BELLE Newsletter Vol 1, No. 3, March 1993

STRESS PROTEINS AND ADAPTIVE RESPONSES

This issue of the BELLE Newsletter includes a paper by Professor Joan Smith-Sonneborn on the role of stress proteins as a basic mechanism of cellular adaptation to a wide range of chemical stressor agents. The role of stress proteins as a biomarker of chemical exposure as well as a fundamental adaptive response was repeatedly addressed in individual presentations at the recent annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC) held in Cincinnati (November 1992). Likewise, the role of stress proteins as an adaptive response to radiation-induced cellular stress and/or change was again emphasized at the international conference on the Biological Effects of Low Level Radiation held in Kyoto, Japan (July 1992). The challenge of Professor Smith-Sonneborn's paper is that it attempts to provide a cogent synthesis of this rapidly evolving field with the intention of assessing whether the stress protein response not only is fundamental to the process of cellular adaptation, but may also provide the cellular underpinning of many observations referred to as U-shaped or hormetic dose-response relationships.

GETTING INVOLVED WITH BELLE

Many individuals have asked how they could get involved in BELLE activities. This was discussed at the most recent Advisory Committee meeting. While this will be an evolving issue, the Committee offers several specific suggestions.

1. Work with the BELLE Office to get articles on BELLE published in newsletters or other publications that you may be associated with. This would extend the BELLE message to other interested groups. For example, a recent article on BELLE was published in the Epidemiology Monitor.
2. The BELLE Finance Committee is developing a strategy to yield stable and adequate funding for BELLE. If you have suggestions that should be considered by the Finance Committee contact the

BELLE Office.

3. Work with the BELLE Office to develop a mini-symposium on BELLE at regional conferences that relate to your specialty.

BELLE IN THE CLASSROOM

We have a number of extra newsletters. If you would like to use the newsletter articles for classroom reading assignments, we are able to supply available newsletters on a first-come basis.
Book Reviews

BOOK REVIEWS

Two books are reviewed that discuss topics involving the biological effects of low level exposure to chemicals (Ultra Low Doses) and radiation (Radiation Hormesis).

Ultra Low Doses

Edited by C. Doutremepuich, Taylor and Francis, Ltd., 4 John St. London WC1N 2ET, UK; Taylor and Francis, Inc., 1900 Frost Road, Suite 101, Bristol, PA 19007, USA; 162 pp., $59.00, ISBN 0-7484-0021-4.

The first International Congress on Ultra Low Doses was convened on September 1990 in Bordeaux, France. The purpose of this assembly was to bring together researchers from multidisciplinary areas to discuss the effects and applications of ultra low doses of chemicals. Ultra Low Doses is a summary of the proceedings of this Congress organized into five chapters according to discipline: Experimental Pharmacology, Biophysics, Biochemistry-Toxicology, Cell Biology, and Clinical Pharmacology. The chapter on "Experimental Pharmacology" presents data covering a wide range of biological responses including intestinal transit time, platelet aggregation, and lymphatic vessel contractions that are affected by homeopathic doses of various chemicals or regulatory peptides. Studies involving the in vivo modulation of carcinogenesis by ultra low doses of carcinogens and the in vitro reduction of tumor cell drug resistance with combination doses of immune cytotoxic factors and chemo-therapeutic drugs are also reported and discussed.

The development of experimental protocols to evaluate the influence of ultra low doses of chemicals and to aid in the design of new anti-inflammatory agents are presented in the chapter devoted to "Biophysics". The creation of a

predictive model derived from detailed biophysical characteristics of molecules is also proposed to identify other compounds with anti-inflamatory properties.

The chapter entitled "Biochemistry-Toxicology" focuses on two diagnostic approaches for the early detection of renal dysfunction or toxicity based on corpuscular derangement and degranulation of mesenteric mast cells. It is suggested that these methods could provide sensitive in vitro screening tools for identifying effects of low and ultra low doses of chemicals.

The discussion of potential models for the screening of low and ultra low doses of drugs is continued within the context of "Cell Biology" in the subsequent chapter. The effects of multiple ultra low doses of agonists and inhibitors on oxidative metabolism and adhesion of human neutrophils are evaluated and the advantages of the simultaneous measurements of two cell functions over other methodologies are examined. Analysis of the effects of ultra low doses of histamine on human basophil degranulation by two different protocols are also reported and compared. The hypothesis that dilutions of a given substance can modulate the biological effects exhibited by this same substance at higher concentrations was evaluated with the experimental model of concanavalin A (Con A)-dependent mitogenesis of mononuclear cells. This study showed that doses of Con A too low to induce a direct mitogenic effect were capable of modulating a response induced by a higher concentration of the same substance.

The final chapter examines the efficacy of ultra low doses in relation to "Clinical Pharmacology". The use of ultra low doses of copper in the treatment of hemodialysis-related muscle cramps is described and the therapeutic effectiveness of homeopathic medicine is discussed. The phenomenon of reduced bleeding time induced by ultra low doses of aspirin, contrary to results observed with higher doses of aspirin, was demonstrated in one study involving adult males. The clinical implications of these findings are addressed and future studies are proposed.

Overall, this book represents a broad scope of experimental research involving the effects and possible mechanisms of ultra low doses of chemicals. These proceedings provide a rare opportunity to compare in one source the recent advancements, applications, and problems encountered in the study of ultra low doses over a wide range of disciplines.

The following book review by David S. Gooden, Saint Francis Hospital, Tulsa, OK, is reproduced from the journal

Health Physics (Volume 63, pp. 240-241, 1992) with permission from the Health Physics Society.

Radiation Hormesis
By T.D. Luckey
CRC Press, Inc., 2000 Corporate Blvd., NW, Boca Raton, FL 33431, 1991, 239 pp., $139.00, ISBN 0-8493-6159-1.

Two contradictory theses of radiation injury exist today. The zero thesis states that all radiation exposure is harmful. The linear, nonthreshold model characterizes the zero thesis. Most, if not all, of the world's advisory and regulatory organizations embrace this model.

The hormesis theory, on the other hand, holds that large and small exposures to radiation produce opposite results large exposures are harmful but small exposures to radiation stimulate beneficial results in all living things (including humans!). Many consider a single individual, Dr. T.D. Luckey, to be the champion of the hormesis theory. Luckey states that the purpose of this new book "is to review all available evidence and present a basis for the conclusion that hormetic exposure to ionizing radiation is beneficial to humans."

Luckey states that after the holocaust at Hiroshima and Nagasaki, the world "was mesmerized" into accepting the thesis that all doses of ionizing radiation are harmful. Luckey seeks to replace the myth with the concept that "low doses of ionizing radiation are beneficial." Luckey devotes an entire chapter to reporting studies that support his premise that exposures to low levels of ionizing radiation provide increased growth rates, development, and survival.

In his chapter on "Radiation Hormesis and Reproduction," Luckey quotes a study involving 44,000 pregnant Brazilian women exposed to 5-10 times the average background radiation. This study identified no unusual congenital abnormalities, stillbirths, or change in live births. Another study suggests fewer spontaneous abortions and neonatal deaths among Chinese peasants living in high background radiation areas. Luckey claims that even the Hiroshima and Nagasaki data suggest a hormetic effect on stillbirths, general defects, and neonatal deaths at doses below 0.1 Gy.

Luckey's chapter on "Radiation Hormesis in Immunity" reports many studies that show both the cellular and hormonal

elements of the immune system that are stimulated by low doses of ionizing radiation. Luckey proposes a biological explanation for hormesis in the immune system: A disproportionate reduction in the suppressor T cells at low doses of ionizing radiation allows helper and functioning T cells to operate more effectively.

"Radiation Hormesis in Cancer" is an exciting 80-page chapter with more than 70% of the space devoted to information detailing human studies. Luckey takes data that most of us have seen before and looks at it absent the paradigm of the linear, nonthreshold model. With no apparent scientific or mathematic "sleight of hand," he uses the Hiroshima and Nagasaki studies, the uranium miner studies, nuclear facility studies, above-ground atomic testing studies, and other studies to support the hormesis theory.

In the final chapter, Luckey sums up data in support of the hormesis theory. He states, "The dose is everything! This maxim of toxicology applies to ionizing radiation. Large doses are harmful; small doses are stimulatory. Dose and dose rates are cardinal issues in our evaluation...many of the reports cited (here) provide irrefutable evidence that high and low doses of ionizing radiation produce biometrically opposed effects. Biopositive effects are consistently reported when low doses of ionizing radiation were used."

Does this work prove radiation hormesis? No, Luckey does not aspire to the lofty goal of proof. Does this work describe a viable basis for a theory of radiation injury that competes with the linear, nonthreshold model? Yes, Luckey appears to meet this goal. He seems to do so without taking greater liberties with data and conclusions than we routinely accept in our BEIR, NCRP, and ICRP committee reports. As servants of the people and advisors to the regulatory process, scientists must carefully consider the implications of this important work.

BELLE BOOK
The telephone number listed in the last issue of the BELLE Newsletter to order your copy of the book entitled Biological Effects of Low Level Exposures to Chemicals and Radiation is incorrect.

The correct number for Lewis Publishers, Chelsea, MI is 1-800-272-7737, ISBN 0-87371-665-5.

Primer on Stress Proteins
Organisms exposed to a variety of physical, chemical and biological stresses respond by synthesizing a unique set

of polypeptides termed "shock" or "stress" proteins. This is a highly conserved, rapidly inducible phenomenon observed in a wide variety of procaryotic and eucaryotic organisms.

The first stress response was discovered in the salivary gland of fruit flies exposed to heat. Hence the initial term "heat shock proteins" and the grouping of these proteins into families based on molecular weight (e.g., hsp 100, hsp 90, etc.). Stress proteins are also expressed in response to a wide variety of stressors, including radiation, anoxia, ethanol, oxidizing agents and heavy metals.

The functions of stress proteins are implicated in diverse cellular processes and their role as an adaptive, protective response has been proposed and expanded upon in the following article, which was previously published in the book Biological Effects of Low Level Exposures to Chemicals and Radiation, Lewis Publishers, 1992.

THE ROLE OF THE "STRESS PROTEIN RESPONSE" IN HORMESIS

Joan Smith-Sonneborn
Zoology and Physiology Department, University of Wyoming, Laramie, Wyoming

Hormesis refers to the phenomenon of induction of beneficial effects by low doses of otherwise harmful physical or chemical agents: [1] "a little bit of bad can be good for you." That the hormetic response may operate by a common mechanism already has been proposed [2, 3] but this review is the first to propose the hypothesis that the common pathway is a heat shock-like response. The heat shock response is a model for a more general phenomenon, called "the stress response." The stress response is characterized by increased synthesis of a family of stressor specific proteins with concomitant reduction of synthesis of most of the proteins transcribed prior to the exposure to the toxic agent.[4] The stress response has been characterized using heat, radiation, heavy metals, and oxidizing agents as the stressors.[5] To develop the hypothesis that the hormetic response may operate through the stress response, this chapter includes

1. identification of agents known to induce both the stress response and hormetic phenomena

2. a description of the unique and common pathways in the stress response to three stressors: heat, DNA-damaging agents, and teratogens
3. the stress response as a model for teratogen-induced damage
4. a theory explaining the paradoxical beneficial response to low doses of an otherwise harmful agent via a stress-response pathway

Hormetic agents and stress inducers

Hormetic agents are highly diverse, including heavy metals, polychlorinated biphenyls, insecticides, alcohol, oxygen poisoning, cyanide, antibiotics[6] ionizing radiation[7] cosmic or gamma radiation,[8,9] electromagnetic radiations,[10,11] and ultraviolet plus photoreactivation.[12] Examples of beneficial biological responses include increased life span, cell division rate,[10,12] accelerated maturation time, and acclimation (see Table 2.1).[6,7,12-21]

Table 2.1. Agents Identified As Both Hormetic and Heat Shock Agents

Hormetic and Heat Shock	Agents Beneficial Response	References
Cadmium	increased survival	6,13
	increased growth	6
	increased hormone secretion	6
	increased acclimation	14
Mercury	increased survival	6,13
	increased growth	6
	increased ATPase	6

Copper	increased survival	6,13
	increased growth	6,13
Zinc	increased survival	6,13
	increased growth	6,13
Ethanol	behavior	6,13
Oxygen poison	vital signs improved	6,13
Chloramphenicol	increased growth	6,13
X-rays	Increased mean life span	7,15
	faster seed growth	16
Ultraviolet radiation	life span (UV + PR)	12,17,18
Heat	suboptimal benefit	19,20,21

Agents identified as both hormetic agents and inducers of the stress response are listed in Table 2. 1. There is an impressive overlap between the hormetic and stress response inducers, though no experiment was designed to correlate induction of the stress response and the onset of a beneficial biological effect. However, a biomarker of the stress response is induced resistance to the stressor. The induction of resistance or acclimation to challenge with higher doses or prolonged exposure to the stressing agents include the following:

1. Prior heat treatment can induce survival of organisms to higher temperatures.[20,22]
2. Prior heat treatment can prevent heat-induced developmental defects.[21]
3. Hormetic agents like cadmium and ethanol can induce cross-resistance to other environmental stressors like heat-inducing

thermotolerance.[20]

4. In E. coli a chemical mutagen, MNNG, can induce resistance after the first hour of treatment by induction of a novel form of repair.[23]
5. A more youthful resistance to ultraviolet irradiation was found in Paramecium exposed to a prior regime of ultraviolet and photoreactivation treatment at doses that affected an increased mean life span.[12]
6. Fish with prior exposure to cadmium could regenerate clipped fins faster than nonexposed organisms in cadmium-contaminated water.[14]

Since not all stressors induce the same transcripts or metabolic changes (see below), all stressors need not be hormetic agents. However, this proposal predicts that seemingly unrelated agents that induce the same stress response should stimulate the same biological effects and induce cross-resistance. Likewise, stressors with opposing alterations in chromatin should increase sensitivity, not resistance, to the stressing agent. Available data on the molecular biology of the stress response to various stressors is reviewed below as a potential model of a hormetic pathway.

The stress response

The stress proteins are divided into two groups: those referred to as the heat shock proteins, first found induced by nonphysiological exposure to heat; and those called the glucose-regulated proteins, which exhibit increased synthesis when cells are deprived of glucose or oxygen, or when calcium homeostasis is disrupted. Members of the two families exhibit considerable homology.[4]

Heat

The first stress response detected was appearance of puffs induced in the salivary gland of fruit flies by heat and dinitrophenol.[24] The heat shock response is universal from bacteria to humans.[13,20,25] The stress response induced

by heat is characterized by transcription of a coordinately regulated subset of induced proteins, repression of the transcription, and translation of previously active genes and preexisting messages.[13] The heat shock proteins are members of families of proteins with species-related molecular weight range classes. In higher organisms, the high molecular weight families are Hsp 110, a normal nucleolar protein found in vertebrates; the Hsp 100 family (Mr 92-102), a phosphoprotein normally present in the plasma membrane; Hsp 89 (Mr 83-95), found in the soluble protein in all animal cells; and the Hsp 70 family (Mr 68-78).[4,25] The multigene Hsp 70 family has members in the cytoplasm, in the lumen of the endoplasmic reticulum, and in the matrix of mitochondria which function in protein translocation across membranes.[26]

The heat shock protein Hsp 70 is a major factor in the heat response since

1. mammalian cells in which Hsp 70 is not made or is inactivated by antibody binding cannot develop thermotolerance[27]
2. cycloheximide can induce tolerance to higher temperatures, but heat shock proteins are required for full protection[28]
3. the thermolability of mouse oocytes is due to the lack of expression and/or inducibility of Hsp 70[29]

Smaller Hsp's (15-28 kd) bind reversibly to the nuclear skeleton during heat shock and form higher order aggregates. A common central domain of the four small Drosophila Hsp's[22,23,26,28] show great similarity to alpha crystallin.[21,30]

The smallest Hsp's are the ubiquitin family (7-8 kd),[31] which have been implicated as regulator molecules in chromatin, DNA repair, meiosis, sporulation, degradation of abnormal proteins,[32] ribosome biogenesis,[33] and facilitation of transposition.[34]

Besides the induction of heat shock proteins, other metabolic changes found in response to nonphysiological heat

exposure, which impact on chromatin structure, include

1. increased levels of high molecular weight ubiquitin conjugates and decreased ubiquitinated histone in HeLa cells[35,36] (ubiquinated DNA is associated with active expression)[37]
2. hypermethylation of H2 B and decreased methylation of H3[38]
3. the ubiquinated form of histones in yeast when grown under mildly stressful but not lethal temperature[35]

Topological changes in chromatin are typical of the heat shock response,[39] and are assumed to participate in the changes in heat-induced gene expression and repression.

A presumed physiological consequence of heat-induced alteration of chromatin is heat-induced radiosensitivity of cancer cells.[40,41] Heat induces a dramatic increase of nonhistone protein content, resulting in a reduced affinity to repair enzymes.[40]

Heat also causes conformational changes of membrane lipids and proteins,[42,43] excessive fluidization of the plasma membrane, and leakage of required low molecular weight components.[43] Low doses of local anesthetics procaine and lidocaine, known to decrease membrane viscosity, increase neoplastic killing.[43] The membrane defects may cause release of polyamines and disturb DNA replication.[40,41]

DNA-Damaging Agents

In prokaryotes' response to a given stressor, unlinked and individually controlled genes can be coordinately controlled by common regulator genes called regulons.[5] The damage response in bacteria to ultraviolet irradiation is the "SOS" response;[44,45] to reactive oxygen species, the oxy R response;[46] and specialized responses to other environmental stresses, like cold, heat, nutrient limitation, salinity, and osmolarity, are well characterized.[47,48] Different stressors are related in the sense that they share member genes or protein products that interact. For

example, in Escherichia coli, both heat and ethanol initiate the same response (i.e., solely a heat shock response). On the other hand, both hydrogen peroxide and 6 amino-7-chloro-5, 8-dioxoquinoline (ACDQ) stimulate an oxidation stress response and a secondary SOS response; nalidixic acid and puromycin, an SOS and heat shock response; isoleucine restriction, a poor heat shock response; and cadmium chloride strongly induces all three stress responses.[5] The regulon typical response to ACDQ, cadmium chloride, and hydrogen peroxide was a minor response; these agents stimulated the synthesis of another 35 proteins by 5- to 50-fold. Another accumulated product of exposure to certain stressors are adenylated nucleotides, which are candidates as alarmones.[5]

Thus, general and specific cellular responses are triggered by different stressors. Ultraviolet- or carcinogen-related DNA damage-induced expression of the stress response does not appear to conform to the prokaryote SOS model.[48] DNA-damaging agents induce a spectrum of molecular responses, including the production of proteases, DNA repair agents, onco- genes, and chromatin changes (Table 2.2).[18,49-63] One gene product induced, extracellular inducing factor (EPIF), can induce the ultraviolet spectrum of proteins in untreated cells.[57,58] The induction of a hormetic effect by EPIF would shed light on the participation of these gene products in the protective pathway. Besides the induction of specific identified (and unidentified) proteins, a major change induced by DNA damage is alteration of the chromatin structure involving increased synthesis of poly (ADP-ribose),[50] alteration of histone methylation patterns,[55,63] and dependence on the presence of ubiquitin-histone conjugants.[31] In contrast with heat shock, DNA- damaging agents inhibit rather than increase DNA methylation,[63] and/or induce demethylation.[55] The ubiquitin conjugating enzyme is essential for DNA repair since loss of the ubiquitin-conjugating enzyme, E3, results in slow growth; sensitivity to UV, X-rays, and chemical mutagens; retrotrans- position; and inability to sporulate.[31] A suggested role for the ubiquitin- conjugating enzyme is to mediate changes in chromatin by patched degradation of chromosomal proteins to allow access for repair.[31]

Table 2.2. DNA Damage Response

Induced Proteins
Plasminogen activator, a protease[49]
PolyADP ribose[50]

DNA ligase[51,52]
Metallothione53[55]
H2 antigen[56]
Extracellular inducing factor[57,58]
Collogenase[53]
c fos[53]
cmyc[59]
p53 tumor antigen[6]
DNA p polymerase [61,62]
Hsp 28[18]

Metabolic Changes
Inhibition of DNA methylation[63]
Demethylation[55]

Degradation of Abnormal Proteins Produced by Stressors
When cells are exposed to heat and other toxic agents, abnormal proteins accumulate. The abnormal proteins signal expression of heat shock proteins, which can directly interact with the protein for "protein repair" by catalyzing ATP-driven refolding.[64] The unrepaired proteins are eliminated by a second major pathway of the response, an ATP-driven elimination of abnormal proteins mediated by the ubiquitin system. But imbalances in the protein degradation system, perhaps induced by an overload of abnormal proteins, can result in premature degradation of necessary regulatory molecules.[65] With respect to hormesis, the beneficial stress response may be protein repair and the elimination of abnormal proteins. The detrimental response may be the inappropriate destruction of short-lived essential regulator molecules when a threshold level of abnormal proteins are produced by toxic agents, radiation damage, aging, or age-related diseases. In addition to imbalance in the degradation pathway at higher doses when abnormal proteins accumulate, changes in the fundamental structure of the essential ubiquitin-conjugating enzymes is dosage dependent, at least with respect to heat.[31] The ubiquitin-conjugating enzymes, essential for survival to the

stressing agent, have introns. Since splicing of introns is blocked at higher temperatures,[66,67] the introns could serve to restrict function of protective enzymes to moderate, not severe stress.[31]

Heat Shock Genes in the DNA Damage Response

The role of heat shock genes in the DNA damage response is not known. But heat shock genes do appear in the DNA damage response. Hsp 70 expression is temporarily correlated with maximal survival of viruses after UV irradiation of viral infected cells,[15] and small Hsp's were induced by UV and teratogenic agents.[15,68] Ubiquitin was induced after treatment with mutagens and teratogens.[69-71]

Using ionizing irradiation of rat embryos in utero, enhanced expression of Hsp 70 and c-myc was increased 4 or 5 days after treatment, and c-fos increased only after the embryos were incubated in vitro.[15,72] Coordinate expression of Hsp 70 and c-myc have been detected during heat shock.[73]

Chemical teratogens showed enhanced induction of small heat shock proteins in embryos when cultivated in vitro72 and induced a subset of small heat shock protein in flies,71,74 and ubiquitin in mammalian cells.[75,76]

Since the ubiquitinated Rad6 DNA repair enzyme is a ubiquitin- conjugating enzyme essential for normal growth, sporulation, and repair,[31] ubiquitin may be a key regulatory molecule in the stress response. Changes in metabolism of ubiquitin, as well as increased synthesis of unique forms of ubiquitin gene family members, may shed light on controlling elements.

Teratogenic Agents and Heat Shock Agents

The common pathway of several apparently unrelated chemicals (and heat) to induce the teratogenic response was reviewed.[2l] The known teratogenic agents ‹ heat, ethanol, arsenite, cortisone, retinoic acid, valproic acid, cadmium, diazepam, verapamil, and phenobarbital -all induce some or all members of the so-called heat shock proteins.[21] Cadmium and ethanol are also hormetic agents. The type of defect induced during development by the teratogenic agent depends critically on the timing of the environmental insult.[77] Defects induced by heat in flies can be induced only at specific times during development. Heat treatment can alter the order of development time. During recovery,

heat shock proteins are synthesized first, then synthesis and decay of messages involved in the developmental program.[78] The interruption in the development and delay in resumption can cause the failure to complete one process before the next process begins.[79] In mammals, teratogens induce heat shock protein and affect differentiation of nerve and muscle in Drosophila embryonic cells.[78,79]

Molecular Models of Developmental Defects

The common pathway then is the interruption of an ordered series of events by any chemical or physical agent that induces a stress response. The stress response is a cessation of the synthesis of normal proteins with the selective production of the proteins required to cope with the specific toxin. The interruption, not the agent, triggers the defect. The timing of the insult dictates the defect. Recovery depends on the length and severity of treatment as well as on whether the temperature is raised slowly or abruptly.[22]

Stress Proteins: Resolution of the Paradox

The hypothesis that the stress protein response is the common pathway for hormetic agents is supported by the following findings:

1. The same agents identified as hormetic also induce the stress response.
2. Some hormetic agents with molecular responses common to the heat shock response can induce thermotolerance, while others with known differences in induction of methylation patterns induce sensitivity.
3. The stress response includes preferential synthesis of products that repair both protein and DNA, which could stimulate growth and longevity.
4. The alterations in the chromatin structure could facilitate derepression of growth-promoting products or provide access to DNA for repair.

There is a model for a biphasic response using heat as the stressor. In moderate doses, the protective molecular reactions progress. But at higher temperatures, intron splicing is inhibited, and therefore production of the needed protective response. Other as yet unknown important differences in molecular responses at low and high doses may be uncovered in the future. In summary, the stress response could provide an explanation for a beneficial response to an otherwise harmful agent. The potential for a theoretical biological beneficial response stems from the induction of cellular repair processes. The protective responses include

1. expression of "protein repair" proteins, like the heat shock proteins, which can monitor proper folding of denatured proteins
2. stimulation of elimination of abnormal proteins that cannot be repaired
3. induction of increased DNA repair and replication molecules
4. alteration of chromatin structure to facilitate repair of regions previously refractory to repair and/or alter gene expression to accelerate growth and maturation
5. induction of cross-resistance to other environmental toxins, thereby increasing tolerance to the same or apparently unrelated environmental toxins that are life-shortening agents

Why the beneficial response is effected only at low doses cannot yet be explained, but the inability to remove introns from gene transcripts required for survival, at moderate but not high temperatures, and changes in histone-ubiquitin conjugates may provide a clue to explain cytotoxic and genotoxic responses after a threshold limit for a beneficial response.

Since different stressors have specific responses, not all stressors are expected to be beneficial -or beneficial with respect to the same parameter.The hormetic response may not be an "overcorrection" response to the damaging agent, but rather a benefit derived from the "stress" response (i.e., repair or removal of accumulated age or environmental induced cellular damage in proteins, genes, and cell membranes; chromatin changes to accelerate

seed maturation; or cross-resistance to certain other environmental toxins).

REFERENCES

1. Health Phys. 52:517-680 (1987).
2. Luckey, T. D. "Ionizing Radiation Promotes Protozoan Reproduction," Radiat. Res. 108:215-221 (1986).
3. Stabbing, A. R. D. "Growth Hormesis: A Byproduct of Control," Health Phys. 52:543-548 (1987).
4. Welch, W. J., L. A. Mizzen, and A. P. Arrigo. "Structure and Function of Mammalian Stress Proteins," in Proteins, M. L. Pardue, J. R. Feramisco, and S. Lindquist, Eds. (New York: Alan R. Liss, 1989), p. 187.
5. VanBogelen, R., P. M. Kelley, and F. C. Neidhardt. "Differential Induction of Heat Shock, SOS and Oxidation Stress Regulons and Accumulation of Nucleotides in Fscherichia coli," BacterioL 169:26-32 (1987).
6. Calabrese, E. J., M. E. McCarthy, and E. Kenyon. "The Occurrence of Chemically Induced Hormesis," Health Phys. 52:531-542 (1987).
7. Congdon, C. C. "A Review of Certain Low-Level Ionizing Radiation Studies in Mice and Guinea Pigs," Health Phys. 52:93-598 (1987).
8. Planel, H., R. Soleilhavoup, and R. Tixador. "Influence of Cell Proliferation on Background Radiation or Exposure to Very Low Chronic Gamma Radiation," Health Phys. 52:571-578 (1987).
9. Tixador, R., G. Richoiley, E. Monrozies, H. Planel, and G. Tap. "Effects of Very Low Doses of Ionizing Radiation on the Clonal

Life-Span in Paramecium tetraurelia," Int. J. Radiat. Biol. 39:47-54 (1981).

10. Darnell, C. "Effects of Extremely Low Electromagnetic Radiation on Paramecium Life Span and Ion Conductance," MS Thesis, University of Wyoming, Laramie, WY (1988).
11. Dihel, L., and J. Smith-Sonneborn. "Effects of Low Frequency Electromagnetic Field on Cell Division and the Plasma Membrane," Bioelectromagnetics 6:61-71 (1985).
12. Smith-Sonneborn, J. "DNA Repair and Longevity Assurance in Paramecium tetraurelia," Science 203:1115-1117 (1979).13. Nover, L. Heat Shock: Response of Eukaryotic Cells (New York: SpringerVeriag, 1984), pp. 1-82.
14. Weis, P., and J. S. Weis. "Cadmium Acclimation and Hormesis in Fundulus heteroclitus During Fin Regeneration," Environ. Res. 39:356-363 (1986).
15. Higo, H., J. Y. Lee, Y. Satow, and K. Higo. "Elevated Expression of Proto- Oncogenes Accompany Enhanced Induction of Heat-Shock Genes after Exposure of Rat Embryos In Utero to Ionizing Irradiation," Teratogenesis, Carcinogenesis, and Mutagenesis 9:191-198 (1989).
16. Sheppard, S. C., and P. J. Regitnig. "Factors Controlling the Hormesis Response in Irradiated Seed," Health Phys. 52:599-606 (1987).
17. Williams, K. J., B. Landgraf, N. L. Whiting, and J. Zurio. "Correlation Between the Induction of Heat Shock Protein 70 and Enhanced Viral Reactivation in Mammalian Cells Treated with Ultraviolet Light and Heat Shock," Cancer Res. 49:2735-2742 (1989).

18. Vivino, A. A., M. D. Smith, and K. W. Minton. "A DNA Damage-Responsive Drosophila metanogaster Gene Is Also Induced by Heat Shock," Mol. Cell Biol. 6:4767-4769 (1986).
19. Strehler, B. J. "Further Studies on the Thermal Induced Aging of Drosophila melanogaster," J. GerontoL 17:347 (1962).
20. Lindquist, S. "The Heat Shock Response," Ann. Rev. Biochem. 55:1151-1191 (1986).
21. Petersen, N. S. "Effects of Heat and Chemical Stress on Development," Advances in Genetics 28:275-296 (1990).
22. Li, G. C. "Induction of Thermotolerance and Enhanced Heat Shock Protein Synthesis in Chinese Hamster Fibroblasts, Sodium Arsenite and Ethanol," J. Cell Physiol. I 1 5:116-122 (1983).
23. Samson, L., and Cairns. "A New Pathway for DNA Repair in E. con," Nature 267:281 (1977).
24. Ritossa, F. M. "A New Puffing Pattern Induced by Heat Shock and DNP in Drosophila," Experientia 18:571-573 (1961).
25. Lindquist, S., and E. A. Craig. "The Heat Shock Proteins," Ann. Rev. Genet. 22:631-677 (1988).
26. Craig, E., P. J. Kang, and W. A. Boorstein. "A Review of the Role of 70 koaHeat Shock Proteins in Protein Translocation across Membranes,' Antonia van Leeuwenhoek 58:137-146 (1990).
27. Riabowol, K. T., L. A. Mizzen, and W. J. Welch. "Heat Shock Is Lethal to Fibroblasts Microinjected with Antibodies against HSP 70," Science 243:433-436 (1988).
28. Haliberg, R. I., K. W. Kraus, and E. M. Hallberg. "Induction of

Acquired Thermotolerance in Tetrahymena thermophile Can Be Achieved without the Prior Synthesis of Heat Shock Proteins," Mol. Cell Biol. 5:2061-2070.

29. Hendrey, J., and 1. Kola. "Thermolability of Mouse Oocytes Is Due to the Lack of Expression and/or Inducibility of Hsp 70," Mol. Reproduc.Devel.28:18(i99,1).
30. Tuite, M. F., N. J. Bentley, and Bossier. "The Structure and Function of Small Heat Shock Proteins: Analysis of the Saccharomyces cerevisiae Hsp 26 Protein," Antonia van Leeuwenhoek 58:147-154 (1990).
31. Jentch, S., W. Seufert, and T. Sommer. "Ubiquitin-Conjugating Enzymes: Novel Regulators of Eukaryotic Cells," TIBS 15:195-198 (1990).
32. Dice, J. F., and S. A. Goff. "Error Catastrophe and Aging: Future Directions of Research," in Modern Biological Theories of Aging, H. R. Warner, R. N. Butler, R. L. Sprott, and E. L. Schneider, Eds. (New York: Raven Press, 1987), pp. 155-168.
33. Finley, D., B. Bartel, and A. Varshavsky. "The Tails of Ubiquitin Precursors Are Ribosomal Proteins Whose Fusion to Ubiquitin Facilitates Ribosome Biogenesis," Nature 338:394-401 (1989).
34. Picologou, S., N. Brown, and S. W. Liebman. "Mutations in RAD6, a Yeast Gene Encoding a Ubiquitin-Conjugating Enzyme, Stimulate Retrotransposition," MoL Cell Biol. 10(3):1017-1022 (1990).
35. Pratt, G., Q. Deveraux, and M. Rechsteiner. "Ubiquitin Metabolism in Stressed Cells,' in Stress Induced Proteins, M. L. Pardue, J. R. Feramisco, S.Lindquist, Eds., UCLA

Symposium on Molecular and Cellular Biology %:149-162 (1989).

36. Bonner, W. M. "Metabolism of Ubiquitinated H2A," in The Ubiquitin System, M. Schlesinger and A. Hershko, Eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1988), pp. 155 158.
37. Davie, J. R., S. E. Nickel, and J. A. Ridsdalc. "Ubiquitinated Histone H2B Is Preferentially Located in Transcriptionally Active Chromatin," in The Ubiquitin System, M. Schlesing@r and A. Hershko, Eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1988), pp. 159 163.
38. Desrosiers, R., and R. M. Tanguay. "Methylation Histones at Proline, Lysine, and Arginine Residues During Heat Shock," J. Biol. Chem. 468@92.
39. Higgins, C. F. "DNA Supercoiling, Chromatin Structure and the Regulation of Gene Expression," Antonia van Leeuwenhoek 58:51-55 (1990).
40. Carper, S. W., P. M. Harari, and D. J. M. Fuller. "Biochemical and Cellular Response to Hyperthermia in Cancer Therapy," in Stress-Induced Proteins, M. L. Pardue, J. R. Feramisco, S. Lindquist, Eds., UCLA Symposium on Molecular and Cellular Biology 96:247-256 (1989).
41. Hahn, G. M., M. K. 1. Adwankar, and V. S. Basrur. "Survival of Cells Exposed to Anticancer Drugs after Stress," in Stress-Induced Proteins, M. L. Pardue, J. R. Feramisco, S. Lindquist, Eds., UCLA Symposium on Molecular and Cellular Biology %:223-234 (1989).
42. Lepock, J. R., K. H. Cheng, and J. Kruuv. "Thermotropic Lipid

and Protein Transitions in Chinese Hamster Lung Cell Membranes: Relationship to Hyperthermic Cell Killing," Can. J. Biochem. Cell BioL 61:421-427 (1983).

43. Yatvin, M. B. "The Influence of Membrane Lipid Composition and Procaine on Hyperthermic Death of Cells," Int. J. Radiat. BioL 32:513-521 (1977).
44. Witkin, E. M. "Ultraviolet Mutagenesis and Inducible DNA Repair in Escherichia coli," BacterioL Rev. 40:869-907.
45. Walker, G. C. 'Mutagenesis and Inducible Responses to Deoxyribonucleic Acid Damage in Escherichia coli, " 48:60-93 (1984).
46. Storz, G., L. A. Tartaglia, and B. N. Ames. "The OxyR Regulon," Antonia van Leeuwenhoek 58:157-161 (1990).
47. Bhagwat, A. A., and S. K. Apte. "Comparative Analysis of Proteins Induced by Heat Shock, Salinity and Osmotic Stress in the Nitrogen-Fixing Cyanobacterium Anabaena sp. Strain L3l," J. Bacteriology 171:5187-5189 (1989).
48. Elespuru, R. K. "Inducible Responses to DNA Damage in Bacteria and Mammalian Cells," Environ. MoL Mutagen. 10:97-116 (1987).
49. Miskin, R., and E. Reich. "Plasminogen Activator: Induction of Synthesis by DNA Damage," Cell 19:217-224 (1980).
50. Ueda, K., and 0. Hayaishi. "ADP-Ribosylation," Ann. Rev. Biochem. 54:73-100 (1985).
51. Mezzina, M., and S. Nocentini. "DNA Ligase Activity in UV-Irradiated Monkey Kidney Cells," Nucl. Acids Res. 5:43174334 (1978).

52. Sarasin, A. "SOS Response in Mammalian Cells," Cancer Invest. 3(2):163-174 (1985).
53. Herrlich, P., P. Angel, and H. J. Rahmsdorf. "The Mammalian Genetic Stress Response," Adv. Enzyme ReguL 25:485-504 (1986).
54. Herrlich, P., U. Mallick, and H. Ponta. "Genetic Changes in Mammalian Cells Reminiscent of an SOS Response," Hum. Genetics 67:360-368 (1984).
55. Lieberman, M. W., L. R. Beach, and R. D. Paimiter. "Ultraviolet Radiation Induced Metallothionein-I Gene Activation Is Associated with Extensive DNA Demethylation," Cell 35:207-214 (1983).56. Rahmsdorf, H. J., N. Koch, and U. Mallick. "Regulation of Class I Invariant Chain Expression: Induction of Synthesis in Human and Murine Psmocytoma Cells by Arresting Replication," EMBO J. 2:811-816 (1983).
57. Schorpp, M., U. Mallick, and H.-J. Rahmsdorf. "UV-Induced Extracellular Factor from Human Fibroblasts Communicates the UV Response to Nonirradiated Cells,' Cell 37:861-868 (1984).
58. Emerit, I., and P. A. Cerruti. "Tumor Promoter Phorbol 12-Myristate 137, Acetate Inducts Clastogenic Factor in Human Lymphocytes," Proc. Nat. Acad. Sci. 79:7509-7513 (1982).
59. Ronal, Z. A., E. Okin, and I. B. Weistein. "Ultraviolet Light Induces the Expression of Oncogenes in Rat Fibroblast and Human Keratinocyte Cells," Oncogene 2:201-204 (1988).
60. Maltzman, W., and L. Czyzyk. "UV Irradiation Stimulates Levels of p53 Cellular Tumor Antigen in Nontransformed Mouse Cells," MoL Cell. Biol. 4:1689-1694 (1984).

61. Williams, T. J. "Determination of Clonal Life Span in Paramecium," PhD Thesis, University of Wyoming, Laramie, WY (1980).
62. Keiding, J., and 0. Westergaard. "Induction of DNA Polymerase Activity in Irradiated Tetrahymena Cells," Exp. Cell Res. 64:317-322 (1971).
63. Wilson, V. L., and P. A. Jones. "Inhibition of DNA Methylation by Chemical Carcinogens in Vitro," Cell 32:239-246 (1983).
64. Rothman, J. E. "Polypeptide Chain Binding Proteins: Catalysts of Protein Folding and Related Processes in Cells," Cell 59:591-WI (1989).
65. Dice, J. F. "Lysosomal Pathways of Protein Degradation," in The Ubiquitin System, M. Schicsinger and A. Hershko, Eds. (Cold Spring Harbor: Cold Spring Harbor Laboratory, 1988), pp. 141-146.
66. Yost, H. J., and S. Linquist. "RNA Splicing Is Interrupted by Hcat Shock and Is Rescued by Hcat Shock Protein Synthesis," Cell 45:185-193.
67. Yost, H. J., and S. Lindquist. "Translation of Unspliced Transcripts after Heat Shock," Science 242:1544-1548.
68. Buzin and N. Bournias-Vardiabasis. "Teratogens Produce a Subset of Small Hcat Shock Proteins in Drosophila Primary Embryonic Cell Cultures," Proc. Nat. Acad. Sci. 81:4075-Q79 (1984).
69. Friedbcrg, E. C. "Deoxyribonucleic Acid Repair in the Yeast Saccharomyces cerevisiae,' Microbial. Rev. 52:70-102 (1988).
70. Bournias-Vardiabasis, N., R. L. Teplitz, G. F. Chernoff, and R. L. Scccof. 'Detection of Teratogens in Drosophila Embryonic

Cell Culture Test Assay of 100 Chemicals," Teratology 28:109-122 (1983).

71. Tregar, J. M., K. A. Heichman, and K. McEntee. "Expression of the Yeast UB14 Gcnc Increases in Response to DNA-Damaging Agents and in Meiosis," MoL Cell. BioL 8:1132-1136 (1988).
72. McCianahan, T., and K. McEntree. "DNA Damage and Hcat Shock Dually Regulate Genes in Saccharomyces cerevisiae," MoL Cell. BioL 6:90-96 (1986).
73. Higo, H., K. Higo, and J. Y. Lee. "Effects of Exposing Rat Embryos in Utero to Physical or Chemical Teratogens Are Expressed Later as Enhanced Induction of Heat Shock Proteins When Embryonic Hearts Are Cultivated In Vitro," Teratogenesis, Carcinogenesis, and Mutagenesis 8:315-328 (1988).
74. Kingston, R. E., A. S. Baldwin, Jr., and P. A. Sharp. "Regulation of Heat Shock Protein 70 Gene Expression by c-myc," Nature 12:280-282 (1984).
75. Fornace, A., 1. Alamo, and C. M. Hollander. "Induction of Heat Shock Protein Transcripts and B2 Transcripts by Various Stresses in Chinese Hamster Cells," Exp. Cell Res. 182:61-74 (1989).
76. Fornace, A., Jr., A. Isaac, Jr., and C. Hollander. "Ubiquitin MRNA is a Major Stress-Induced Transcript in Mammalian Cells," Nucl. Acid Res. 17:1215-1229 (1989).
77. Schardcin, J. L. Chemically Induced Birth Defects (New York: Dekkcr, 1985).
78. Petersen, N. S., and H. K. Mitchell. "Effects of Heat Shock on

Gene Expression During Development: Induction and Prevention of the Multihair Phenocopy in Drosophila," in Heat Shock from Bacteria to Man, J. Schicsinger, M. Ashburner, and A. Tissieres, Eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982), pp. 345-352.

79. Mitchell, H. K., J. Roach, and N. Petersen. "Morphogenesis of Cell Hairs in Drosophila," Devel. Biol. 95:387-398 (1983).