain mild regularity conditions. The process $\{Z(t)\}$ is assumed to involve a certain random release mechanism. Here $Z(0)$ is the dose administered at time $t = 0$. Under these assumptions, the joint distribution of $\chi(t)$ and $Z(t)$ is studied for an arbitrary risk function f . In particular with $f(0) = 0$, the probability of no response is obtained. Assuming $f(x) = x$, this probability is then fitted to certain observed data. Finally, an indirect comparison of this model is made with the classical one.

REFERENCES
~~~~~

- [1] Ashford, J.R. (1958). Quantal responses to mixtures of poisons under conditions of similar action - the analysis of uncontrolled data. Biometrika, 45, 74-88.
- [2] Ashford, J.R. and Smith, C.S. (1964). General models for quantal response to the joint action of a mixture of drugs. Biometrika, 51, 413-428.
- [3] Bellman, R. (1960). Some mathematical aspects of chemotherapy - II: The distribution of a drug in a body. Bul. Math. Biophysics, 22, 309-322.
- [4] Bellman, R. (1970). Topics in Pharmacokinetics I. Concentration dependent rates. Math. Biosciences, 6, 13-17.
- [5] Berkson, J. (1953). A statistically precise and relatively simple method of estimating the bioassay with quantal response, based on the logistic function. J. Am. Stat. Asso., 48, 565-599.
- [6] Bliss, C.I. (1952). The Statistics of Bioassay with Reference to the vitamins. Reprinted with additions from Vitamin Methods, Vol. II, Academic Press, N.Y.
- [7] Finney, D.J. (1952). Statistical Method in Biological Assay. Charles Griffin, London.
- [8] Finney, D.J. (1947). Probit Analysis. A Statistical Treatment of the Sigmoid Response Curve. Cambridge Univ. Press, Cambridge.
- [9] Gani, J. (1955). Some problems in the theory of provisioning and of dams. Biometrika, 42, 179-200.
- [10] Martin, J.T. (1942). The problem of the evaluation of rotenone - containing plants VI. The toxicity of  $\delta$ -elliptone and of poisons applied jointly with further observations on the rotenone equivalent method of assessing the toxicity of derris root. Annals of Appl. Biology, 29, 69-81.
- [11] Moran, P.A.P. (1969). A Theory of dams with continuous input and a general release rule. J. Appl. Prob., 6, 88-98.
- [12] Neyman, J. and Scott, E.L. (1967). Statistical Aspect of the Problem of Carcinogenesis. Proc. 5th Berkeley Symp. Math. Statist. Prob., University of California Press, Berkeley.
- [13] Plackett, R.L. and Hewlett, P.S. (1967). A comparison of two approaches to the construction of models for quantal responses to mixtures of drugs. Biometrics, 23, 27-44.
- [14] Plackett, R.L. and Hewlett, P.S. (1963). A unified theory for quantal responses to mixture of drugs: the fitting of data of certain models for two non-interactive drugs with complete positive correlation of tolerances. Biometrics, 19, 517-531.

- [15] Prabhu, N.U. (1965). Queues and Inventories. John Wiley, N.Y.
- [16] Puri, P.S. (1967). A class of stochastic models of response after infection in the absence of defense mechanism. Proc. 5th Berkeley Symp. Math. Statist. Prob., University of California Press, Berkeley.
- [17] Puri, P.S. (1969). Some new results in the mathematical theory of phage reproduction. J. Appl. Prob. 6, 493-504.
- [18] Puri, P.S. (1971). A quantal response process associated with integrals of certain growth processes. Proc. Symp. Mathematical aspects of life sciences, Queens Univ. Press, 95-125.
- [19] Puri, P.S. (1971). A method for studying the integral functionals of stochastic processes with applications: I. Markov Chain Case. J. Appl. Prob. 8, 331-343.
- [20] Puri, P.S. (1970). A method for studying the integral functionals of stochastic processes with applications: II. Sojourn time distributions for Markov chains. Submitted for publication .
- [21] Puri, P.S. (1970). A method for studying the integral functionals of stochastic processes with applications: III. Birth and death processes. To appear in Proc. 6th Berkeley Symp. Math. Statist. Prob.
- [22] Sapirstein, L.A.; Vidt, D.G.; Mandel, J.J., and Hanusek, G. (1955). Volumes of distribution and clearances of intravenously injected creatinine in the dog. Am. J. Physiol. 181, 330-36.
- [23] Senturia, J. (1971). On a Mathematical Theory of Quantal Response Assays and Some New Models in Dam Theory. Ph.D. Thesis, Purdue University.