Developing Insights on the Nature of the Dose-Response Relationship in the Low Dose Zone: Hormesis as a Biological Hypothesis

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Introduction

The concept of hormesis has had a long and often controversial history. In fact, there appears to be a lack of general consensus within the scientific community of whether hormesis is a reproducible biological phenomenon with an underlying evolutionary/genetic foundation or merely an artifact of endpoint selection and study design limitations. However, the hormetic hypothesis is an important issue to resolve since, if true, broad acceptance of hormesis would have potentially substantial implications for the risk assessment process as well as for important biomedical and therapeutic applications. Consequently, an effort was made to evaluate hormesis as a biological hypothesis based on *a priori* study design, endpoint response, characterization, statistical assessment, and reproducibility of the findings.

Development of the Hormesis Hypothesis

The assumption that an agent is inactive below the toxicity threshold may not be an accurate description of what actually occurs in cells and whole organisms at subtoxic exposure levels. For example, it has long been known that elements such as minerals and vitamins are toxic at high doses, but essential in lower amounts. Also, pharmaceutical agents such as aspirin have an optimal therapeutic zone: too high a dose causes toxicity, while too low a dose renders the drug ineffective.

The hormesis hypothesis states that most, if not all, chemical and physical agents, such as radiation, have the capacity to stimulate biological effects at doses below the toxicity threshold, while causing toxicity at doses above the threshold. Such low-dose stimulatory effects have been referred to as 'hormetic' responses from the Greek word meaning 'to excite'. The shape of the dose-response curve depicting the maxim 'the dose determines the poison' is seen in the high-dose range of the two graphs in Figure 1. The hormesis hypothesis (i.e., that doses below the toxic threshold may be stimulatory) yields a different dose-response relationship. When the response refers to factors such as growth, longevity, fecundity, and weight gain, the curve displays what is called a 'b'-curve (i.e., a low-dose stimulation followed by inhibition of the stimulated response at higher doses). However, if the response at low doses were to diminish effects such as mutations, background cancer, or birth-defect incidence, the dose-response curve would be a 'U'- or 'J'-shaped curve. In such cases the low-dose treatment group would display less damage than the unexposed control group. The phenomenon of low-dose stimulatory effects was first reported in 1888 by Hugo Schulz[1] from studies on yeast fermentation. These findings were integrated with similar observations by Rudolph Arndt in what became known as the Arndt-Schulz Law, which stated that all poisons are stimulatory in low doses. This so-called law was believed to be applicable to most, if not all, stressor agents, such as toxic chemicals, medications, and radiation, and to most biological systems.

While the origin of modern-day hormetic research can be traced back to the work of Schulz over a century ago, other investigators independently reported comparable effects. The well known bacteriologist, Ferdinand Hueppe, an associate of the Nobel Laureate Robert Koch, extended the observations of Schulz to bacteria. In fact, this phenomenon has also been widely referred to as Hueppe's Rule. The University of Wisconsin professor Louis Kahlenberg, a former Ph.D. student of the Nobel Laureate Wilhelm Ostwald, reported that low doses of toxic substances stimulated plant growth within the context of assessing the biological effects of dilute solutions. In addition, the Nobel Laureate Charles Richet, at the University of Paris, who developed the concept of anaphylaxis, also published extensively on this topic. Early findings likewise published by well-established academic researchers with fungi (e.g., B.L. Duggar at Cornell University/University of Wisconsin; H.M. Richards at Columbia University) and bacteria (C.E.A. Winslow with a series of his Ph.D. students at Yale University), along with numerous other respected investigators, helped to establish the generalizable, reproducible and quantitative nature of the hormetic response over the early decades of the 20th century. In fact, as early as 1905 the basic description (e.g., b-curve) of the hormetic dose-response (as seen in Figure 1b) was clearly established in the scientific literature by Rodney True, a former colleague of Kahlenberg (see [2] for a review).

Despite these developments in the field of low dose hormetic effects through the early decades of the 20th century the Arndt-Schulz Law became progressively marginalized from the 1920's onward.[4] One reason is that it failed to offer a convincing underlying mechanism of how the agent induced the hormetic response. Also, the low-dose stimulatory effects were often seen as an artifact of the study design or normal variation that at times disappeared on further or more rigorous testing. The hormesis concept also fell victim to the historical conflict between homeopathy and 'traditional' medicine since many homeopaths viewed the Arndt-Schulz

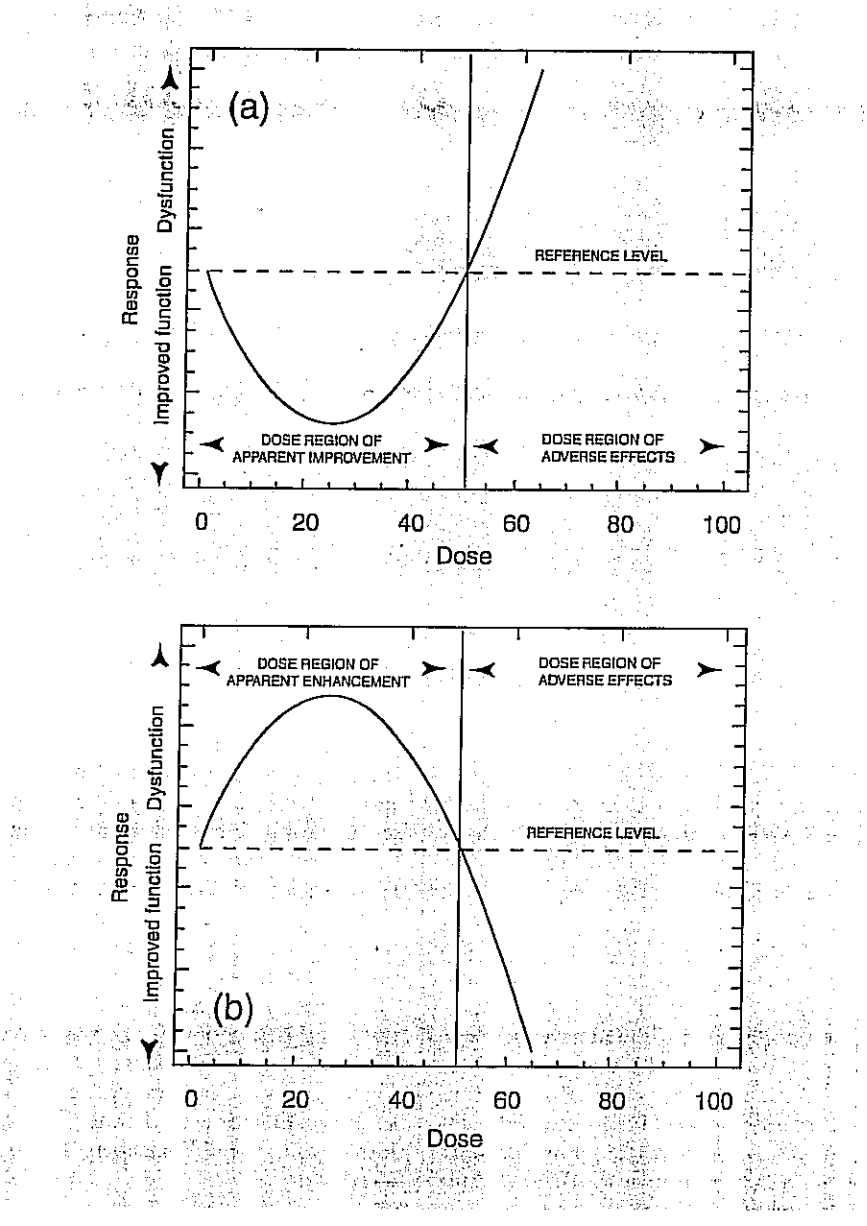

Figure 1. (a) General form of U-shaped dose-response curve showing response relative to a reference level, with a region of apparent improvement (e.g., reduction in dysfunction) as well as a region of toxic or adverse effects. (b) Reciprocal of the same curve showing a region of apparent enhancement (e.g., increase above normal level of function) as well as a region of toxic or adverse effects.[3]

Law as a supporting principle of homeopathic practices. But perhaps the most compelling explanation is that the percentage of studies capable of accurately studying hormesis as a biological hypothesis has been too few.

Traditional toxicologists have generally overlooked the potential for hormesis, focusing instead on regulatory driven hazard assessment experimentation, which emphasizes defining the high-dose component of the dose-response curve. Consequently, there has been a limited amount of experimentation to assess responses at doses below the threshold of toxicity. In fact, it is probably fair to say that most toxicologists not only have failed to study hormesis, but have not even observed bona fide examples of this phenomenon.

Hormetic Mechanisms

When the imposition of an external stressor agent (e.g., pollutant exposure) challenges the adaptive capacity of a biological system, the system typically more than compensates for the initial disruption and/or damage, leading to the net stimulatory (i.e., hormetic) response. Thus, hormesis represents an overcompensation to an alteration in homeostasis. As the dose progressively increases the system's capacity to compensate becomes overwhelmed, the 'no observed adverse effect level' (i.e., 'NOAEL') is exceeded, and evidence of toxic effects becomes manifest via biochemical and histological techniques.

The strongest effort to explain the mechanism of specific hormetic responses has been made in the area of herbicide-induced stimulatory effects. Herbicide researchers have long recognized hormetic responses to such an extent that they have studied not only the molecular basis for the responses, but also why species differ in responsiveness, how plant age affects the response, the impact of minor structural changes to the molecule and how this impact may alter the hormetic response.[5]

The range of hormetic effects such as increased growth, fecundity, longevity, and decreased disease incidence suggests that these effects are fundamental and affect thousands of genes. This further implies that hormetic mechanisms affect basic biological processes. Nonetheless, investigators often direct their attention to mechanisms closely attuned to aspects through which biological protection may be mediated. For example, there is substantial evidence that very specific alterations in patterns of gene expression in numerous species occur in response to toxicant exposures.[6] Such responses fall into one of two classes: (1) those resulting in an enhanced metabolic capacity for detoxification of the particular toxicant (e.g., the cytochrome P-450 gene family whose products play an essential role in detoxification of a vast array of organic contaminants); or (2) those that

offer more general protection against cellular damage caused by a wide variety of agents (e.g., heat-shock or stress proteins whose expression is mediated by a wide variety of stressors).

The field of molecular biology has recently provided tools to enhance understanding of the mechanistic foundations of hormetic dose-response relationships. Of particular interest is the 'adaptive response' phenomenon that occurs in both radiation and chemical toxicity. In general, if a low, and often nontoxic, exposure to radiation or a toxic chemical is administered either to cells in culture or to whole organisms, and is then followed by a massive exposure to the agent that would normally seriously injure or even kill the cells or organisms, the preexposed cells or organisms display remarkable protection from toxicity and lethality.[7] This phenomenon has been intensely studied for over a decade, resulting in several hundred research papers providing important insights into how the protection occurs, the nature of the dosing that elicits the response, why some cells and organisms differ in their responsiveness, and the overall generalizability of the phenomenon.[8] Although the adaptive-response phenomenon technically differs from hormesis as described in this dialogue, the induction of molecular adaptive responses at the low levels used in these experiments may provide a sound model for how hormetic processes are triggered.

Assessing the Viability of the Hormetic Hypothesis

Despite the lack of mainstream acceptance of hormesis, various groups and individuals have tried to document the phenomenon and highlight its implications for society. Over the years, evidence of hormetic responses has been reported in peer-reviewed journals, summarized in books, and the subject of national and international conferences. For example, Dr. Thomas Luckey, professor emeritus of the University of Missouri, wrote two books extensively documenting radiation hormesis.[9, 10] Investigators in the area of plant biology published a newsletter from 1970-1981 on the capacity of low doses of radiation to enhance plant growth and yield.[11] Independent groups of researchers have organized international scientific conferences in China, Japan, Russia, and the United States on chemical and radiation hormesis.[12, 13] Of particular importance with respect to the scientific dimensions of hormesis are recent advances concerning adaptive-response mechanisms, such as induction of detoxification mechanisms, DNA repair, heat-shock protein, acute-phase protein, and other responses that have been shown to alter cellular and organismal responses to toxic substances.[6]

A research group at the University of Massachusetts at Amherst, of which the author is a part, recently set forth to assess the viability of hormesis as a scientific hypothesis.[14] The group used as a guide the operational definition that hormesis is characterized by low-dose stimulation and inhibition at higher doses, as seen in the ß- and U/J-shaped dose-response curves discussed above. A search of various computer databases yielded over 8,000 studies potentially relevant to hormesis. Approximately 1,500 of these appeared sufficiently relevant to obtain and carefully assess with objective criteria to screen out those articles that did not present evidence of hormesis, as well as provide a quantitative ranking of agents with respect to degree of evidence and documentation to support a hormetic hypothesis. The research team developed the following criteria to determine whether a study presented evidence of hormesis:

(1) the nature of the study design (e.g., the total number of doses, the number of doses below the toxic threshold, the presence of an adequate control group)

(2) the type of effects the study measured

(3) the magnitude and statistical significance of the responses and

(4) the capacity of data replication.

These criteria were assigned point values, which were then applied to the studies. The results provided the basis for determining whether a given study displayed no, low, moderate, or high evidence of hormesis. Using this assessment nearly 500 of the nearly 1,500 articles the group has reviewed to date have shown evidence of hormesis to some degree.[15]

This assessment has revealed much about the types of endpoints (i.e., effects) that display hormesis, the kinds of organisms and agents used in the studies, what may actually be a low dose, the range and magnitude of hormetic responses, and how studies should be designed if the investigator's intent is to study low-dose phenomena. The findings indicate that low-dose stimulatory responses are not restricted to any particular taxonomic group but are observed broadly across the microbial, plant, and animal kingdoms. Though not unexpected, this is highly significant because it illustrates the broad generalizability of the hormetic phenomenon. Likewise, the types of agents shown to cause hormesis are also without apparent restriction, consisting of chemicals of seemingly all chemical classes and different types of physical stressors, including various kinds of radiation. The range of biological effects observed with respect to hormesis is also widespread and includes growth, longevity, reproduction, disease incidence, and behavioral aspects. Thus, with respect to generalizability to species, agent, and endpoint, hormesis is potentially far-reaching, as is evident from the following examples.

Antibiotics are expected to kill and/or prevent bacteria from reproducing. Studies have shown, however, that low doses of antibiotics such as streptomycin actually enhance reproduction of certain harmful strains of bacteria at low doses, while killing these strains at higher doses.[16] In fact, administering low doses of streptomycin can actually enhance the capacity of the microbe to kill the host.[17] FDA researchers recognized this phenomenon over 50 years ago. It has also been observed with penicillin and other antibiotics.

In the area of plant biology, research shows that certain agents can stimulate growth at low doses, while retarding growth at higher doses. For example, numerous herbicides have been shown to induce stimulation of both root and stem growth at low levels.[18] Of particu-

lar significance is the substantial body of work dealing with the triazine herbicides, which include the agents atrazine and simizine. Low doses of these agents have been repeatedly shown in greenhouse and field studies to enhance growth and yield of various plants in the absence of competing weeds.[19] How these low-level exposures enhance the growth of economically important plant varieties has become a major area of research.

In addition, low doses of inorganic substances (such as cadmium, fluoride, and mercury), organic substances (including a wide range of pesticides), and radiation have been shown to enhance the fecundity of a variety of organisms, including crustaceans, insects, worms, fish, and mammals.[20,21,22,23,24] This is particularly relevant to entomologists and others concerned with insect control. The observation that low levels of environmental toxins can enhance reproductive performance runs counter to the prevailing concept that a reproductive toxin is simply that, and its capacity to affect the biological system is merely proportional to dose. In these instances, the investigators clearly show the paradoxical influence of dose in affecting the final outcome of the study.

Finally, a well-studied example of the J-shaped curve in humans is the relationship between ethanol consumption and the risk of cardiovascular disease. Numerous reports from the epidemiological literature indicate that persons who consume several alcoholic drinks per day have a demonstrably lower risk of heart attack than both individuals who consume excessive amounts of alcohol and those who abstain from drinking alcohol.[25] Of importance is that the response seems to have a reasonably solid underlying mechanistic explanation involving the enhancement of the HDL proteins, the so-called good cholesterol, which are known to protect against cardiovascular disease.

Despite the evidence, why does accepting hormesis remain a stumbling block for many scientists? First, although there are many examples of hormetic dose-response relationships, such studies actually constitute a very small percentage of the whole toxicological database. For example, about 500,000 toxicology studies have been published in the peer-reviewed literature since 1900 (Index Medicus, Chemical Abstracts, Biosis). Yet, in order to have a reasonable chance of obtaining a high score for evidence of hormesis based on the group's evaluation system, a study must have at least six total doses, with at least three doses in the subtoxic threshold range. Studies so designed are relatively few: only about 1 out of every 500 published studies would satisfy the dose conditions.[14] This alone is sufficient to explain why many toxicologists know little about the concept of hormesis.

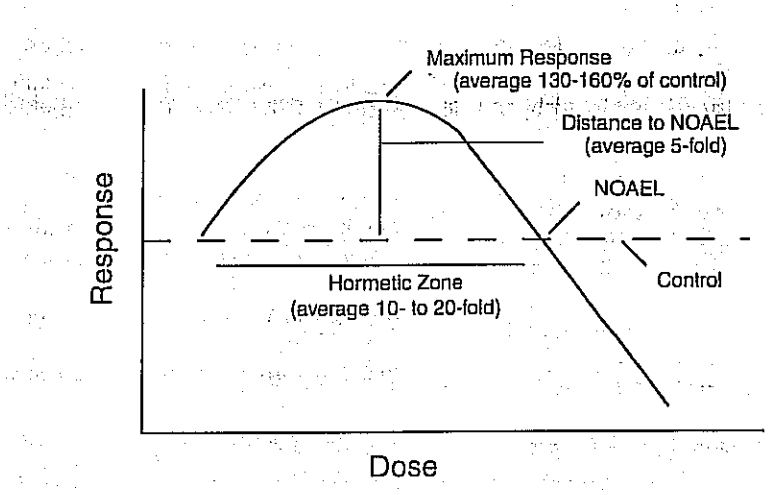

Figure 2. Dose-response curve depicting characteristics of the chemical hormesis zone. Note that the magnitude of stimulation is typically 30-60% greater than control values while the zone of stimulation extends on average approximately over a 10- to 20-fold range.[26]

Second, as seen in Figure 2, hormetic responses have been repeatedly shown to occur over a limited dosage range and response magnitude. For example, the average maximum stimulatory response is about 30 to 60 percent greater than the control. When responses are seen in the 'percentage' increase zone rather than in the '-fold' increase zone, the stimulatory response may often be interpreted as normal variability rather than a real stimulation (i.e., a 'false positive' response). Recognition that hormetic responses are of a generally modest nature places greater experimental design demands on the researchers, since more treatment groups are needed to define the nature of the dose-response relationship, especially in the low-dose zone where the hormetic response would be expected to occur. In addition, the more limited nature of the response requires that even more attention than usual be devoted to sample size and statistical issues. Such constraints suggest that in experiments designed to investigate the low-dose area, it is wise to use more subjects in the low-dose treatment groups than at the higher doses, due to the treatment effects' limited magnitude and the variability of response.

Recent analyses of the developing hormesis database indicates that there is a considerable range in the type of U- or inverted U-shaped dose-response that differ from that offered in Figure 2. Such a recognition has lead to our developing of a recent general classification of U-shaped dose-response.[26] More specifically, reliable evidence indicates that the range of stimulatory response can greatly exceed 10- to 20-fold while the magnitude of stimulatory response can at times approach 10-fold that of controls. In addition, the stimulatory response at times appears to be a compensatory response to a disruption in homeostasis or a direct stimulation. There is clearly much more to learn about the range of hormetic effects and their diverse underlying mechanisms.

Potential Significance of Hormesis to Environmental and Health Related Decision Making

What is the potential significance of hormesis, especially to federal regulators and environmental health scientists? Although it is too early to say precisely, the possibilities are intriguing. Federal regulatory agencies, using the concept of threshold responses, have adopted risk assessment procedures that assume that noncarcinogenic toxic substances cause harmful effects above a toxic threshold, below which no adverse effects are expected to occur. While the agencies have focused on adverse effects, they have given virtually no consideration to whether other biologically significant effects might occur below the so-called toxic threshold. However, the generalizability of hormesis to most compounds suggests that agencies such as EPA, the FDA, and OSHA should carefully consider applying this concept to their risk assessment procedures.

Each agency might have a different perspective on evaluating the role of hormesis based on its respective responsibilities. For example, OSHA's occupational health standards are designed to permit higher exposure levels than community exposure standards. EPA's and the FDA's risk assessment procedures are currently designed to protect the public against the harmful effects of toxic substances. In fact, the resultant standards are believed to be sufficiently conservative so as not only to achieve the protection goal, but also to reduce the exposure below the estimated hormetic dose. If the agent produces a significant biological response that is beneficial (such as longevity) at low doses, and such low-level exposures are not permitted, these standards may, in fact, be counterproductive.

Thus, all regulatory decisions on noncarcinogenic chemical and physical agents should, at a minimum, address how the proposed standard affects possible hormetic responses. Every exposure standard should provide a description of the biological and population-based responses for the entire dose-response relationship, not just for the higher (e.g., greater than the NOAEL) doses, which has been the standard practice to date. Regulatory agencies need to provide information on the complete set of public health implications regarding each exposure standard beyond the benefit of avoiding potentially harmful exposures. Moreover, if the standard, by preventing low-level exposure to a regulated agent, has eliminated the attainment of potentially beneficial effects, agencies need to recognize and justify this decision in future standard-setting activities.

Risk assessment regarding exposure to carcinogenic agents could also explicitly address possible hormetic responses. Federal agencies generally assume that there is no safe level of exposure to carcinogens. However, the hormesis concept, which is more supportive of the threshold phenomenon, suggests that this is incorrect. In fact, a number of cancer-related bioassays that cover a broad dose range and have a modest to high tumor background incidence in controls display decreased foci formation, hyperplasia and/or tumor incidence at low doses as predicted by the hormesis hypothesis.

Insect Control

Several factors contribute to insect outbreaks, including the destruction of competitive species and the development of resistance in certain species.[21] Entomologists are seeking to determine whether low-level exposures to certain insecticides can provide a low-dose physiological stimulus that actually enhances insect fecundity. There is a growing body of evidence that this phenomenon has contributed to some insect outbreaks. Such evidence suggests the necessity of reevaluating the strategies for pesticide use in insect control and eradication programs.

Weed Control and Plant Growth

The potential of low levels of herbicides to enhance plant growth should also be given serious consideration. Hormetic effects may be relevant not only to weed control, but to growing plants for commercial purposes.

Global Warming

A recent study from the Netherlands[27] suggests that widespread low-level contamination of soil may enhance the metabolism of soil microbes, thereby increasing the release of carbon dioxide into the atmosphere. Increased levels of atmospheric carbon dioxide are believed to contribute to global warming. The Netherlands findings have led to the preliminary hypothesis that hormetic responses thus contribute to factors enhancing global warming.

Endocrine Disputing Agents

There has been a major debate regarding chemicals that have the potential to cause disruption of the endocrine system. Within this context, it is important to note that a highly diverse set of chemical agents known to cause alterations in reproductive performance at high doses are also known to enhance fecundity at lower doses.[20,21,22,23,24] These observations suggest the need to consider carefully the issue of dose and underlying mechanisms of biological effects at high and low doses, and how these factors may affect regulatory strategies in dealing with these agents.

Adaptive Response

In the wake of the Chernobyl nuclear reactor accident, many individuals developed radiation poisoning from entering the contaminated facility. The research on adaptive response[6,7,8] suggests that, if time had permitted, it may have been wise to have preexposed those individuals with a low dose of radiation or some other stressor agent. While this is speculative to some extent, the foundations for this type of action are substantial.[7] Clearly, this type of emergency procedure should be better understood in order to reduce risk from exposure under similar circumstances.

Conclusion

The journey to explain what chemicals may be doing to cells and whole organisms at low doses has been a toxicological

'road less taken.' Despite the developing database on chemical and radiation hormesis and the growing confidence in the phenomenon as a broadly generalizable hypothesis, hormesis still suffers from lack of adequate recognition within the scientific and regulatory community. It is vital, however, for society to better understand how hormesis works. Recognition of such a generalizable phenomenon has far-reaching scientific and societal implications with the potential to affect the:

1) evaluation of all drugs and chemicals
2) establishment of exposure standards
3) cost of regulatory activities
4) optimization of strategies for microbial, insect, weed and other pest control activities;
5) development of more effective therapeutic strategies including improved dosing regimens and temporal treatment sequencing and
6) establishment of optimization strategies for multi-physiologic system (e.g., immune, central nervous system, etc.) functioning and other system stress modulation.

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