SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 2, pp. 334–343, 2005

Printed in the USA
0730-7268/05 $12.00 + .00

HORMETIC EFFECTS OF GAMMA RADIATION ON THE STRESS AXIS OF NATURAL POPULATIONS OF MEADOW VOLES (*MICROTUS PENNSYLVANICUS*)

RUDY BOONSTRA,*† RICHARD G. MANZON,‡ STEVE MIHOK,§ and JULIE E. HELSON†
†Department of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, Ontario M1C 1A4, Canada
‡Department of Biology, University of Regina, Regina, Saskatchewan S4S 0A2, Canada
§Canadian Nuclear Safety Commission, P.O. Box 1046, Station B, Ottawa, Ontario K1P 5S9, Canada

(*Received* 26 *March* 2004; *Accepted* 12 *July* 2004)

Abstract—We tested the hypothesis that low doses of gamma radiation have beneficial, hormetic effects on the stress axis (the hypothalamic-pituitary-adrenocortical axis) of free-ranging meadow vole populations (*Microtus pennsylvanicus*). Voles were exposed to chronic gamma radiation from a ^{137}Cs field irradiator. In isolated populations, voles received one of three treatments over a four-year period: Controls (0.19–0.42 μGy/h − levels that were 2–5× above background levels [0.1 μGy/h] and live-trapped in all years − 1982–1985), low doses (22.6 μGy/h − 50–200× background, live-trapped from November 1982–April 1985), or high doses (3,840 μGy/h − 40,000× background, live-trapped from November 1983–April 1985). Voles exposed to a low dose had levels of free and total corticosterone that were significantly higher than those in the control or high-dose groups. Differences in response to radiation between the sexes were apparent for maximum corticosterone-binding capacity, with females exposed to low doses having higher binding capacity than control or high-dose females, whereas males exposed to low doses had lower binding capacity than control or high-dose males. Low-dose voles had higher counts of neutrophils than either the controls or high-dose voles; hematocrit was greater in the controls than in irradiated voles. These results indicate that voles display a hormetic response to radiation, wherein low doses of an otherwise harmful agent produce a beneficial effect. The stimulation of the stress axis resulting in the increased secretion of glucocorticoids, which may protect against the excessive actions of the immune and inflammatory responses, may be a key mechanism producing this effect.

Keywords—Hormesis Gamma radiation Stress axis Hyperadrenocorticism Hematology

INTRODUCTION

The effects of ionizing radiation on the environment have been of interest since the 1940s [1]. However, despite considerable early research, several areas of uncertainty exist, particularly in regards to the health effects of low doses [2]. Most studies have followed four, often independent, experimental protocols: Acute doses in the laboratory, chronic doses in the laboratory, acute doses in the field, and chronic doses in the field [3,4]. This has made it difficult to extrapolate most laboratory work, typically involving acute doses, to natural field situations where chronic exposure generally occurs [3]. In nature, animals also are challenged simultaneously with additional stresses involved in day-to-day living such as having to deal with changing environmental conditions, competition from conspecifics, parasites, and predators. Any one of these factors could exacerbate responses to radiation stress. Hence, relevant information on the impact of radiation stress on natural populations is scanty and is difficult to interpret beyond a qualitative level for environmental protection purposes [5].

Toxicology and radiation biology traditionally have focused on high exposures and detrimental effects that can be quantified with short-duration experiments. Thus, few studies have been conducted at sufficiently low exposures or for long enough periods to detect possible beneficial effects of exposure (i.e., hormesis [6,7]). Hormesis is defined as a phenomenon where low doses of an otherwise harmful agent can result in stimulatory or beneficial effects [8,9]. Hormesis has been observed for a broad range of chemicals including alcohol and its metabolites, antibiotics, auxin-related compounds, hydrocarbons, metals, herbicides, insecticides, and fungicides, as well as physical processes, such as radiation exposure and caloric restriction. Moreover, effects have been observed in a diverse range of organisms, such as microbes, fungi, plants, and animals [6,8,10,11]. Hormetic responses take many forms, including increased longevity, growth responses, metabolic effects, reproductive responses, and physiological responses. This tremendous diversity suggests the presence of a fundamental underlying process, possibly acting to alter the expression of a variety of genes [6,8,11,12]. A common finding is that organisms perform better at low exposures of ionizing radiation compared with exposures approaching zero [4,7,12–16]. However, despite the established literature supporting radiation hormesis, there is still only limited acceptance for it. This is partly because of the difficulty of establishing a common, plausible, underlying mechanism [12].

Ionizing radiation is known to act as a stressor. The most typical response to stressors in vertebrates is the release of glucocorticoids. This response is ultimately controlled by the hypothalamic-pituitary-adrenocortical (HPA) axis (i.e., the stress axis). A major function of glucocorticoids is to protect against the excessive actions of primary defense systems, such as the immune and inflammatory responses. Although these defenses provide protection against the damaging effects of metabolic processes and environmental agents, they can be damaging on their own [17,18]. Masoro [9] suggested that moderate hyperadrenocorticism (an increased secretion of adrenal cortisol, found in humans, rabbits, and squirrels, or corticosterone, found in mice, rats, and voles), produced in response to low-level stressors offers a protective or beneficial

* To whom correspondence may be addressed (boonstra@utsc.utoronto.ca).

advantage not present in the absence of the low-level stressor. This effect has been documented for caloric restriction [9,19,20], which results in moderate hyperadrenocorticism, in conjunction with increased longevity, retardation of age-associated physiological deterioration, and either delays in or the prevention of age-associated diseases [13,15,16,20]. Could hormetic effects, therefore, simply be the result of increased levels of glucocorticoids associated with low-level stressors, such as low-level radiation?

This study was designed to test the hormetic hypothesis using samples from a radioecological study of free-living meadow vole (*Microtus pennsylvanicus*) populations at the Zoological Environment Under Stress (ZEUS) facility in Manitoba, Canada [21]. Specifically, the glucocorticoid stress response was studied in free-ranging populations exposed in the field with a gamma irradiator for yearlong periods to contrast the impact of low and high chronic doses of gamma radiation. Free and total plasma corticosterone concentrations, a measure of corticosteroid-binding globulin maximum (maximum corticosteroid-binding capacity), and routine diagnostic hematological indices (white and red blood cells), were measured to assess the effects of low- and high-dose gamma irradiation to test the hormetic hypothesis.

Biology of meadow voles

The meadow vole is the ubiquitous field mouse of North America. Its range extends from Georgia, throughout northern and eastern United States, across Canada from Newfoundland to British Columbia and on into the Yukon and Alaska, USA. It is a seasonally reproducing small mammal with breeding concentrated in spring through autumn and little or no reproduction occurring in winter. In Manitoba, the ground is snow-covered from November to late April and the breeding season typically begins in May and ends in September [21]. These voles have a high reproductive rate, with females becoming pregnant at 25 d of age, having a gestation of 21 d and five to six young per litter, and engaging in postpartum estrus. Thus, adults can be both pregnant and lactating for most of the breeding season. Under field conditions, most voles live considerably less than one year and virtually no animal that breeds in one year is alive the next (for a review [22]). Spring- or early summer–born females can mature and breed in the year of their birth but die before the next breeding season, whereas late summer– and autumn-born young delay maturing until the next breeding season and, thus, must survive winter first [22].

MATERIALS AND METHODS

Experimental design

Details of the ZEUS facility and the sequence of experiments conducted to expose wild populations of small mammals to chronic gamma radiation are in Mihok [21]. Briefly, the facility was established in deciduous forest at the Whiteshell Nuclear Research Establishment near Pinawa, Manitoba, Canada (50°N, 96°W) in 1974 [3]. It consisted of six 1-ha island grassland grids (100 m × 100 m) that were cleared of trees in 1974 and seeded with grasses and clovers to provide habitat for voles, which rapidly colonized the area. The grids were set up in a 2 × 3 array with each grid separated from the next by 100 m of forest. Grid 1 was the permanent control throughout the experiment and was trapped from 1980 to 1986. Wild meadow voles were exposed to gamma radiation from a ^{137}Cs field irradiator on a rotating basis on single grids, with those on other grids and on a nearby grassland grid (~1 km away) serving as controls. Medium (experiment 1), low (experiment 2), and high (experiment 3) fixed dose rates were used in three consecutive experiments conducted between 1981 and 1985. Irradiation was continuous during each experimental period (79–88%), with interruptions required simply to provide access to the grid. Vole demography and health were monitored by biweekly live-trapping throughout the year, winter weather permitting. Experiments were started in November, after the end of reproduction. This timing ensured that the starting generation consisted of a reasonably homogeneous group of late-season young-of-the-year, with few early-season voles present, and likely no overwintered voles from the previous year still alive. This parental generation was then irradiated for about six months before breeding commenced the next spring.

A random sample of frozen blood plasma collected in association with routine diagnostic hematology [23] was available for glucocorticoid analysis from two of the three experiments. Plasma was not available from the medium dose–rate experiment (experiment 1, in 1981–1982) because samples had already been used for genetic studies [24]. Mean ambient background in the region varies from about 0.05 to 0.11 μGy/h across years [21]. In the low dose–rate experiment (experiment 2, November 1982–October 1983), voles on the irradiated area (grid 4) lived in a gamma field of about 200× background (22.6 ± 2.6 μGy/h) versus a dosage for controls of 0.19 to 0.42 μGy/h. The low-dose animals received a measured, absorbed dose in air of 23.8 ± 1.8 μGy/h (calculated per hour of irradiation). In the high dose–rate experiment (experiment 3, November 1983–April 1985), voles on the irradiated grid (grid 5) were exposed to about 40,000× background (3,840 ± 167 μGy/h) versus a dosage for controls of 0.22 μGy/h. They received a dose of 2,099 ± 72 μGy/h. This value was lower than expected due to the presence of numerous large boulders on grid 5. Doses received by irradiated voles within the experiments did not differ significantly between individuals or by sex, reproductive condition, or season [21].

During the high dose–rate experiment (experiment 3, grid 5), the adjacent grid 6 received irradiation above background. Voles on this grid also were sampled to provide simultaneous information on low dose–rate effects. These voles lived at about 50× background (5.2 ± 0.3 μGy/h) and received a dose of 7.2 ± 0.6 μGy/h. Data from these voles were pooled with those from voles living on the low dose–irradiated grid at 200× background in experiment 2. This provided a low-dose group (50 or 200× background, $n = 70$) for contrast with the high-dose group (40,000× background, $n = 83$) and the control group living at or near background ($n = 101$).

Cumulative doses from first capture sampled for the 254 voles in this study were 5.0 ± 0.1 mGy for the low group and 1,505 ± 20 mGy for the high group. Doses from conception were estimated at about 1.9× these measured values [21]. Controls received negligible doses, mostly indistinguishable from background, as estimated by the animal dosimetry system used (Harshaw TLD-100 chips on collars exposed for about 2–4 weeks [21]; Harshaw-Bicron, Newbury, OH, USA). One third of the control sample was obtained from areas at background (old field) and received no experimental radiation, one third from an area approaching background (long-term control ZEUS grid 1), and the remainder from areas exposed to no more than a few times background (other ZEUS control meadows). Thus 67% of the control voles (the latter two groups)

received an incremental radiation dose of about 2 to 5× background.

Animal live-trapping and processing

Monitoring was optimized for meadow voles based on protocols from baseline research (2 d of biweekly capture-mark-release sampling with two Longworth live traps per station with 64 stations arranged in an 8 × 8 grid at 11-m spacing) [21]. Males were considered to be active reproductively when testes were scrotal. Females were considered to be active reproductively if they had a perforate vagina, were visibly pregnant, lactating, or both pregnant and lactating. Movements between grids were rare (1%, 14 of 1,437 voles [21]).

Animals were processed in the laboratory in the late morning, extending into the early afternoon at times of high density. Voles could have spent up to 24 h confined in a trap (with raw oats and fresh carrot for nourishment) prior to sampling, but there was no systematic bias as to when animals were processed relative to experimental treatment. In addition, in a separate study, Fletcher and Boonstra (University of Toronto at Scarborough, Toronto, Ontario, Canada, unpublished data) found no increase in corticosterone levels as length of time in live-traps increased up to 12 h. Blood was collected by suborbital puncture with a heparinized microhematocrit tube, typically within 30 sec of first handling. A portion was used for routine diagnostic hematology [23] and the rest spun for plasma. The plasma was stored in a chest freezer at −20°C initially and then transferred to the University of Toronto on dry ice in spring 1986 and stored at −80°C. As a result of the use of isolated meadows, frequent trapping, and high trappability [21], individual reproductive status (breeding, nonbreeding) could be assigned with confidence based on life histories and external indicators (males, testes abdominal or scrotal; females, vagina perforate or nonperforate, lactating or nonlactating, and obviously pregnant or not).

In the blood, most corticosterone normally is bound to a carrier protein, corticosteroid-binding globulin (CBG). About 5 to 10% of the corticosterone is unbound and free. Only the free form appears to be active biologically [25]. Corticosteroid assays, therefore, involved measurements of total plasma corticosterone and CBG. From these assays, free-plasma corticosterone was calculated. Total plasma corticosterone was measured using radioimmunoassay [26]. Plasma CBG was measured as the maximum corticosterone-binding capacity (MCBC) [26]. Free corticosterone concentrations were calculated by the procedures of Tait and Burstein [27]; for these calculations, three values were required: The albumin concentration in plasma (albumin also binds corticosterone and has high capacity but low affinity), the ratio of albumin-bound to free corticosterone, and the affinity constant of CBG for corticosterone. The procedures used to measure these values are given in Boonstra et al. [28]. We calculated that voles (pooled sample of five animals) have 2.83 g albumin per 100 ml plasma, that the ratio of albumin-bound to free corticosteroid in a 1% solution is 0.72, and that the CBG-binding constant is 6.32×10^7 M^{-1}. The latter constant is comparable to that found in the laboratory rat of 1×10^7 M^{-1} [29]. Plasma analysis for corticosterone and MCBC for all samples were carried out between July and August 1986 and all samples were randomized and run blind.

Hematological analysis [23] included the examination of hematocrit (packed red blood cell volume), normoblasts, and leucocytes, which were further subdivided into basophils, eosinophils, neutrophils, lymphocytes, monocytes, and azurocytes (an immune cell unique to the genus *Microtus* [30]). In addition, neutrophils were classified as immature (band forms) or mature (segmented forms). Several blood parasites also were enumerated, including parasitic bacteria of the genera *Haemobartonella*, *Eperythrozoon*, *Grahamella*, and parasitic protozoa of the genera *Babesia*, *Hepatozoon*, and *Trypanozoon*.

Statistical analyses

Total corticosterone, free corticosterone, MCBC, and principle hematological indices (hematocrit, leucocytes, neutrophils, and lymphocytes) were analyzed using two-way analyses of variance. First, dose (control, low, and high) and sex were examined, and then for each sex, dose, and reproductive condition (breeding, nonbreeding). Data were divided further by sex or reproductive condition, respectively, if significant interaction effects were detected. Normality was achieved through log transformation of corticosterone, MCBC, leucocytes, total neutrophils, mature neutrophils, and lymphocytes. Box Cox transformations were used for free corticosterone (lambda −0.2) and for hematocrit (lambda 3.9). Transformed variables either satisfied the Shakiro-Wilks *W*-test for normality (examined with Statisica®, StatSoft, Tulsa, OK, USA) or visually fit a normal curve for sample sizes >100. Tukey-Kramer multiple comparison post hoc tests were used to examine significant main effects. Three outliers, having extremely high values, were removed from the analysis for total and free corticosterone and MCBC. These voles appeared to be in advanced pregnancy and, hence, had artificially very high corticosterone levels [26].

Hematological indices for less common cells such as normoblasts, basophils, eosinophils, monocytes, immature neutrophils (band forms), and azurocytes, as well as qualitative scores for the presence of various blood parasites [23], were examined using the nonparametric Kruskal-Wallis test. Dose effects were examined using all data, with further subanalyses conducted for each sex or reproductive condition. All analyses of variance and Kruskal-Wallis tests were done using Statview® (SAS Institute, Cary, NC, USA).

RESULTS

Corticosteroids

Free-corticosterone levels were affected by both dose and sex (dose: $F = 13.69$, df 2,245, $p < 0.0001$; sex: $F = 41.0$, df 1,245, $p < 0.0001$; interaction: $F = 0.88$, df 2,245, $p = 0.42$). Overall, voles exposed to a low dose had significantly higher free-corticosterone levels than either control or high-dose animals (Fig. 1). Females had significantly higher free-corticosterone levels than males. Because trends also varied by reproductive condition, separate analyses were carried out to examine the consistency of dose effects in more detail.

In females, free-corticosterone levels (Fig. 1a) were affected by dose and reproductive condition (dose: $F = 5.70$, df 2,115, $p = 0.004$; reproductive condition: $F = 2.89$, df 1,115, $p = 0.008$; interaction: $F = 1.12$, df 2,115, $p = 0.33$). Nonbreeding females ($F = 4.18$, df 2,94, $p = 0.018$) exposed to a low dose had significantly higher free-corticosterone levels than controls or those exposed to a high dose; there was no significant difference between controls and high-dose animals. Among breeding females, free-corticosterone levels were higher in the controls and low-dose group than in the high-dose group ($F = 5.67$, df 2,21, $p = 0.011$); there was no significant difference between the control and the low-dose groups.

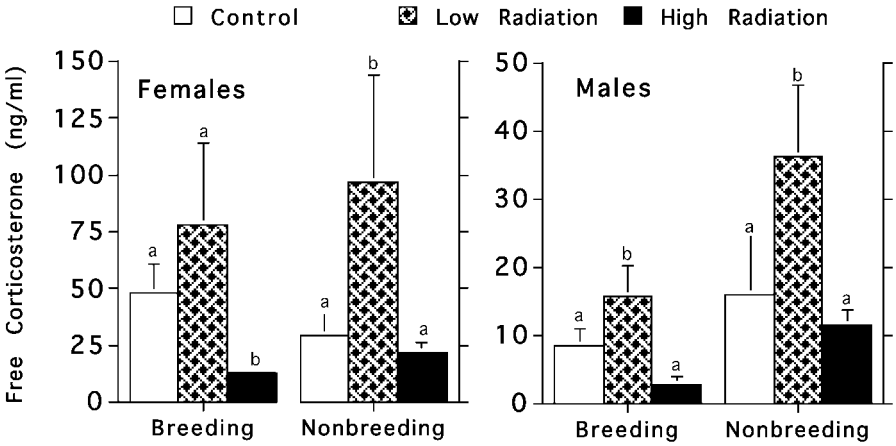

Fig. 1. Response of free corticosterone levels in each sex (**a** = females, **b** = males) of the meadow vole (*Microtus pennsylvanicus*) to differing radiation level treatments (control, low, and high). Data are separated by reproductive condition. Means (±1 standard error of the mean) are presented for all bars. Significant ($p < 0.05$) pair-wise (Tukey-Kramer) differences within a reproductive class (breeding or nonbreeding) are indicated by different letters. ($n = 124$ for females, $n = 130$ for males).

In males, free-corticosterone levels (Fig. 1b) also were affected by both dose and reproductive condition (dose: $F = 9.0$, *df* 2,124, $p = 0.0002$; reproductive condition: $F = 20.84$, *df* 1,124, $p = 0.001$; interaction: $F = 2.15$, *df* 2,124, $p = 0.12$). For both reproductive and nonreproductive males, low-dose animals had significantly higher levels of free corticosterone than control and high-dose animals, but the latter did not differ. In all experimental groups, breeding males had consistently lower levels of free corticosterone than nonbreeding males.

Trends in total corticosterone were similar to those for free corticosterone (Tables 1–3). The major difference was that total corticosterone levels in males were not affected by dose ($F = 0.42$, *df* 2,124, $p = 0.66$). In addition, total corticosterone levels in nonbreeding males displayed a trend opposite to free-corticosterone levels. High-dose animals had the highest total corticosterone levels and low-dose animals had the lowest total corticosterone levels.

Maximum corticosteroid-binding capacity was affected by both dose and sex; however, an interaction was present (dose: $F = 8.20$, *df* 2,245, $p = 0.0004$; sex: $F = 33.93$, *df* 1,245, $p < 0.0001$; interaction: $F = 18.83$, *df* 2,245, $p < 0.0001$) (Fig. 2). Females consistently had higher MCBC than males. Within females, MCBC was affected by both dose and reproductive condition (dose: $F = 4.40$, *df* 2,115, $p = 0.01$; reproductive condition: $F = 10.88$, *df* 1,115, $p = 0.001$; interaction: $F = 0.76$, *df* 2,115, $p = 0.47$). Breeding females had significantly higher MCBC than nonbreeding females. In both breeding ($F = 3.63$, *df* 2,21, $p = 0.04$) and nonbreeding females ($F = 2.03$, *df* 2,94, $p = 0.14$) controls had the lowest MCBC, high-radiation females had intermediate MCBC, and low-radiation females had the highest MCBC. However, a significant difference existed only between control and low-dose breeding females.

Maximum corticosteroid-binding capacity in males was affected by dose ($F = 9.89$, *df* 2,124, $p = 0.0001$), but not by reproductive condition ($F = 0.69$, *df* 1,124, $p = 0.79$); there was no interaction effect ($F = 0.86$, *df* 1,124, $p = 0.42$). Maximum corticosterone-binding capacity in both nonbreeding and breeding males was lowest in voles exposed to a low dose, intermediate in controls, and highest in the high-dose group (all groups significantly different from one another).

Hematology

Leucocytes as a whole were affected by dose ($F = 3.16$, *df* 2,245, $p = 0.04$), but not by sex ($F = 0.6$, *df* 1,245, $p =$

Table 1. Two-way analysis of variance testing the response of total corticosterone levels in the meadow vole (*Microtus pennsylvanicus*) to radiation treatments (control, low, and high). All absent probability values are nonsignificant

Source	*df*	Mean square	*F*	*p*
Total corticosterone				
Treatment	2	0.67	9.21	0.0001
Sex	1	8.25	114.03	0.0001
Treatment × sex	2	1.01	13.96	0.0001
Error	245	0.072	—	—
Female				
Treatment	2	0.49	5.25	0.006
Sexual condition	1	1.38	14.76	0.0002
Treatment × sexual condition	2	0.001	—	—
Error	115	0.093	—	—
Male				
Treatment	2	0.015	—	—
Sexual condition	1	0.92	26.06	0.0001
Treatment × sexual condition	2	0.28	8.04	0.0005
Error	124	0.035	—	—

Table 2. Summary of corticosterone and hematology for female meadow voles. Data are presented as means ± 1 standard error; sample sizes in parentheses. MCBC = Maximum corticosteroid-binding capacity; pcv = packed cell volume

	Control		Low		High	
Parameter	Nonbreeding	Breeding	Nonbreeding	Breeding	Nonbreeding	Breeding
Total corticosterone (ng/ml)	1,045.2 ± 137.3 (43)	1,582.8 ± 96.6 (6)	2,029.6 ± 446.6 (16)	3,318.6 ± 708.0 (12)	1,024.8 ± 108.6 (38)	1,907.3 ± 503.9 (6)
MCBC (ng/ml)	2,106.0 ± 192.7 (43)	2,325.2 ± 257.9 (6)	2,946.1 ± 483.6 (16)	4,645.0 ± 689.7 (12)	2,582.8 ± 216.8 (38)	4,502.3 ± 1,083.1 (6)
Free corticosterone (ng/ml)	29.3 ± 9.0 (43)	48.3 ± 12.8 (6)	96.6 ± 47.2 (16)	78.2 ± 36.1 (12)	21.9 ± 4.6 (38)	12.5 ± 1.8 (6)
Hematocrit (pcv − %)	49.5 ± 0.4 (43)	49.6 ± 0.6 (8)	45.1 ± 1.6 (16)	47.6 ± 0.8 (13)	47.7 ± 0.5 (38)	48.1 ± 1.6 (6)
Leucocytes (per µl)	3.9 ± 0.3 (43)	5.6 ± 1.3 (g)	5.7 ± 0.8 (16)	5.2 ± 0.4 (13)	5.2 ± 0.3 (38)	3.9 ± 0.5 (6)
Neutrophils (per µl)	1.2 ± 0.1 (43)	1.4 ± 0.6 (8)	2.5 ± 0.3 (16)	1.9 ± 0.3 (12)	1.7 ± 0.2 (20)	1.1 ± 0.2 (6)
Normoblasts (per µl)	0.03 ± 0.02 (43)	0.008 ± 0.006 (8)	0.3 ± 0.2 (16)	0.08 ± 0.04 (12)	0.01 ± 0.006 (20)	0.03 ± 0.02 (6)
Basophils (per µl)	0.03 ± 0.008 (42)	0.1 ± 0.06 (8)	0.04 ± 0.01 (16)	0.03 ± 0.01 (12)	0.08 ± 0.02 (20)	0.05 ± 0.04 (6)
Eosinophils (per µl)	0.06 ± 0.02 (42)	0.09 ± 0.05 (g)	0.09 ± 0.03 (16)	0.06 ± 0.02 (12)	0.1 ± 0.03 (20)	0.02 ± 0.02 (6)
Lymphocytes (per µl)	2.4 ± 0.2 (42)	3.3 ± 0.5 (8)	2.6 ± 0.5 (16)	2.6 ± 0.3 (12)	3.2 ± 0.3 (20)	2.2 ± 0.5 (6)
Monocytes (per µl)	0.1 ± 0.02 (42)	0.3 ± 0.06 (8)	0.2 ± 0.07 (16)	0.1 ± 0.02 (12)	0.2 ± 0.04 (20)	0.2 ± 0.05 (6)
Azurocytes (per µl)	0.06 ± 0.03 (42)	0.4 ± 0.2 (8)	0.2 ± 0.07 (16)	0.4 ± 0.09 (12)	0.2 ± 0.06 (20)	0.2 ± 0.2 (6)

0.44) (Tables 2 and 3). However, because there was a significant interaction effect ($F = 3.16$, df 2,245, $p = 0.04$), data were further analyzed separately by sex. Females were not affected by either dose or reproductive condition (dose: $F = 2.02$, df 2,118, $p = 0.14$; reproductive condition: $F = 0.007$, df 1,118, $p = 0.93$), but again, interpretation was confounded by a marginal interaction effect ($F = 2.94$, df 2,118, $p = 0.05$). In the final subanalyses, nonbreeding females, but not breeding females, were affected significantly by dose ($F = 8.19$, df 2,94, $p = 0.0005$ and $F = 1.27$, df 2,24, $p = 0.30$, respectively). Both high- and low-dose animals had significantly higher numbers of leucocytes than controls (Table 2). In males (Table 3), neither dose nor reproductive condition affected leucocyte counts (dose: $F = 0.44$, df 2,121, $p = 0.64$; reproductive condition: $F = 0.001$, df 1,121, $p = 0.98$; interaction: $F = 1.42$, df 2,121, $p = 0.24$).

Neutrophils were affected by dose ($F = 10.30$, df 2,213, $p < 0.0001$), but not by sex ($F = 0.74$, df 1,213, $p = 0.39$; interaction: $F = 1.85$, df 2,213, $p = 0.16$). Counts within the sexes were affected by dose, but not by reproductive condition (males, dose: $F = 2.69$, df 2,109, $p = 0.07$; reproductive condition: $F = 2.77$, df 1,109, $p = 0.10$; interaction: $F = 0.07$, df 2,109, $p = 0.94$; females, dose: $F = 7.20$, df 2,98, $p = 0.001$; reproductive condition: $F = 3.05$, df 1,98, $p = 0.08$; interaction: $F = 0.46$, df 2,98, $p = 0.63$). For both sexes, regardless of reproductive condition, counts were significantly higher in low-dose voles than either control or high-dose voles, but the latter did not differ (Tables 2 and 3). In terms of untransformed cell counts, these results were equivalent to a substantial 54% (significant) relative increase in neutrophil counts in the low-dose group relative to controls, as opposed to an 11% (nonsignificant) increase in the high-dose group. Mature neutrophils showed the same pattern as total neutrophils (dose: $F = 8.88$, df 2,213, $p = 0.0002$; sex: $F = 0.53$, df 1,213, $p = 0.53$; interaction: $F = 2.04$, df 2,213, $p = 0.13$), as few immature cells contributed to total counts. Lymphocytes were not affected significantly by dose, regardless of sex or reproductive condition.

Normoblasts, immature nucleated erythrocytes typically present in very low numbers in the peripheral blood, were affected by dose ($H = 12.39$, df 2,219, $p = 0.002$), with control animals having the lowest mean rank, high-dose animals an intermediate rank, and low-dose animals the highest rank (Tables 2 and 3). When the data were subdivided by sex and reproductive condition, only effects in nonbreeding males approached significance ($H = 5.73$, df 2,82, $p = 0.057$). A similar analysis for immature neutrophils indicated that dose significantly affected counts only in nonbreeding males ($H = 8.27$, df 2,82, $p = 0.016$). Again, control animals had the lowest mean rank, high-radiation animals an intermediate rank, and low-radiation animals the highest mean rank (Tables 2 and 3). Significant differences were found neither in these cell counts, for breeding males, nonbreeding females, or breeding females, nor in other cell counts, where nonparametric statistics were applied.

Hematocrits were affected by both dose and sex (dose: $F = 16.05$, df 2,247, $p < 0.0001$; sex: $F = 7.97$, df 1,247, $p = 0.0052$; interaction: $F = 1.50$, df 2,247, $p = 0.23$). Hematocrits were significantly higher in controls than both low- and high-dose animals, and were higher in high-dose than low-dose animals. Males had higher hematocrits than females. In separate analyses, female hematocrits were affected by dose ($F = 5.5$, df 2,118, $p = 0.005$), but not by reproductive condition

Table 3. Summary of corticosterone and hematology for male meadow voles. Data are presented as means ± 1 standard error; sample sizes in parentheses. MCBC = Maximum corticosteroid-binding capacity; pcv = packed cell volume

	Control		Low		High	
Parameter	Nonbreeding	Breeding	Nonbreeding	Breeding	Nonbreeding	Breeding
Total corticosterone (ng/ml)	562.7 ± 49.8 (30)	376.6 ± 40.5 (20)	476.7 ± 32.0 (29)	469.3 ± 52.4 (12)	732.4 ± 38.6 (35)	252.5 ± 54.0 (4)
MCBC (ng/ml)	1,873.3 ± 128.6 (30)	1,683.1 ± 136.7 (20)	1,276.1 ± 145.2 (29)	1,329.3 ± 146.5 (12)	2,597.7 ± 169.1 (35)	2,137.8 ± 276.0 (4)
Free corticosterone (ng/ml)	15.9 ± 8.6 (39)	8.5 ± 2.7 (20)	36.2 ± 10.4 (29)	15.8 ± 4.6 (12)	11.6 ± 2.2 (35)	2.8 ± 1.2 (4)
Hematocrit (pcv%)	51.0 ± 0.7 (29)	50.2 ± 1.4 (20)	43.7 ± 1.5 (29)	50.1 ± 1.0 (12)	50.2 ± 0.5 (35)	49.3 ± 0.4 (4)
Leucocytes (per μl)	4.6 ± 0.3 (29)	5.7 ± 0.5 (18)	5.7 ± 0.7 (29)	4.9 ± 0.5 (12)	5.1 ± 0.4 (35)	4.2 ± 1.0 (4)
Neutrophils (per μl)	1.3 ± 0.2 (28)	1.7 ± 0.2 (18)	1.9 ± 0.2 (29)	2.3 ± 0.4 (12)	1.4 ± 0.3 (24)	1.5 ± 0.6 (4)
Normoblasts (per μl)	0.03 ± 0.02 (28)	0.03 ± 0.01 (18)	0.3 ± 0.09 (29)	0.07 ± 0.03 (12)	0.1 ± 0.09 (24)	0.01 ± 0.01 (4)
Basophils (per μl)	0.04 ± 0.01 (28)	0.06 ± 0.02 (18)	0.05 ± 0.01 (29)	0.1 ± 0.02 (12)	0.05 ± 0.02 (24)	0.05 ± 0.04 (4)
Eosinophils (per μl)	0.06 ± 0.02 (28)	0.2 ± 0.06 (18)	0.10 ± 0.02 (29)	0.12 ± 0.04 (12)	0.08 ± 0.02 (24)	0.04 ± 0.02 (4)
Lymphocytes (per μl)	2.9 ± 0.3 (28)	3.2 ± 0.3 (18)	3.4 ± 0.5 (29)	2.1 ± 0.2 (12)	3.3 ± 0.4 (24)	2.4 ± 0.8 (4)
Monocytes (per μl)	0.2 ± 0.03 (28)	0.4 ± 0.09 (18)	0.3 ± 0.04 (29)	0.25 ± 0.03 (12)	0.3 ± 0.06 (24)	0.3 ± 0.1 (4)
Azurocytes (per μl)	0.02 ± 0.01 (28)	0.08 ± 0.07 (18)	0.02 ± 0.009 (29)	0.04 ± 0.01 (12)	0.01 ± 0.007 (24)	0.009 ± 0.009 (4)

($F = 0.9$, df 1,118, $p = 0.34$; interaction $F = 0.48$, df 2,118, $p = 0.62$) (Table 2). In females, hematocrits were significantly higher in controls than in high- and low-dose groups. In males, hematocrits were affected by dose ($F = 5.23$, df 2,123, $p = 0.007$), but not by reproductive condition ($F = 1.13$, df 1,123, $p = 0.29$) (Table 3). However, an interaction effect was detected ($F = 3.37$, df 1,123, $p = 0.037$). When examined separately, hematocrits of breeding males were not affected by dose ($F = 0.32$, df 2,33, $p = 0.73$); those of nonbreeding males were affected ($F = 14.87$, df 2,90, $p < 0.0001$). Both control and high-dose, nonbreeding males had significantly higher hematocrits than low-dose animals.

In terms of blood parasites, only *Haemobartonella* infections possibly were related to radiation dose ($H = 9.452$, df 2,220, $p = 0.009$). Control animals had a lower mean rank than both low- and high-exposure animals. *Haemobartonella* is a common, often cryptic blood parasite of voles, but does not appear to be related to any significant pathology [23]. When data were divided by reproductive condition for males and females, there were no effects of radiation on the predominance of any of the parasitic infections.

DISCUSSION

Our results indicate that low, chronic doses of gamma radiation produce an apparent hormetic effect on the stress axis in natural populations of meadow voles. In general, free (Fig. 1, Tables 2 and 3) and total corticosterone levels were higher in low-dose voles than in either control or high-dose voles. Dynamic changes also occurred in corticosteroid-binding capacity, but these differed between the sexes: MCBC was higher in low-dose females than control and high-dose females (Fig. 2a), and lower in low-dose males than control and high-dose males (Fig. 2b). In terms of leucocyte indices (Tables 2 and 3), counts of most cell types were relatively unchanged by dose, with the notable exception of neutrophils. Higher numbers of neutrophils were found in low-dose animals relative to both high-dose and control animals. Hematocrit was the only parameter potentially affected negatively by radiation, with higher packed red blood cell volumes in controls than in irradiated animals.

Corticosteroids

Uniform, chronic exposure to gamma radiation at low-dose rates resulted in a classic stress response, characterized by increases in free and total corticosterone [20]. In both breeding and nonbreeding females, low doses resulted in higher concentrations of both free and total corticosterone relative to both controls and females exposed to high doses (Fig. 1a, Table 2). In males, free corticosterone, the physiologically active form, behaved in a similar fashion (Fig. 1b). Total corticosterone followed these trends in breeding males, but not in nonbreeding males (Table 3).

In contrast, chronic radiation exposure did not result in the expected, stress-related decrease in MCBC in all animals. Chronically stressed animals typically have lower CBG concentrations; decreases in CBG have been observed following the application of stressors both in the laboratory and nature [25,26,30–33]. Corticosterone, when bound to CBG, is inactive biologically; thus its bioavailability is mediated by this key factor [25]. The MCBC concentrations in females were higher in the irradiated groups than the controls (Fig. 2a). Thus, the increase in free-corticosterone levels observed in females exposed to low-radiation dose was not the result of decreased

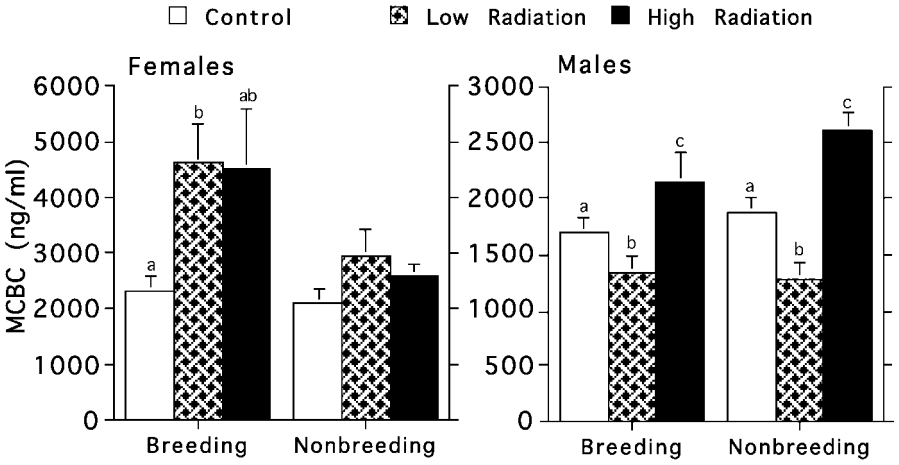

Fig. 2. Response of maximum corticosteroid-binding capacity (MCBC) in each sex (**a** = females, **b** = males) of the meadow vole (*Microtus pennsylvanicus*) to differing radiation level treatments (control, low, and high). Data are separated by reproductive condition. Means (±1 standard error of the mean) are presented for all bars. Significant ($p < 0.05$) pair-wise (Tukey-Kramer) differences within a reproductive class (breeding or nonbreeding) are indicated by different letters. ($n = 124$ for females, $n = 130$ for males).

CBG levels (Fig 1a). High-dose males (both breeding and nonbreeding, Fig. 2b) showed the same response as females, with increased MCBC concentrations. In contrast, low-dose males (both breeding and nonbreeding) do not fit the pattern, having the lowest MCBC. We do not understand why they responded in this way, but suggest that it must be occurring at the level of the liver, where CBG is made, and is not a function of circulating testosterone, as both breeding and nonbreeding males show the same relationship.

Collectively, these results suggest that chronic exposure to gamma radiation at up to 40,000× background does not result in a progressive increase in stress response in meadow voles. Instead, our results illustrate a hormetic response at low-dose rates (50–200× background). These results are consistent with the proposal of Masoro [20] that low-intensity stressors result in a beneficial effect. Consequently, under low-level stress, organisms not only repair damage, but also reduce background damage more effectively [6,10]. Acceptance of the validity of this phenomenon, however, is not universal, largely because of the difficulty in identifying clear underlying mechanisms that could produce it [12]. Our results suggest that the HPA axis likely is involved intimately in mediating hormesis, with moderate hyperadrenocorticism being beneficial.

Presently, a major area of research examining hormesis is life span extension through dietary restriction in rodents [9]. A number of studies [19,34–36] all have reported higher secretion of glucocorticoids (e.g., corticosterone) in food-restricted rodents. These studies suggest that sustained moderate hyperadrenocorticism may be the critical underlying factor, when hormesis leads to increased longevity [9,34]. This relationship could explain why chronic low-level exposure to ionizing radiation resulted in increased, rather than decreased, levels of free (Fig. 1) and total corticosterone (Tables 1–3), even when MCBC increased in females (Fig. 2, Table 2). Mihok (see page 240 in [21]) was not able to obtain an integrated assessment of longevity at the low-level radiation exposure because of small sample sizes and a termination of these study grids in late summer and early fall. However, at the high-level exposure (40,000× background, 44 mGy/d), there was no negative effect on vole longevity [21]. Many, but not all, studies report that small mammal longevity is increased following exposure to low doses of chronic radiation. For example, Caratero et al. [16], found that survival was 22.6% higher in laboratory mice exposed to 7 or 14 cGy/y than in control mice. Luckey [15] discusses numerous studies where chronic low doses of radiation significantly increased longevity in mice and rats. Lastly, an extensive series of studies on mice and guinea pigs exposed to low-level radiation (0.88 mGy per 8-h day until natural death) showed significant increases in survival and growth and a decreased incidence of inflammatory disease [13]. In contrast, French and Kaaz [37] found that low-level radiation (8.8 mGy/d) reduced average life expectancy in deer mice (*Peromyscus maniculatus*).

Exactly how low-level radiation causes a hormetic response remains uncertain because few laboratories have studied the pathology or physiology of mammals exposed throughout life to dose rates below those causing detrimental effects [38]. It has long been known that glucocorticoids play a central role in mitigating the damaging effects of a variety of stressors [17]. Glucocorticoid levels that are too low can be detrimental [39]. At the same time, chronic exposure to excessively high glucocorticoids can be damaging [39], potentially causing an inhibition of growth, impaired disease resistance, infertility, and/or hypertension [32,33]. However, between these two extremes, slightly elevated levels of free corticosterone, causing moderate hyperadrenocorticism, may be beneficial [20].

Munck et al. [17] have suggested that a major role of stress-induced increases in glucocorticoid levels is to protect against excessive primary defense reactions (e.g., immune and inflammatory responses) that respond to both intrinsic (e.g., harmful molecules from normal metabolism such as superoxide, hydrogen peroxide, and hydroxyl radicals) and extrinsic (e.g., infectious agents, toxic substances, and temperature extremes) assaults [9,20]. Glucocorticoids dampen or shut down these reactions after they have accomplished their purpose, thereby preventing damage to the organism and an imbalance in homeostasis. Glucocorticoids also have stimulatory effects on the induction of certain enzymes, such as tryptophan oxygenase, glutamine synthetase, hepatic cytochrome P450, and metallothionein; several of these have detoxifying functions [17]. Consequently, mild stresses, inducing physiologically protective mechanisms, may benefit an organism [9,20].

Another mechanism likely underlying hormesis is the expression of heat-shock proteins (HSPs). Hormetic responses

often occur together with HSPs, which respond adaptively to protect against protein denaturation, as well as to protect cellular components [11,12]. Several studies have found higher levels of HSPs in animals exposed to low-level ionizing radiation, ultraviolet light, and increased temperatures, as well as in calorie-restricted animals [11,12].

In our study, the lack of a stimulatory effect on corticosteroid secretion at high-radiation doses simply could have been due to a negative impact on the functioning of the adrenal gland (Fig. 1). In house mice, significant nonneoplastic pathology occurs with protracted gamma irradiation at levels as low as 22 cGy, despite a lack of evidence for shifts in the age distribution of mortality [38]. Irradiation often ([40], as compared to [13]) has been associated with amyloidosis, a process characterized by the overproduction and deposition of amyloid proteins in the interstitial regions of numerous organs and tissues. Chronic doses below 5 Gy caused an increased rate of deposition of amyloid deposits in the adrenals of both male and female mice [40]. The deposition of amyloid in the intercellular regions can result in the formation of casts that block fluid movements, inhibit normal organ functioning, and eventually cause organ degeneration [40]. In our study, relevant histopathological information was obtained during experiments [41], but unfortunately, this work did not specifically address the highest dose-rate experiment due to an unanticipated population decline that affected all populations throughout the study area (controls at the ZEUS site, those 1 km away, and experimentals on the ZEUS site [21]). Nevertheless, of 136 voles examined (15 low dose, 17 medium dose, 1 high dose, 103 controls), amyloidosis was found only in two from the medium dose rate (experiment 1 in 1981–1982, with voles receiving 15 mGy/d [21]).

The absence of radiation-related pathology in voles during experiments at medium- and low-dose rates [41] may account for why we were unable to detect changes in corticosterone levels in voles living in an ambient radiation field at 40,000× background. An absence of gross negative impacts at a physiological level is consistent with the absence of effects on reproduction, survival, and growth in detailed analyses of vole demography from these experiments [21]. At the highest dose rate received by animals (44 mGy/d), typical mean chronic lifetime doses of 4 to 6 Gy and up to 10 Gy in long-lived animals did not cause any clear effects over about three generations [21]. Similarly, Ross [24], in a study of meadow voles receiving a dose of about 15 mGy/d in the first ZEUS experiment, found no apparent effects on the genetic structure of irradiated populations. This ability of voles to adapt to high levels of chronic radiation exposure without evidence of detrimental effects is reviewed relative to the historical literature on the radioecology of mammals in Mihok [21]. A typical example is the situation within the highly radioactive area of the exclusion zone at Chernobyl, Ukraine. There, Baker et al. [42] found no reduction in the diversity or abundance of small mammals and no gross aberrant morphology or chromosomal rearrangements, eight to nine years after the accident. More recently, Rodgers and Baker [43] found no chromosomal damage in *Clethrionomys glareolus* (bank voles) in the same area. In the only long-term experimental study ever conducted (the Field Irradiator Gamma Forest in Canada), 1,074 red-backed voles (*C. gapperi*) were captured and over 11,178 small mammals were autopsied over 15 years to document radiation effects. Even so, there was no clear evidence for radiation having an effect on *C. gapperi* individuals or populations [44].

Sex differences in corticosteroids

Free and total corticosterone levels, as well as MCBC, were significantly higher in females than males, independent of radiation treatment. Boonstra and Boag [26] reported similar results, with female *M. pennsylvanicus* having corticosterone concentrations and MCBC about twice those of males. McDonald and Taitt [45] also found that female *M. townsendii* had concentrations of corticosterone seven to twelve times those of males. Additionally, breeding females always had higher levels of free and total corticosterone, as well as higher MCBC, than nonbreeding females. Lactating and pregnant females voles had total corticosterone levels and MCBC significantly above those of nonbreeding females [27,45], as well as higher free-corticosterone levels [45]. In contrast, nonbreeding males always had significantly higher free and total corticosterone levels than breeding males, but MCBC was similar. These differences between the sexes and sexual conditions are the result of male and female sex steroid hormones having differential effects on the HPA axis, thus the release of adrenocorticotropin and glucocorticoids. Testosterone is known to be inhibitory, whereas estrogen is stimulatory, especially when females are pregnant or lactating [46]. In addition, testosterone is inhibitory to CBG levels, which are known to decrease when males are in the scrotal state [26].

Hematology

Radiation had only a very subtle effect on white blood cell parameters of meadow voles, but one that is consistent with an hormetic response to low-dose radiation. No suggestion was present of radiation-induced cytotoxicity or immunosuppression in leucocyte counts for various cell types. Neutrophil counts were elevated with low-dose, but not high-dose, exposure in all groups (Tables 2 and 3); lymphocytes were unaffected. This suggests that overall neutrophil homeostasis is mediated by corticosterone. Neutrophils are the first line of defense against infection, and are a pivotal component of the inflammatory response, increasing markedly after the injection of glucocorticoids [47].

Radiation appeared to have a negative effect on the erythropoietic system, with hematocrits being higher in controls than in voles exposed to either low or high doses (Tables 2 and 3). Voles exposed to a low dose had the lowest hematocrits. Consistent with this trend, immature erythrocyte counts (normoblasts) were highest in the low-dose group and lowest in controls. In a chronic, as opposed to an acute, stress situation [23], this pattern of mild anemia suggests an increased demand on erythropoiesis in the low-dose group that could not be met adequately in terms of maintaining normal packed cell volume, and in terms of adequate maturation of erythrocytes before release from the bone marrow. Erythrocyte counts, hematocrit, total hemoglobin, and beta globulin were also significantly lower in deer mice exposed to low-level radiation than controls [48]. However, given the considerable dynamic variability in hematocrits in wild voles [23], caution should be used when interpreting these results.

CONCLUSION

The findings of this study suggest that a moderate increase in glucocorticoid levels, associated with low-level radiation, could be an important factor underlying the increase in longevity that has been seen in other studies on small mammals exposed to low-level radiation. This relationship between mod-

erate hyperadrenocorticism and increased life span also has been documented in small mammals put on calorie-restricted diets. As with caloric restriction, low-level radiation is considered to be a mild stressor [19,36]. However, the findings of this research bring up a number of unresolved questions. First, what are the precise mechanisms by which hormesis augments the stress axis? Second, what are the precise mechanisms stimulated by moderately increased glucocorticoid levels that may translate into increased life span? Third, what are the tradeoffs that occur as a result of increased longevity; for example, what are the effects on reproduction? Thus, more research is required to determine how low-level stressors produce hormetic effects that slow the aging process.

Acknowledgement—We thank the numerous Atomic Energy of Canada Limited staff, colleagues, and students for assisting in the design and execution of the ZEUS experiment. We thank B. Schwartz and T. Lawton for helping to collect the field data and G. Alexopoulus for helping with the radioimmunoassays. The Natural Sciences and Engineering Research Council of Canada provided funding to R. Boonstra and Atomic Energy of Canada provided funding for the ZEUS experiment. This article reports the results of research only. The information presented does not constitute an endorsement or recommendation by the Canadian Nuclear Safety Commission.

REFERENCES

1. Turner FB. 1975. Effects of continuous irradiation on animal populations. *Adv Radiat Biol* 5:83–144.
2. Sagan LA, Cohen JJ. 1990. Biological effects of low-dose radiation: Overview and perspective. *Health Phys* 59:11–13.
3. Turner BN, Iverson SL. 1976. Project ZEUS: A field irradiator for small-mammal population studies. Report AECL-5524. Atomic Energy of Canada, Pinawa, MB, Canada.
4. Keirim-Markus IB. 2002. Radiation exposure normalization taking account of specific effects at low doses and dose rates. *Atom Energy* 93:836–844.
5. United Nations Scientific Committee on the Effects of Atomic Radiation. 1996. Sources and effects of ionizing radiation. UNSCEAR 1996 report to the general assembly, with scientific annex. Annex, Effects of radiation on the environment. United Nations Publications Sales E.96.IX.3. UNSCEAR, New York, NY, USA.
6. Chapman PM. 2002. Ecological risk assessment (ERA) and hormesis. *Sci Total Environ* 288:131–140.
7. Sagan LA. 1987. What is hormesis and why haven't we heard about it before. *Health Phys* 52:521–525.
8. Calabrese EJ, Baldwin LA. 1998. Hormesis as a biological hypothesis. *Environ Health Perspect* 106:357–362.
9. Masoro EJ. 1998. Hormesis and the antiaging action of dietary restriction. *Exp Gerontol* 33:61–66.
10. Stebbing ARD. 1998. A theory for growth hormesis. *Mutat Res* 403:249–258.
11. Parsons PA. 2002. Radiation hormesis: Challenging LNT theory via ecological and evolutionary considerations. *Health Phys* 82: 513–516.
12. Parsons PA. 2001. Radiation hormesis: An ecological and energetic perspective. *Med Hypotheses* 57:277–279.
13. Congdon CC. 1987. A review of certain low-level ionizing radiation studies in mice and guinea pigs. *Health Phys* 52:593–597.
14. Zukhbaya TM, Smirnova OA. 1991. An experimental and mathematical analysis of lymphopoiesis dynamics under continuous irradiation. *Health Phys* 61:87–95.
15. Luckey TD. 1991. *Radiation Hormesis.* CRC, Boca Raton, FL, USA.
16. Caratero A, Coutade M, Bonnet L, Planel H, Caratero C. 1998. Effect of a continuous gamma irradiation at a very low dose on the life span of mice. *Gerontology* 44:272–276.
17. Munck A, Guyre P, Holbrook N. 1984. Physiological functions of glucocorticoids during stress and their relation to pharmacological actions. *Endocr Rev* 5:25–44.
18. Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21: 55–89.
19. Masoro EJ. 1995. Dietary restriction. *Exp Gerontol* 30:291–298.
20. Masoro EJ. 2000. Caloric restriction and aging: An update. *Exp Gerontol* 35:299–305.
21. Mihok S. 2004. Chronic exposure to gamma radiation of wild populations of meadow voles (*Microtus pennsylvanicus*). *J Environ Radioactiv* 75:233–266.
22. Boonstra R. 1994. Population cycles in microtines: The senescence hypothesis. *Evol Ecol* 8:196–219.
23. Mihok S, Schwartz B. 1989. Anemia at the onset of winter in the meadow vole (*Microtus pennsylvanicus*). *Comp Biochem Physiol A* 94:289–304.
24. Ross HA. 1986. Genetic changes in an irradiated population of wild meadow voles (*Microtus pennsylvanicus*). *Can J Zool* 64: 698–703.
25. Rosner W. 1990. The functions of corticosteroid-binding globulin and sex hormone-binding globulin: Recent advances. *Endocr Rev* 11:80–91.
26. Boonstra R, Boag PT. 1992. Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones. *J Anim Ecol* 61: 339–352.
27. Tait JR, Burstein S. 1964. In vivo studies of steroid dynamics in man. In Pincus G, Thiman KV, Astwood EB, eds, *The Hormones*, Vol 5. Academic, New York, NY, USA, pp 441–557.
28. Boonstra R, McColl CJ, Karels TJ. 2001. Reproduction at all costs: How breeding compromises the stress response and survival in male Arctic ground squirrels. *Ecology* 82:1930–1946.
29. Westphal U. 1967. Steroid-protein interactions. XIII. Concentration and binding affinities of corticosteroid-binding globulin in sera of man, monkey, rat, rabbit, and guinea pig. *Arch Biochem Biophys* 118:556–567.
30. Mihok S, Schwartz B. 1991. Origins and maturation patterns of azurocytes in the meadow vole (*Microtus pennsylvanicus*). *Comp Biochem Physiol C* 99:219–230.
31. Fleshner M, Deak T, Spencer RL, Laudenslager ML, Watkins LR, Maier SF. 1995. A long-term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. *Endocrinology* 136:5336–5342.
32. Boonstra R, Hik DS, Singleton GR, Tinnikov A. 1998. The impact of predator-induced stress on the snowshoe hare cycle. *Ecol Monogr* 68:371–394.
33. Hik DS, McColl CJ, Boonstra R. 2001. Why are Arctic ground squirrels more stressed in the boreal forest than in alpine meadows. *Ecoscience* 8:275–288.
34. Sabatino F, Masoro EF, McMahan A, Kuhn RW. 1991. Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. *J Gerontol* 46:B171–179.
35. Han E, Levin N, Bengani N, Roberts JL, Yongman S, Karelus K, Nelson JF. 1995. Hyperadrenocorticism and food restriction–induced life extension in the rat: Evidence for divergent regulation of pituitary proopiomelanocortin RNA and adrenocorticotropic hormone biosynthesis. *J Gerontol* 50:B288–B294.
36. Duffy PH, Leakey JEA, Pipkin JL, Turturro A, Hart RW. 1997. The physiologic, neurologic, and behavioral effects of caloric restriction related to aging, disease, and environmental factors. *Environ Res* 73:242–248.
37. French NR, Kaaz HW. 1968. The intrinsic rate of natural increase of irradiated *Peromyscus* in the laboratory. *Ecology* 49:1172–1179.
38. Carnes BA, Gavrilova N, Grahn D. 2000. Pathology effects at radiation doses below those causing increased mortality. *Radiat Res* 158:187–194.
39. Masoro EJ. 1995. Glucocorticoids and aging. *Aging Clinical and Experimental Research* 7:407–413.
40. Lesher S, Sacher GA, Grahn G, Hamilton K, Sallese A. 1965. Survival of mice under duration-of-life exposure to gamma rays. *Radiat Res* 24:239–277.
41. Descôteaux JP, Mihok S. 1986. Serologic study on the prevalence of murine viruses in a population of wild meadow voles (*Microtus pennsylvanicus*). *J Wildl Dis* 22:314–319.
42. Baker RJ, Hamilton MJ, van den Bussche RA, Wiggins LE, Sugg DW, Smith MH, Lomakin MD, Gaschak SP, Bundova EG, Rudenskaya GA, Chesser RK. 1996. Small mammals from the most-radioactive sites near the Chernobyl nuclear power plant. *J Mammal* 77:155–170.

43. Rodgers BE, Baker RJ. 2000. Frequencies of micronuclei in bank voles from zones of high radiation at Chornobyl, Ukraine. *Environ Toxicol Chem* 19:1644–1648.
44. Mihok S, Schwartz B, Iverson SL. 1985. Ecology of red-backed voles (*Clethrionomys gapperi*) in a gradient of gamma radiation. *Ann Zool Fenn* 22:257–271.
45. McDonald IR, Taitt MJ. 1982. Steroid hormones in the blood plasma of Townsends's voles (*Microtus townsendii*). *Can J Zool* 60:2264–2269.
46. Handa RJ, Burgess LH, Kerr JE, O'Keefe JA. 1994. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Horm Behav* 28:464–476.
47. Mihok S, Schwartz B. 1991. Artificial induction of azurocytes in the meadow vole (*Microtus pennsylvanicus*). *Comp Biochem Physiol C* 99:213–218.
48. Arthur WJ, Markham OD, Groves CR, Keller BL, Halford DK. 1986. Radiation dose to small mammals inhabiting a solid radioactive waste disposal area. *J Appl Ecol* 23:13–26.