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Response to First BELLE Newsletter

The first BELLE Newsletter was sent to over 6,000 people including the entire memberships of the Society of Toxicology, the Society of Environmental Chemistry and Toxicology, the International Society of Regulatory Toxicology and Pharmacology, the International Society for Environmental Epidemiology, and the National Council for Radiation Protection. In addition, the newsletter was sent to the 300 attendees of the recent conference in Japan on the biological effects of low level exposure to radiation.

An enthusiastic response to the first newsletter was received by the BELLE office which included numerous calls for more information on the organization and the upcoming BELLE conference, requests for additional copies of the newsletter, and examples of U-shaped dose-response curves. We want to thank the readers for taking the time not only to compile information packages of publications relevant to BELLE, but to offer suggestions for topics and areas of investigation for consideration by BELLE. The publications are currently being added to the BELLE database and we look forward to continued readership response.

A "Letters to the Editor" section has been incorporated into the BELLE Newsletter to facilitate readership participation.

Low Dose, Low Doses, Low Dosages

BELLE is dedicated to assessing the effects of low levels of chemicals and radiation. However, one of the critical issues is how does one define the term "low" with respect to toxicology in general and dose response relationships in particular. In an effort to gain some insights into how various investigators use the term "low", we conducted a computer search of the Medline database through Dialog Information Services, Inc. This database contains numerous journals covering a broad range of medical topics, such as clinical medicine, nutrition, pathology, radiation, and toxicology. We selected for articles published during the years 1966 through 1992 which contained the words low dose, low dosage or low doses in their title and/or abstract.

The search yielded 4452 citations for low dose, 284 citations for low dosage, and 1190 citations for low doses. Over

the next several months we will be assessing how these terms have been used in the various disciplines.

CONFERENCE REVIEW -

Leonard Sagan, MD,
Electric Power Research Institute, Palo Alto, CA.

International Conference on Low Dose
Irradiation and Biological Defense Mechanisms
Kyoto, Japan, July 12-16, 1992.

Over the past fifty years, toxicologists and risk assessors have adopted the practice of predicting risks at low levels of exposure on the basis of extrapolating from observations at high levels of exposure, whether in human studies or in studies of laboratory animals. Such a strategy ignores the possibility that defense mechanisms may operate at low levels of exposures in such a manner as to protect the individual against harm. Yet, many laboratory scientists and epidemiologists have frequently observed phenomena at low doses of either radiation or chemicals suggesting that such mechanisms do operate. Until recently, no systematic attempt has been made to carefully examine these phenomena or to understand the mechanisms with which they operate. Judging from the international meetings being held, or planned, that situation is apprently changing.

In 1979, T. Don Luckey of the University of Missouri published a book entitled "Radiation Hormesis", in which the author had collected some 1200 references on what he called "radiation hormesis", apparently paradoxical effects of low level radiation exposures. Six years later, the first conference to examine the validity of radiation hormesis was called in Oakland, California. Now, after another seven years, another meeting has been held, this one considerably larger than the first.

Sponsored by three Japanese research agencies, and co-sponsored by the International Atomic Energy Agency, the meeting focused on several questions: What is "adaptive repair?" Does background radiation play any important role in normal biological functioning? and, is there epidemiological evidence of adaptation to radiation in humans?

To address these questions, some 250 speakers and participants travelled from throughout the world to present

papers and talk with colleagues; half of these were Japanese, the other half represented both North American, European, and Asian countries. Strong interest in the subject by scientists in Eastern Europe and the former Soviet Union was evident. Both papers and posters were used to present studies on cells, animals, and humans. While most of the studies discussed were observational, some also attempted to investigate mechanisms.

Participants at this meeting were impressed by the high level of science presented. Great care was used in avoiding the value judgements implied in such words as "beneficial effects," or in the term "hormesis". The preferred terms were "adaptive response", or "stimulatory response".

The following is an overview of highlights:

(1) Evidence for biological effect of background radiation. Professor Hubert Planel of the medical school in Roulouse, France, has been investigating the effects of reduced background radiation on the viability and survival of a variety of single cell organisms. He has consistently shown a deleterious effect at reduced background levels of exposure, and a stimulatory effect at slightly enhanced background levels. Preliminary work from the University Sapienza in Rome also showed an increase in sensitivity to radiomimetic drugs in yeast raised at sub-background radiation environments.

(2) Immune stimulation. Several authors demonstrated a stimulatory effect on the immune system with low doses of radiation. These effects were transient and dissipated following termination of the radiation exposure. Planel has now begun work on mice, where he has found a statistically increased immune function among male animals, but not females, an observation that others have also made.

(3) Priming doses and adaptive responses. That animals will respond with decreased sensitivity to fractionated doses of radiation than to single doses, suggesting adaptation, has long well been known. So

called "priming doses", which must be small (0.5 -2 cGy) have also been shown to increase the LD50 of animals. More recently Wolff and others have shown that a priming dose will also reduce the number of chromosome breaks, mutations, sister chromatid exchanges, and micronuclei formation in cells exposed to subsequent toxic exposures. These effects can be found in a wide variety of cell lines, including human cells, as well as in drosophila, but, they are not universally seen. The development of this adaptive response is under investigation; some hours are required. The response can be transmitted through at least three generations of cells.

A number of studies were concerned with the evaluation of radical formation/scavenging rate under conditions of adaptive response.

(4) Effects of low doses on gene expression. A number of papers reported an increase in DNA transcripts following low dose exposures; however, such responses were not universal. Under which conditions these occur is not yet clear. Some investigators are calling these induced proteins "stress proteins", but whether these proteins are responsible for increased survival or decreased DNA damage is not clear. Radiation induced proteins appear to differ from those induced by heat or other stressors.

(5) It has been known for some time that exposure of animals to any stressor (e.g., chemicals, cortisone) will protect animals against lethal doses of radiation. A number of papers presented data on interactions between low dose radiation and other agents, including radioprotective agents. Several of these showed that the adaptive response induced by one agent reduced the toxicity of the other;

However, this was not consistent, nor are the conditions necessary for this phenomenon well characterized. Two papers showed both synergistic and protective effects (radiation and microwaves, heavy metals), depending on the timing and sequence of exposures.

(6) An adaptive response was sometimes seen in whole animals, but strain differences are important.

(7) Epidemiological studies showed mixed responses to low dose radiation. While some studies (British nuclear workers, patients treated with thorotrast) were consistent with a linear cancer response even at low doses, one study of populations exposed to residential radon showed a protective effect of low doses on the frequency of lung cancer.

A-bomb survivor studies show different dose responses; some cancers (e.g., leukemia) demonstrate a linear-quadratic relationship (with possibly a threshold for some types of leukemia), others a linear response (again, with possibly a threshold for some). In still other cancer types, no effect from radiation is demonstrable at all, even at elevated doses. One attempt at therapy utilizing relatively low doses showed a good response in a small minority of patients with non-Hodgkins lymphoma.

One conclusion appears to be justified; radiation produces an adaptive response, just as do many other toxic agents. But, the Conference raised more questions than it answered. Some of these are:

(1) What is the mechanism of this adaptive response, and under what conditions will it appear? To what extent does this reflect the stimulation of stress proteins, immune phenomena, or other possible mechanisms such as endocrine mediation?

(2) Does this adaptive response occur in whole animals, including human beings, and under what conditions might it be of any clinical significance?

(3) Why do some epidemiological studies of populations exposed to low doses of radiation show responses which appear to depart from the conventional linear or linear quadratic models? Are these differences due to difference in exposure conditions, and if so, what are those exposure conditions?

Answers to these questions could have important implications for radiation therapy as well as cancer prevention.

What is needed to answer these questions is the development of explanatory hypotheses which can be tested, in addition to further observational studies.

Proceedings will be published.

Modeling U-Shaped Dose-Response Curves

One topic presented at the first BELLE workshop held April 30 and May 1, 1991 was devoted to the biostatistical design and interpretation of studies involved with biological responses to low doses of chemicals and radiation. Dr. Tom Downs, professor of biostatistics at the University of Texas, School of Public Health, Houston, Texas, was the guest speaker. His presentation covered biostatistical considerations and suggestions for development of more general models suitable for assessing non linear dose-response data. His report has been published as Chapter 7 in the workshop proceedings ("Biological Effects of Low Level Exposures to Chemicals and Radiation", Lewis Publishers, 1992). With kind permission of the author the section on Quantitative Dose-Response Models is reprinted here. (ref. 15-23)

QUANTITATIVE DOSE-RESPONSE MODELS

Tom Downs, Biostatistical Approaches for Modeling U-Shaped Dose-Response Curves and Study Design Considerations in Assessing the Biological Effects of Low Doses, in "Biological Effects of Low Level Exposures to Chemicals and Radiation", Lewis Publishers, 1992.

QUANTITATIVE DOSE-RESPONSE MODELS

Introduction

Almost without exception the dose-response models studied to date have focused on harmful effects. Current models thus have limited flexibility. Some contain mathematical restrictions prohibiting a decrease in response whenever there is an increase in dose. In such cases the existence of a threshold or of beneficial effects are excluded automatically from consideration. Some suggestions are proposed for development of more general models suitable for hormetic dose-response studies.

Model Criteria

The diversity of carcinogenic agents and responses, the variety of exposure settings and routes of administration, and the lack of detailed scientific knowledge about fundamental cellular processes in cancer all combine to make it unlikely that a single dose-response model can suffice for all situations in which a hormetic dose-response model might be required. Still, some general suggestions about models for U-shaped responses can be put forth:

1. The model should be flexible, adaptive, and parsimonious. If the parameters of the model are sufficiently versatile (e.g., capable of being scalar values or mathematical expressions of unspecified form), then these may be useful for looking at data in new ways and suggesting future avenues of research. Flexible models might be useful as guides for hypothesizing responses to low doses, thus aiding in designing dose regimens for hormetic studies, and in deciding whether a projected response is such that a proposed study will have sufficient statistical power. The flexibility requirement automatically rules out the one-hit linear model whose response Px and slope dPx/dx are given, for an administered dose x, by

$$P_x = 1 - \exp[-a - bx],\ dP_x/dx = b \exp[-a - bx]]$$

because the family of such curves is inflexible: All the curves in the

family are convex upward at all doses for all positive values of the potency parameter b.

2. Above all, a model must be capable of exhibiting a U-shaped response. In particular, it must be possible for the slope dPx/dx of the dose-response curve Px to be negative for some small doses x, while eventually becoming positive for larger x. These are essential requirements. They automatically rule out virtually all the common models in use today. This includes the linearized multistage model, popular with regulatory agencies,16 whose response has the form:

$$P_x = 1 - \exp[-q_0 - q_1 x - q_2 x^2 - \ldots]$$

since this model requires that the fitted coefficients q0, q1, q2, ... All be nonnegative.16 Such nonnegativity forces the slope

$$dP_x/dx = (q_1 + 2q_2 x + \ldots) \exp[-q_0 - q_1 x - q_2 x^2 - \ldots]$$

to be positive for all non-zero doses x.

3. The hormetic components of a model must be scientifically verifiable. Models with a U-shape imposed on them by restricting parameters and mathematical forms should be avoided since implicit in such models is the assumption that there is a beneficial effect at all sufficiently low doses (I.e., there is no hormetic threshold). A quantitative measure of the hormetic effect should be attainable from the fitted model, and the hypothesized existence of a hormetic effect should be testable by statistical techniques.

4. Models should be capable of incorporating into their structure pertinent pharmacokinetic and biologic data. The parameters and mathematical forms of the model should be biologically interpretable.

5. Assumptions should be minimized. If made, they should be justified and checked whenever possible.
6. The suggestions above should not be unduly restrictive. The models ought to be applicable to a reasonably wide set of data, otherwise their usefulness is limited.

Adaptive Models

Adaptive Repair

Downs and Frankowski15 employed Michaelis-Menten kinetics to develop a model with the potential for adaptive repair. For dose x, the probability R of repair has the form of a linear ratio:

$$R = \frac{px + q}{(p - r)x + (q + s)}$$

where the parameters p, q, r, and s are all nonnegative, insuring that R lies between 0 and 1. The number of "hits" from particles of the test substance or its metabolites on susceptible portions of DNA is assumed to be Poisson distributed, with mean the linear ratio

$$H = \frac{ax + b}{cx + d}$$

where here also all four coefficients are nonnegative. Note that when x is 0, the spontaneous repair rate is equal to q/(q + s), and the spontaneous hit rate is equal to b/d. The spontaneous rates of hits and repairs are here neither independent of nor additive with the rates induced by the test substance.

It was further assumed that the number of hits that were repaired followed a binomial distribution, with n equal to the number of hits and with the above R being the probability that any particular hit would be repaired. Then it is readily

shown that the number of unrepaired hits follows a Poisson distribution, with mean equal to

$$y = H(1 - R)$$

Then y is a more valid measure of effective dose than the administered dose x. It is entirely possible that y can decrease as x increases, and in fact this will be the case whenever

$$\frac{dy}{dx} < 0$$

$$\frac{dR/dx}{1 - R} > \frac{dH/dx}{H}$$

In such case the repair is adaptive, with the probability of repair increasing with increasing dose. Eventually though, H may become sufficiently large to overwhelm the enhanced repair (for a thorough discussion of these matters see Downs and Frankowski15).

The multistage model given by

$$P_x = 1 - \exp[-q_1 y - q_2 y^2 - \ldots]$$

which uses y instead of x for the dose parameter, will exhibit hormetic behavior whenever dy/dx < 0 at small doses. Another model employing y instead of x would be the (now nonlinear) one-hit model

$$P_x = 1 - \exp[-y]$$

Graphs of this one-hit model are shown in Figure 7.2, where the hit rate H is given for each of the three curves by

$$H = \frac{10x + 10}{x + 10}$$

while the repair rate is givenfor the top, middle, and bottom curves, respectively ‹ by

$$R = \frac{85x + 9}{100x + 10}$$

$$R = \frac{95x + 9}{100x + 10}$$

$$R = \frac{99x + 9}{100x + 10}$$

Figure 7.2 illustrates the flexibility and adaptivity of this repair-modified one-hit model. In the top curve the repair rate is actually impeded by the test substance, and the response Px increases dramatically at low doses. In the middle curve moderate doses are clearly beneficial, and in the bottom curve repair has been increased so much that the response Px never gets back up to its spontaneous rate.

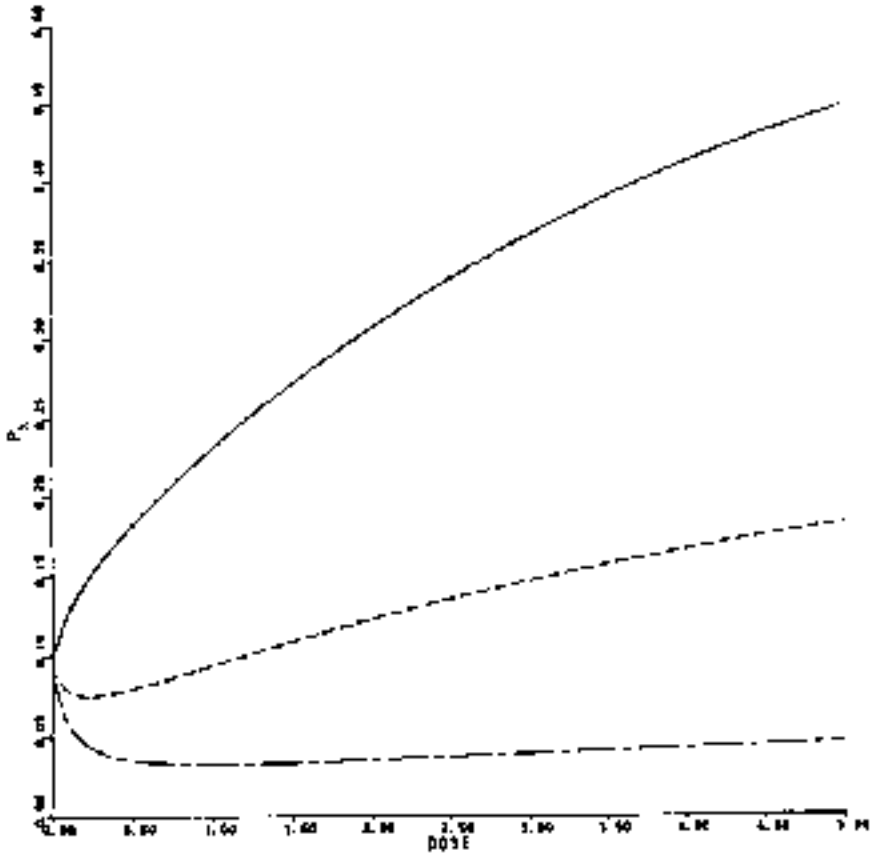

Figure 7.2. Three dose-response curves illustrating the versatility of the one-hit model with adaptive repair.

Any model, such as the k-hit, multistage, or probit,17 can be modified to accomodate nonmonotonic dose-response data by using nonmonotonic functions of the dose, like y above, instead of the administered dose x.

Modified Linear Multistage Model
The multistage model given by

$$P_x = 1 - \exp[-q_0 - q_1 x - q_2 x^2 - \ldots]$$

can be modified directly to accomodate the possibility of beneficial effects by merely allowing the linear coefficient q1 to be negative. This would result in a beneficial effect whenever the dose x satisfies the inequality $q1x + q2x2 < 0$, or equivalently, $x < -q1/q2$. The optimal dose in this case is that value of x that minimizes Px. It is obtained by setting the derivative of P, equal to zero, solving the resulting equation for x, and verifying that the second derivative is positive when evaluated at this value of x.

The multistage model has the advantage of being characterized by a small number of parameters. These parameters may also be readily estimated from long-term animal bioassay data. This may also be a disadvantage, though, because it may be difficult to incorporate pharmacokinetic knowledge into the estimation process. Nevertheless, in the absence of additional biological data, the multistage model will often be the default choice in model-fitting because of its simplicity and parsimony.

Stochastic Two-Stage Model

Moolgavkar and Venzon developed a two-stage stochastic model for carcinogenesis, in which the stages may be thought of as mutations, though not necessarily so.18 A schematic diagram of the model appears in Figure 7.3. Subsequently, Moolgavkar and Knudson expanded the scope of the model, showing how it could be interpreted biologically to explain or help explain such diverse phenomena as age-specific cancer rates, hereditary factors, and environmental agents in the etiology of cancer.19

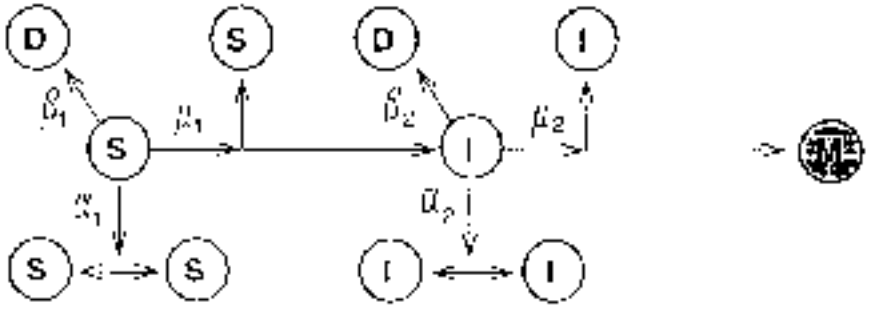

Figure 7.3. Two-stage model for carcinogenesis. S = normal stem cell, I = intermediate stage cell, D = differentiated or dead cell, and M = malignant cell, μ1 = rate at which I cells are formed

from S cells, and μ2 = rate at which M cells are formed from I cells. The rates $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$ of cell formation are as indicated. It is assumed that a single M cell can give rise to a tumor.

When the mutation rate μ2 of the second stage is small, the model implies that the age-specific incidence function I(t) for a tissue in a subject of age t is approximately given by

$$I(t) \sim \mu_1 \mu_2 \int_0^t N(s) \exp[(\alpha_2 - \beta_2)(t - s)] ds$$

where μ1, μ2, $\alpha 2$, and $\beta 2$ are as in Figure 7.3, and N(s) is the number of normal stem cells in the tissue at time s. This approximation was employed by Moolgavkar20 to give precise meanings to the words *initiator* and *promoter*. Later authors have expanded the scope of the model's usefulness in providing a framework for reviewing known facts.21-23

One of the strengths of this model is its versatility: The mathematical expressions for the mutation and birth and death rates of cells have been deliberately left unspecified, so that appropriate forms for such may be derived and tested to fit a variety of circumstances. Thus, while the model specifically requires both stages to be irreversible,22 linear ratios incorporating cell repair notions, like R above, can still be employed and tested in the model. This requires some modification of the terminology: thus, μ1 is now considered as the mutation rate for generating unrepaired intermediate cells from the normal stem cells. Hormetic effects are obtained from this model whenever the net change in cell growth is negative. This model, and variations of it, can be expected to play an important role in the understanding of hormesis.

Table 7.9. Sample Data for Test for Linear Trend

Dose	0	1	2
Number at risk	50	50	50
Number of cases	10	0	20
Percent cases	20	0	40

DISCUSSION

The demonstration of hormetic effects is rendered difficult for a number of reasons: The spontaneous rate must be large enough for a difference to be detectable. In contrast with detrimental effects, there is a limited range of doses over which beneficial effects are likely to be found. Publication bias not only hampers publication of low-dose beneficial effects, but discourages research in the area by not providing sufficient motivation for scientific investigators. Some scientists actually believe that hormetic effects are contrary to reason. All these factors contribute to lessen the chances of detecting hormetic effects through synthesis of the scientific literature.

The extra statistical power obtained from mathematical modeling is not available for hormetic studies when appropriate models are not available. Even a simple statistical device such as a test for linear trend does not work well for U-shaped data. For example, with a control group and two treated groups with dose levels of 1 and 2 units, and with 50 animals per group, a test for linear trend on the data in Table 7.9 results in a statistically significant positive trend ($P < 0.02$).

The range of doses exhibiting hormetic effects will generally be too small and too ill-defined to accomodate a test for negative linear trend, since virtually every substance becomes toxic as the dose increases. Yet data such as the above strongly suggest a hormetic effect may exist.

Nevertheless, there are a wide variety of tools available for studying nonmonotonic dose-response curves. As work in this area progresses, more and more substances will be found which exhibit hormetic behavior with one type of health outcome, and perhaps detrimental behavior with another. The situation will become more complex as our knowledge of synergistic and antagonistic relationships between hormetic and nonhormetic substances increases.

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