AN ABSTRACT OF THE THESIS OF

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Title: The Lethal Response of the Roughskin Newt, Taricha granulosa (Skilton), to Ultraviolet-B Radiation at Three Fluence Rates.

Abstract Approved: Redacted for Privacy

Donald J. Kimeldorf

Roughskin newts, Taricha granulosa (Skilton), were exposed to UV-B (280-320nm), UV-A (321-380 nm), and visible radiation from fluorescent sunlamps (Westinghouse FS40) that were filtered with cellulose acetate film. UV-B controls were exposed to irradiation from sunlamps that were filtered with Mylar-D, a polyester film that effectively absorbed UV-B radiation while allowing the penetration of UV-A and visible wavelengths. UV controls were irradiated with Vita-Lite lamps (Duro-Test Co., North Bergen, NJ) that produce relatively high intensity visible radiation, but only minimal amounts of UV-A and UV-B. The effects of total accumulated UV-B fluences between 300 and 1300 kJm^{-2} were examined at three fluence rates (0.337, 0.646, and 1.179 Wm^{-2} UV-B).

Lethality was found to be a dose-dependent phenomenon that occurred at total accumulated UV-B fluences above a threshold dose of approximately 400 kJm^{-2}. Exposure at all three fluence rates resulted in mortality. Thus, even the lowest fluence rate was above the fluence rate threshold for Taricha. A dose rate effect appears to exist, with respect to LD50 and threshold lethal dose, between the low and intermediate fluence rates.

In addition to the lethal effect, UV-B exposure resulted in

excessive skin sloughing, blanching, and ulceration of the dorsal skin and the development of a darkly pigmented mid-dorsal stripe in some individuals. At the microscopic level, UV-B irradiated skin showed edematous changes and cytopathology. It is proposed that UV-B induced skin damage in Taricha leads to a deficiency in cutaneous respiration that may ultimately result in death. Other potentially lethal systemic effects, that may result from a breakdown in skin integrity, include infection and osmoregulatory dysfunction.

The Lethal Response of the Roughskin Newt, *Taricha granulosa* (Skilton), to Ultraviolet-B Radiation at Three Fluence Rates

by

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Typed by Cheryl McLean for Barbara Michaelis

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I would like to dedicate this work to the memory of my mother, and to my father, who has not only taught me the value of academic pursuits, but has also given me both moral and financial support in these endeavors.

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INTRODUCTION

Ozone in the earth's atmosphere effectively attenuates solar ultraviolet-B (280-320 nm) radiation (Gates, 1966; Green et al., 1974). Halomethanes and other chemicals can catalyze the destruction of atmospheric ozone (Johnson, 1971; Molina and Rowland, 1974). The relatively high levels of these chemicals entering the atmosphere from man-made sources has recently led to concern about global ozone reduction (CIAP, 1974; NAS, 1979; Maugh, 1979). The result of a small decrease in the amount of atmospheric ozone is a relatively large increase in the amount of biologically effective ultraviolet-B (UV-B) radiation reaching the earth's surface (CIAP, 1974; Geise, 1976). Such an increase in UV-B levels may have significant biological consequences.

Excessive UV-B exposure can be detrimental to biological systems. Absorption of UV-B wavelengths by nucleic acids, proteins, and lipids can result in dimer formation, cross-linkage, chain breaks, and lipid peroxidation (Jagger, 1973; Johnson and Daniels, 1969). The most common of these detrimental effects is the formation of pyrimidine dimers in nucleic acids. Extensive damage to important biological molecules can disrupt higher levels of organization and may result ultimately in cell death.

In vertebrate organisms, UV-B irradiation has been associated with erythema (Breit and Kligman, 1969; Geise, 1976; Epstein, 1977), sunburn (Daniels et al., 1968; Geise, 1976; Epstein, 1977), immunosup-

pression phenomena (DeFabo and Kripke, 1980), carcinogenesis (Epstein et al., 1969; Freeman, 1975), as well as developmental distortion and lethality in larval forms (Worrest and Kimeldorf, 1976; Hunter et al., 1979). Lethality has also been reported in adult, unshaved, albino mice exposed to ultraviolet radiation at wavelengths between 200 and 313 nm (Reick and Carlson, 1955). Lethality in the European crested newt, Triturus cristatus carnifex, as a result of UV-B exposure has recently been reported (Zavanella and Losa, 1981).

In adult vertebrates, UV-B damage primarily involves the integument where most, if not all, of the energy is absorbed. The skin is an extremely important organ, and skin damage can conceivably be potentially lethal. The amphibian appears to be an interesting model for the study of UV-B induced lethality. Their relatively thin skin, lack of integumental appendages, and dependence on cutaneous respiration to fulfill a large part of their oxygen requirement ought to make amphibians especially susceptable to UV-B induced damage. The purpose of this study was to examine quantitative changes in the lethal response of the newt, Taricha granulosa (Skilton), with respect to total UV-B fluence and fluence rate.

MATERIALS AND METHODS

Experimental Animals

Roughskin newts, Taricha granulosa (Skilton), were collected throughout the year from permanent ponds in the vicinity of Corvallis, Oregon. During the breeding season, male newts acquire secondary sexual characteristics, including smooth, turgid skin (Pimentel, 1960). After breeding, but while still in the aquatic environment, the males assume their normal rough-skinned morphology. Since the skin is undoubtedly a target for ultraviolet-B damage, males in breeding dress were excluded in order to minimize the variability between individuals and groups. Female T. granulosa do not undergo a change in skin morphology during the breeding season, and are always rough-skinned. Gravid females were excluded in an effort to reduce physiological variability.

The newts were distributed so that all experimental and control groups had approximately the same mean body weight (mean of means = 10.3 $\pm$ 0.5 grams). Since female T. granulosa are generally smaller than the males, an effort was made to insure that the females were distributed evenly among the various groups. A relatively small number of females were collected, so each group consisted of approximately 80 to 90% males. Experimental groups usually contained 20 to 25 individuals, and control groups contained 14. Group sizes varied somewhat according to the relative success of collecting expeditions. Group sizes greater than 25 represent pooled data from replicated experiments. Newts were held at the experimental water temperature (13 $\pm$ 2°C) for at least three days before the start of the experiments.

Lethality Studies

Three different UV-B fluence rates were used. Experimental groups were irradiated for 24 hours per day with either 2, 4, or 8 fluorescent sunlamps (Westinghouse FS40) in the low, intermediate, and high fluence rate studies, respectively. The sunlamps were filtered with 0.25 mm cellulose acetate film that effectively absorbed ultraviolet wavelengths below 280 nm. Thus, the experimental groups were subjected to UV-B (280-320 nm), UV-A (321-380 nm), and visible (above 380 nm) radiations produced by the sunlamps. These lamps produce relatively little radiation in the UV-C region (below 280 nm), and the cellulose acetate (CA) served to attenuate these wavelengths to the point of virtual nonexistence.

Cellulose acetate becomes somewhat more opaque with accumulated exposure. The partial degradation of the filter is rapid at first and then becomes relatively stable (Sisson and Caldwell, 1975). Therefore, CA filters were presolarized for 24 hours under eight sunlamps at a distance of 40 cm in order to stabilize transmission characteristics. The filters were changed every 14, 4, or 3 days in the low, intermediate, and high fluence rate studies, respectively. The UV-B fluence rates reported here are averages of the rates through presolarized filters and the filters after 14, 4, or 3 days of use. Fluence rates in the UV-A and visible regions of the spectrum, in addition to the UV-B rates, are summarized in Table 1.

TABLE 1. Exposure Conditions

Fluence rates (Wm^{-2}) in the UV-B, UV-A, and visible regions of the spectrum for the experimental and control groups. SL = sunlamps, VL = Vita-Lites, CA = Cellulose acetate, MY = Mylar-D.

TABLE 1. EXPOSURE CONDITIONS

GROUP DESIGNATION	LAMPS	FILTER	FLUENCE RATE (Wm^{-2}) UV-B (280-320nm)	UV-A (321-380nm)	VISIBLE (381-800nm)	TOTAL (280-800nm)
LOW UV-B	2 SL	CA	0.337	0.444	0.650	1.431
INTERMEDIATE UV-B	4 SL	CA	0.646	0.874	1.086	2.606
HIGH UV-B	8 SL	CA	1.179	2.050	2.488	5.717
UV-B CONTROL INTERMEDIATE	4 SL	MY	0.034	0.802	1.731	2.567
UV-B CONTROL HIGH	8 SL	MY	0.125	1.358	3.537	5.020
UV CONTROL	2 VL	NONE	0.027	0.439	15.249	15.715

The experiments were designed so that the effect of increasing the total accumulated fluence could be examined at three different fluence rates (Table 2). Groups L-300, L-500, L-650, L-900, and L-1100 were exposed to UV-B irradiation at the lowest fluence rate (0.337 Wm^{-2}). The number in the group designation corresponds to the approximate total accumulated UV-B fluence (in kJm^{-2}) that the group received. Each group was exposed for the appropriate number of successive days (24 hours per day) required to accumulate their respective fluences. Similarly, groups I-300, I-500, I-650, I-900, I-1100, and I-1300 were exposed to irradiation at the intermediate UV-B fluence rate (0.646 Wm^{-2}), and groups H-300, H-650, H-900, and H-1100 were exposed to irradiation at the highest UV-B fluence rate (1.179 Wm^{-2}).

The UV-B exposure took place in aerated, temperature-controlled, 110x56x36 cm acrylic aquaria. A presolarized cellulose acetate filter was placed over the top of each aquarium. Acrylic legs were attached to the fixtures so that the sunlamps were supported about 4 cm above the filter. Opaque black plastic was placed on the sides of the aquaria, and draped over the top of the sunlamp fixtures in order to exclude extraneous room light. Experimental groups remained under continuous UV-B radiation for the number of days required to achieve their designated total fluence, and were then transferred to smaller acrylic aquaria in a temperature-controlled room. Here they were observed for thirty days, during which time they received illumination from two unfiltered Vita-Lite fluorescent lamps (described below) on a 12L:12D cycle. These smaller tanks were covered with nylon mesh screen in order to prevent the newts from escaping. The animals were

TABLE 2. Experimental Groups

A summary of the experimental groups. The letter in the group designation indicates the fluence rate (L = low, I = intermediate, and H = high), and the number corresponds to the total accumulated UV-B fluence in kJm^{-2} at the end of the exposure period. The number of animals in each group is in parentheses.

TABLE 2. Experimental Groups

UV-B FLUENCE RATE		
LOW ($0.337\ Wm^{-2}$)	INTERMEDIATE ($0.646\ Wm^{-2}$)	HIGH ($1.179\ Wm^{-2}$)
L-300 (n=25)	I-300 (n=25)	H-300 (n=25)
L-500 (n=25)	I-500 (n=46)	---
L-650 (n=25)	I-650 (n=69)	H-650 (n=50)
L-900 (n=25)	I-900 (n=66)	H-900 (n=50)
L-1100 (n=25)	I-1100 (n=25)	H-1100 (n=50)
---	I-1300 (n=25)	---

checked daily for mortality, and dead newts were removed from the tanks. Percent survival was calculated, and the mean survival time of the decedent animals was determined at the end of the thirty-day observation period.

A chronic exposure study was also carried out. In this study, experimental animals were exposed to UV-B irradiation at either the intermediate (group I-CH) or the high (group H-CH) fluence rate until all animals died. This experiment was used to determine the mortality distributions under conditions of continuous exposure at the intermediate and high fluence rates.

Two types of control groups were utilized in the lethality studies: UV-B control and UV control. The UV-B controls were subjected to 45 successive days of constant (24 hours per day) irradiation from a bank of eight sunlamps filtered with 0.18 mm Mylar-D polyester film. The Mylar-D film absorbed ultraviolet wavelengths below 320 nm, and thus effectively attenuated UV-B radiation (to about 10% of the energy transmitted through cellulose acetate) while allowing the penetration of UV-A and visible wavelengths. Mylar filters were changed every 14 days, and were not presolarized since their transmission characteristics remain relatively constant with accumulated exposure. The fluence rates reported in Table 1 are averages of the rates through new and 14 day old Mylar filters.

The UV control group was the second type of control used in these experiments. UV controls were subjected to 45 successive days of constant irradiation from two unfiltered Vita-Lite fluorescent lamps (Duro-Test Co., North Bergen, NJ). These lamps have a high total fluence

rate that is primarily due to visible wavelengths (Table 1). Vita-Lites produce relatively little radiation in the UV-B and UV-A regions of the spectrum. The UV-B and UV controls were maintained in acrylic aquaria in a temperature-controlled room under their respective irradiance conditions.

Experimental and control group aquaria were filled to a depth of 5 cm with tap water that had been aged at least three days. The water temperature in all aquaria was maintained at 13 $\pm$ 2°C. Newts were fed Tubifex or red worms every three weeks, and were checked daily for symptoms and mortality.

Chi-square contingency table statistics were employed to test for differences in survival between various groups. Differences were considered to be significant if $X^2 > X^2_{.05}$.

Histological Study

In order to examine the microscopic effects of UV-B radiation on the skin, a histological study was carried out. Newts were irradiated, sacrificed, fixed, and then sent to Dr. C.L. Sanders of Battelle Northwest Laboratories (Richland, WA) for histological examination.

Newts were exposed to continuous UV-B irradiation at the intermediate fluence rate (0.646 Wm^{-2}) for accumulated fluences of approximetely 150, 300, 500, 650, or 900 kJm^{-2}. These newts were sacrificed immediately after receiving their respective fluences. Other newts were exposed to continuous UV-B irradiation at the intermediate fluence rate for accumulated fluences of 300, 500, or 650 kJm^{-2}, and were then held for five days before being sacrificed. During the 5-day holding

period these animals were kept under two unfiltered Vita-Lites on a 12L:12D cycle. The 5-day holding period was used to determine whether there was recovery or continued degradation of the skin when newts were placed under Vita-Lites after their UV-B exposure.

UV-B controls were exposed to 15 days of constant irradiation from four sunlamps filtered with Mylar-D. UV controls were exposed to 15 days of constant illumination from two unfiltered Vita-Lites.

Each experimental and control group contained three animals, and eight dorsal skin samples were taken from each group. The samples were fixed and stained by conventional methods, and examined under TEM, SEM, and light microscopy.

Dosimetry

For exposure field geometry, broad spectrum irradiance was measured at forty-four points (approximately 15 cm apart) on the aquarium bottom with a standardized thermopile system (YSI Radiometer Model 65). One location was found that received the same irradiance as the average of all forty-four points, and the probe was placed at this position for all subsequent radiometric measurements. The fluence rates reported for the UV-B, UV-A, and visible spectral bands were determined in empty exposure tanks with an Optronics Model 742 spectrometer. For wavelengths below 360 nm a teflon diffuser was employed for better cosine response. Energy measurements were made at 2 nm intervals between 360 and 800 nm, and at 1 nm intervals between 270 and 360 nm. The source to probe distance was equivalent to the source to tank bottom distance that was used in the experiments. No correction

was made for any attenuation of the radiation by the 5 cm of water present in the aquaria during actual experiments.

RESULTS

Controls

All UV-B control groups, protected from UV-B wavelengths by Mylar-D film, showed 100% survival. All UV control groups, exposed to the primarily visible wavelengths produced by Vita-Lites, also exhibited 100% survival.

The normally dark brown dorsal skin of T. granulosa gradually became lighter in both the UV-B and UV control groups. By the end of the 45-day exposure period, the dorsal skin of these animals ranged from brown to tan. The color was homogeneous over the whole dorsal surface of any one individual, but the intensity of the effect varied between individuals of the same group. In general, the UV control animals appeared to remain slightly darker than the UV-B controls. Ventral skin, which is normally orange to yellow in color, did not appear to be affected. No other macroscopically visible effects were observed in either of the control groups, except perhaps an increased amount of skin sloughing.

Low Fluence Rate Study

In the low fluence rate study, both groups L-300 and L-500 showed 100% survival. Group L-650 had a survival rate of 96%, which was not significantly different from groups L-300, L-500, or either of the control groups. In group L-900 survival declined significantly to 36%. None of the newts in group L-1100 survived the 30-day observation period. Thus, at the low fluence rate there appears to be a lethal threshold somewhere between 500 and 650 kJm^{-2}, and at higher fluences

the percent lethal response is dose-dependent. Survival curves of the low fluence rate groups appear in Figure 1, mortality distributions appear in Figure 2A, and mean survival time of decedent animals can be seen in Table 3.

As in the control groups, the dorsal skin of the newts irradiated at the low fluence rate gradually blanched. In the group that received the lowest total fluence (L-300), the animals lightened to about the same extent as the control newts. At higher total fluence levels the blanching effect was more dramatic. In most individuals the dorsal skin became tan, and in some cases a light greenish-gray color. The groups receiving the highest total fluences generally exhibited the most discoloration, but there was a fair amount of variability among individuals in any one group.

In some of the newts that received high total fluences, a narrow, darkly-pigmented, mid-dorsal stripe developed despite the discoloration of the rest of the dorsal surface. This stripe usually extended from the tip of the snout to the base of the tail, and was especially prominent as a darkly pigmented splotch on the head. None of the control animals ever exhibited this stripe or any similar pigmentation.

Ulceration of the dorsal skin occurred in all the low fluence rate groups except group L-300. The size and severity of the ulcerations, rather than the number of individuals affected, increased with increasing total fluence. In the groups were ulcerations appeared, 1 to 3 individuals (4 to 12%) were affected. The ulcerations usually occurred as single lesions on the head, but sometimes occurred on the back. The ulcers were less than one centimeter in length.

FIGURE 1

Survival curves of the low fluence rate (0.337 Wm^{-2}) exposure groups: L-300, L-500, L-650, L-900, and L-1100. The number in the group designation corresponds to the total accumulated fluence in kJm^{-2}. Day = the number of days after the start of UV-B exposure.

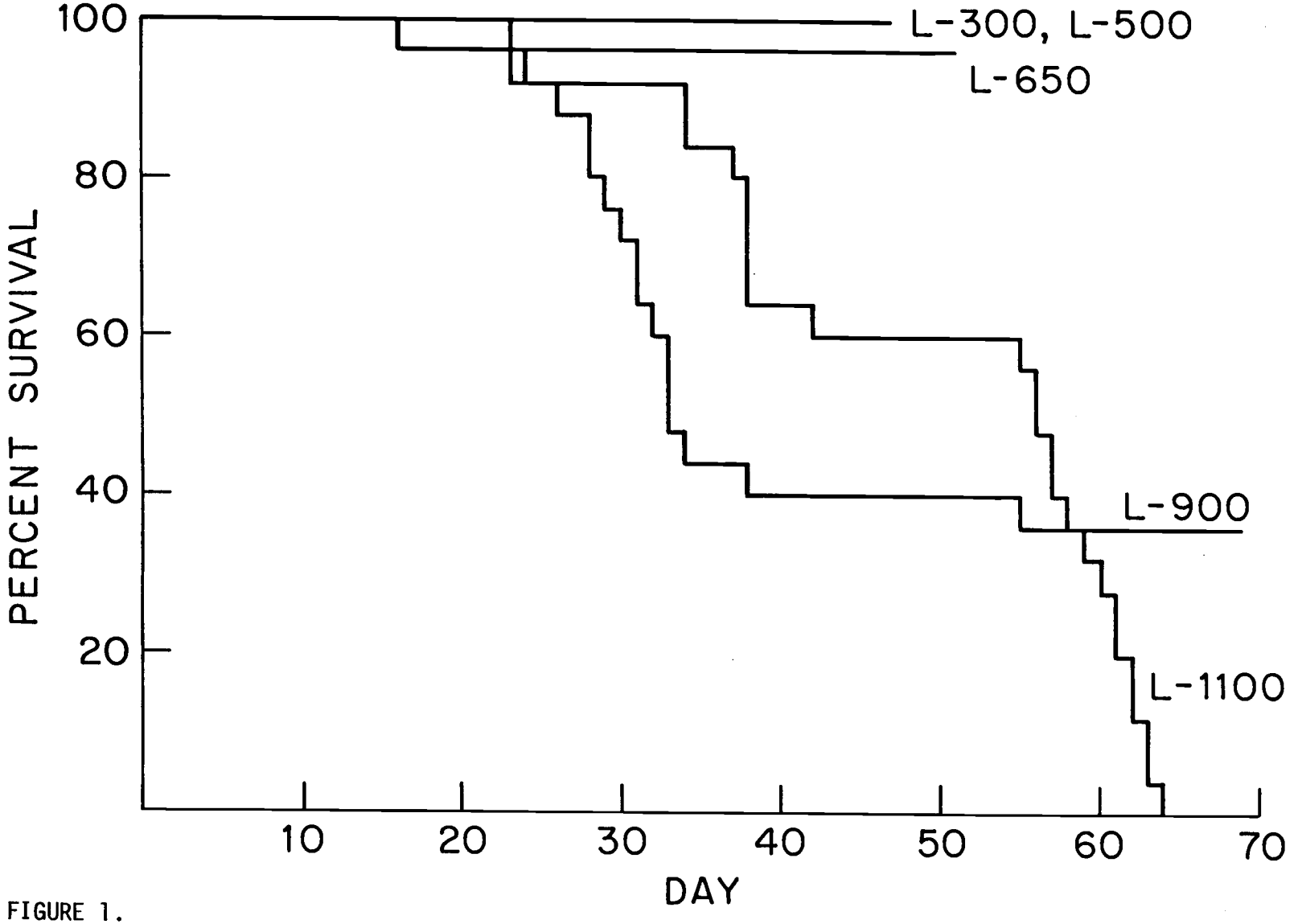
100
80
60
40
20
PERCENT SURVIVAL
L-300, L-500
L-650
L-900
L-1100
10
20
30
40
50
60
70
DAY

FIGURE 1.

FIGURE 2

Mortality distributions of the groups in the low (A), intermediate (B), and the high (C) fluence rate studies. Arrows indicate the last day of UV-B exposure.

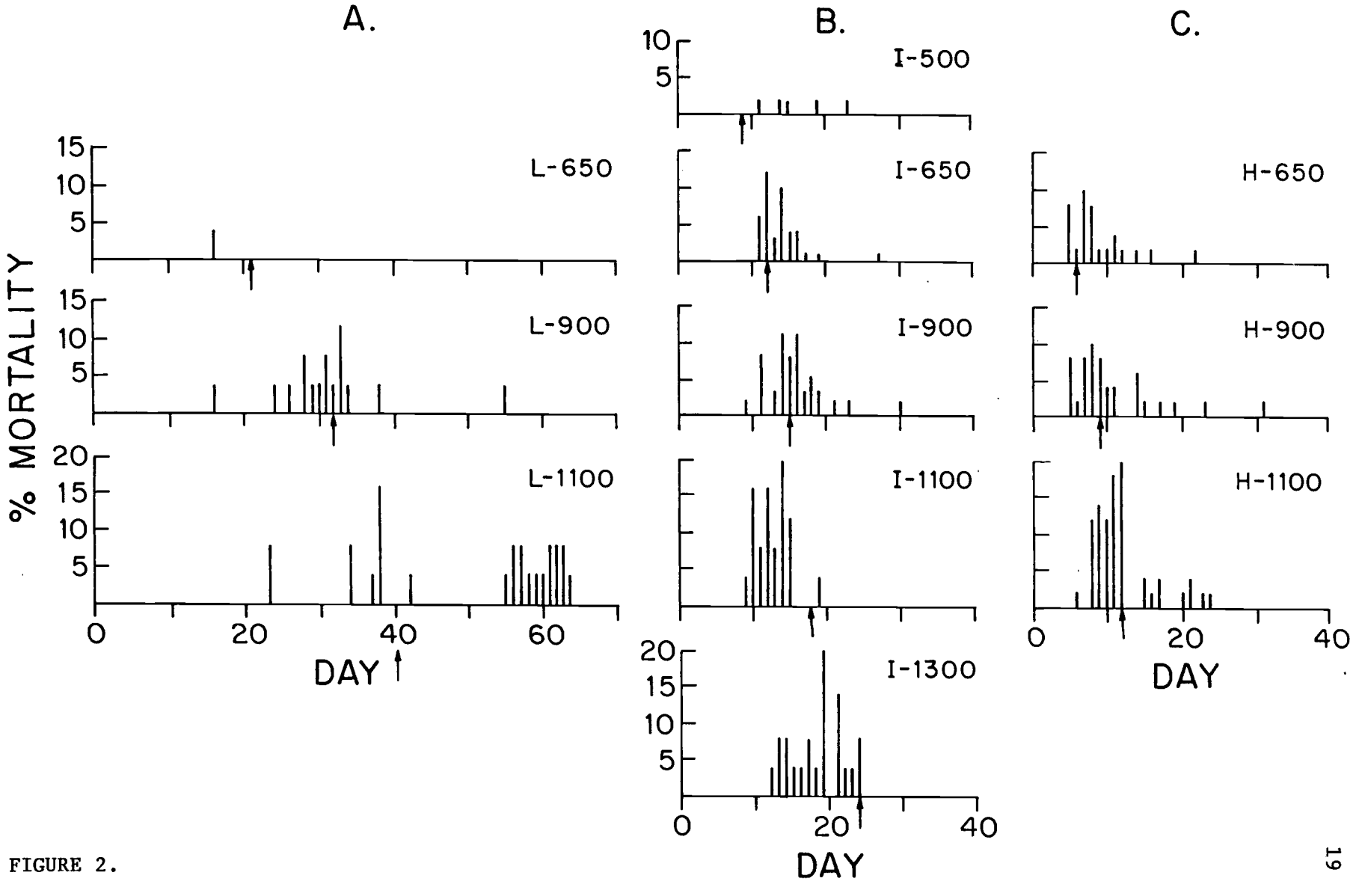
A.
B.
C.
% MORTALITY
L-650
L-900
L-1100
I-500
I-650
I-900
I-1100
I-1300
H-650
H-900
H-1100
DAY
DAY
DAY

FIGURE 2.

TABLE 3. Mean Survival Time of Decedent Animals

Mean survival time in days $\pm$ S.D. for each fluence level in the three fluence rate studies and the chronic exposure study. The asterisk indicates that no animals died.

TABLE 3. MEAN SURVIVAL TIMES (DAYS ± S.D.) OF DECEDENT ANIMALS

TOTAL UV-B FLUENCE (kJm^{-2})	UV-B FLUENCE RATE		
	LOW ($0.337\ Wm^{-2}$)	INTERMEDIATE ($0.646\ Wm^{-2}$)	HIGH ($1.179\ Wm^{-2}$)
300	*	*	*
500	*	16.4 ± 4.1	---
650	16	14.0 ± 3.1	9.0 ± 4.1
900	31.3 ± 8.0	15.6 ± 4.0	10.6 ± 5.8
1100	49.6 ± 13.0	12.7 ± 2.3	11.9 ± 4.1
1300	---	18.5 ± 3.7	---
CHRONIC	---	22.0 ± 5.2	13.5 ± 3.1

The one newt in group L-500 that developed a skin ulcer survived the 30-day observation period, as did two of the three ulcerated individuals in group L-650. Two animals in group L-900, and two in group L-1100 developed relatively severe ulcers and died before the end of the experiment. Thus, five of the eight individuals that developed skin ulcerations died within 30 days after UV-B exposure.

Sloughing of the skin, although not quanitified, appeared to occur to a greater extent in the UV-B exposed animals than in either of the control groups. In some cases, the shed skin accumulated on the bottom of the aquaria to such an extent that it had to be cleaned out three or four times during the 30-day observation period. Newts were occasionally seen consuming their shed skin.

Intermediate Fluence Rate Study

In the intermediate fluence rate (0.646 Wm^{-2}) study there were no deaths in group I-300. Groups I-500, I-650, I-900, I-1100, and I-1300 all had survival rates that were significantly different from the controls and from group I-300. The survival rates of groups I-650 and I-900 were not significantly different from one another, nor were those of groups I-1100 and I-1300. The survival curves of the intermediate fluence rate groups are shown in Figure 3. As in the low fluence rate study, there is a lethal threshold above which the response is dose-dependent. However, the threshold lethal dose appears to be somewhat lower in the animals exposed to UV-B irradiation at the intermediate fluence rate (between 400 and 500 kJm^{-2} as opposed to between 500 and 650 kJm^{-2} in the low fluence rate study).

FIGURE 3

Survival curves of the intermediate fluence rate (0.646 Wm^{-2}) exposure groups: I-300, I-500, I-650, I-900, I-1100, and I-1300. The number in the group designation corresponds to the total accumulated fluence in kJm^{-2}. Day = the number of days after the start of UV-B exposure.

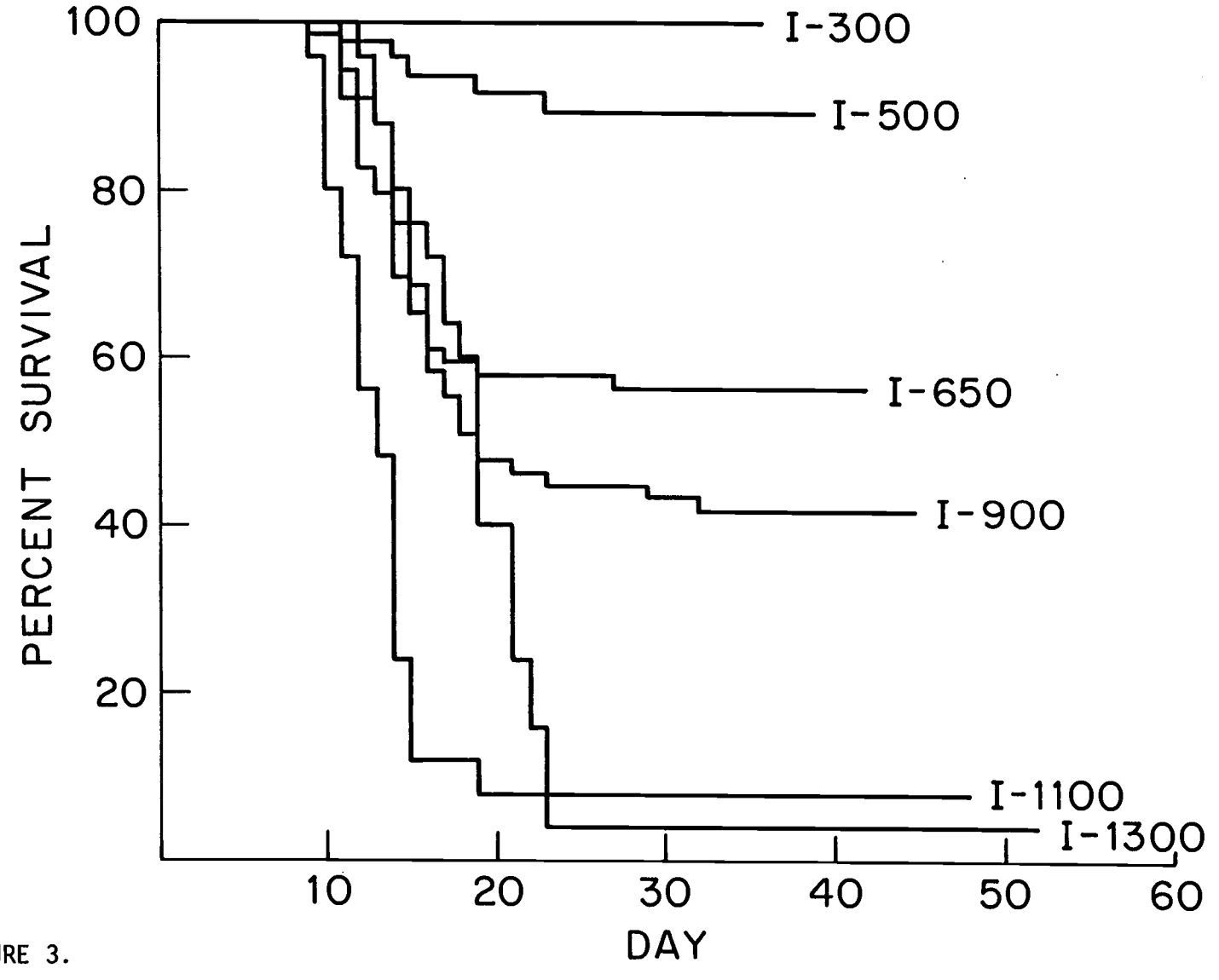
100
80
60
40
20
PERCENT SURVIVAL
I-300
I-500
I-650
I-900
I-1100
I-1300
10
20
30
40
50
60
DAY

FIGURE 3.

The mean survival times of decedent animals in the intermediate fluence rate study can be seen in Table 3. The mean survival times of the groups irradiated at this fluence rate were shorter than those of groups exposed to corresponding fluences at the low fluence rate. This is expected, since any given total fluence is accumulated in a shorter amount of time at the intermediate fluence rate. Mortality distributions can be seen in Figure 2B.

Discoloration was seen in all intermediate fluence rate groups. The degree of blanching appeared to increase with total fluence. The mid-dorsal pigmented stripe, described previously, occurred in all groups except the one that received the lowest total fluence (I-300). The frequency of occurrence increased from about 20% to about 80% as the total fluence increased.

In the intermediate fluence rate study, each group contained individuals that developed skin ulcerations. The anatomical occurrence of the lesions was on the head predominantly, and also on the back. The number of animals affected increased slightly as the total fluence increased. Also, at any given total fluence, the frequency of occurrence was higher than in the low fluence rate study.

The severity of the lesions was greater at the higher total fluences. The ulcerations appeared to be more severe than those observed at equivalent doses in the low fluence rate study. The lesions were often more than one centimeter long and relatively deep.

A large amount of skin sloughing was observed in the intermediate fluence rate groups.

High Fluence Rate Study

In the high fluence rate (1.179 Wm^{-2}) study, there were no deaths in group H-300. Groups H-650, H-900, and H-1100 exhibited survival rates that were significantly different from the controls (Figure 4). The H-650 and H-900 survival rates were not significantly different from one another. As in the other studies, there was a threshold lethal dose and dose-dependent survival at higher total accumulated fluences. Since there was no H-500 group, it is difficult to accurately estimate the threshold dose. However, it appears from Figure 5 that the lethal threshold is approximately the same as that for the animals exposed at the intermediate fluence rate. Thus, it appears that there is no dose rate effect between the intermediate and high fluence rates.

The mean survival times of the decedent animals in groups H-650, H-900, and H-1100 appear in Table 3. The mean survival times were shorter than those of comparable groups in the two lower fluence rate studies. Mortality distributions can be seen in Figure 2C.

Discoloration of the dorsal skin occurred in all groups, but was most severe in newts exposed to the highest total fluences. Most individuals became light greenish-gray in color. The mid-dorsal pigmented stripe appeared in some individuals in all fluence groups except H-300. The frequency of its occurrence increased with increasing fluence, and the stripe stood out distinctly against the blanched skin. The mid-dorsal stripe occurred more frequently in the high fluence rate study than in either of the two lower fluence rate studies.

Ulcerations appeared in all groups, with frequency and severity increasing somewhat with increasing total fluence. At the highest

FIGURE 4

Survival curves of the high fluence rate (1.179 Wm^{-2}) exposure groups: H-300, H-650, H-900, and H-1100. The number in the group designation corresponds to the total accumulated fluence in kJm^{-2}. Day = the number of days after the start of UV-B exposure.

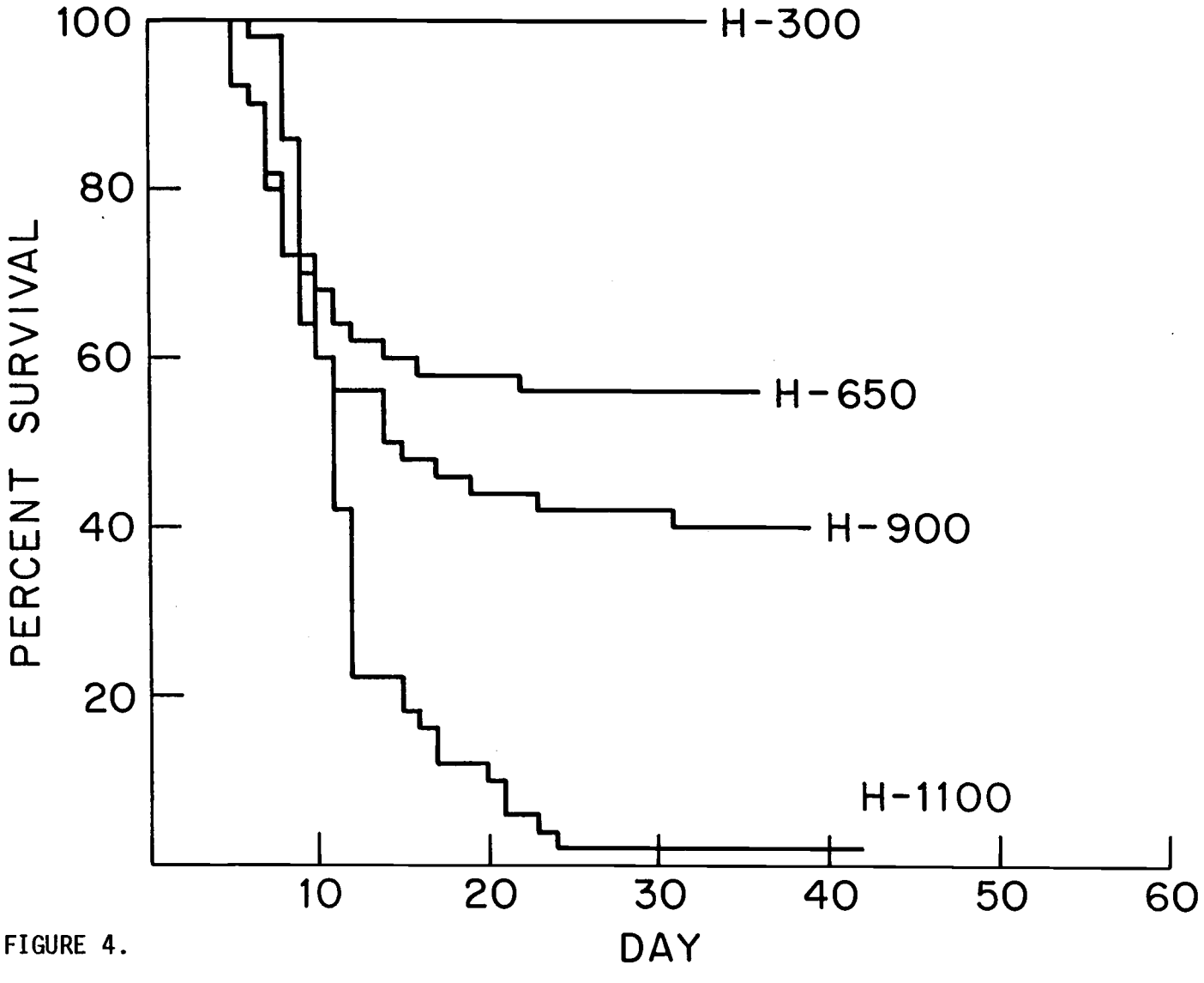
100
80
60
40
20
PERCENT SURVIVAL
H-300
H-650
H-900
H-1100
10
20
30
40
50
60
DAY

FIGURE 4.

FIGURE 5

Survival curves for the low, intermediate, and high fluence rate studies, plotted as percent survival versus the total accumulated fluence that each exposure group received.

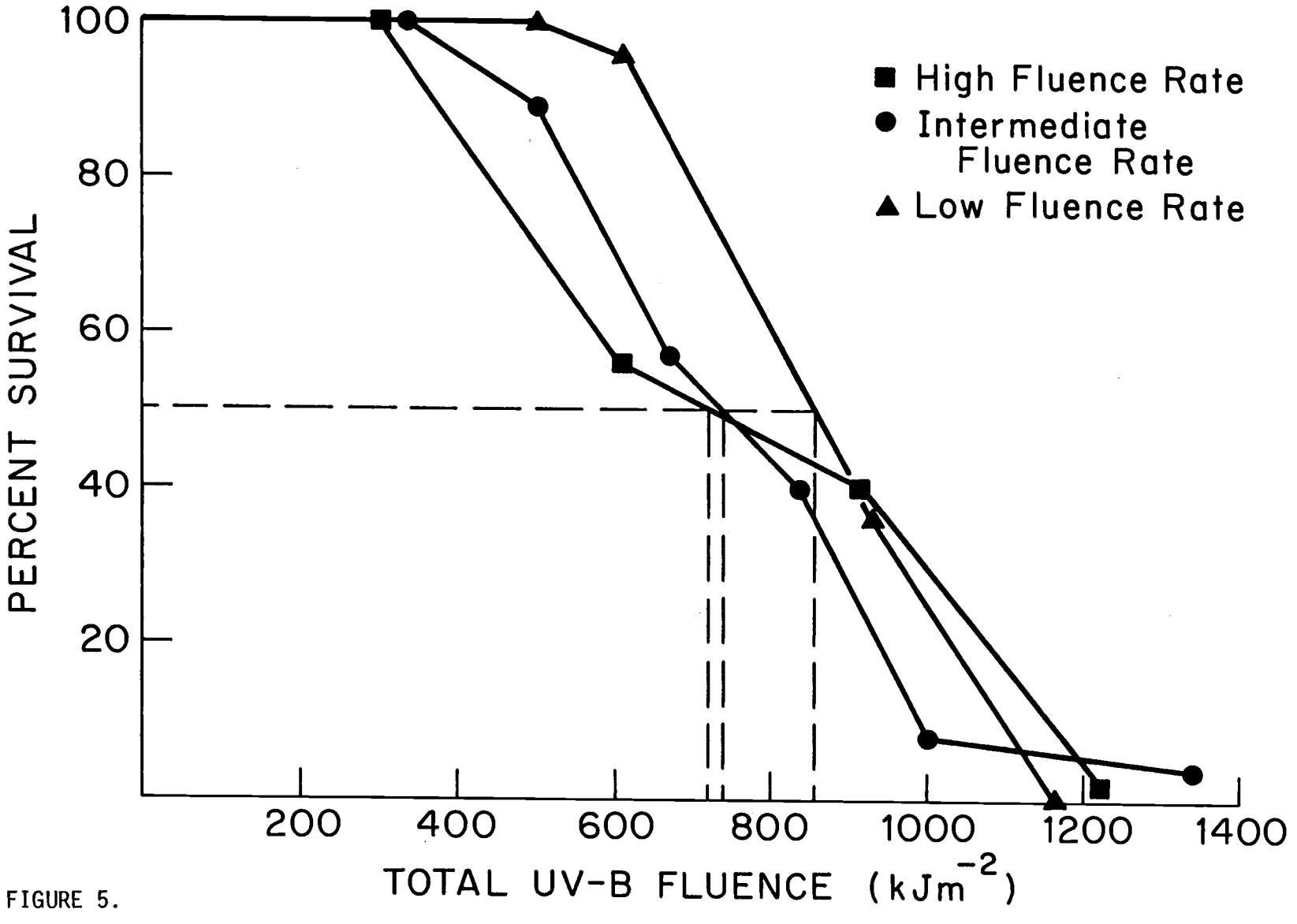
100
80
60
40
20
PERCENT SURVIVAL
200
400
600
800
1000
1200
1400
TOTAL UV-B FLUENCE (kJm^{-2})
High Fluence Rate
Intermediate Fluence Rate
Low Fluence Rate

FIGURE 5.

total accumulated fluence approximately 25% of the individuals were affected. The wounds were generally larger and deeper than those that occurred at the lower fluence rates. One individual developed a lesion that was about 5 cm in length and covered a large portion of the dorsal trunk and head.

Skin sloughing was extensive in the high fluence rate groups, expecially in those that received the highest total fluences.

Chronic Exposure Study

The chronic exposure study was carried out in an attempt to determine the pattern of mortality during continuous UV-B exposure, either at the intermediate (0.646 Wm^{-2}) or the high (1.179 Wm^{-2}) fluence rate, groups I-CH and H-CH, respectively.

Mortality distributions of groups I-CH and H-CH can be seen in Figure 6. The first deaths occurred on day 10 in group I-CH and on day 4 in group H-CH, corresponding to accumulated fluences of approximately 550 and 400 kJm^{-2}, respectively. The mean survival time was about 40% shorter in group H-CH than in group I-CH (Table 3). The survival curves of the chronic exposure groups are represented in Figure 7. The LD50's of groups I-CH and H-CH were 1280 and 1430 kJm^{-2}, respectively (Figure 7). About 65% of the newts in both groups died at accumulated fluences between 1101 and 1500 kJm^{-2}, and approximately 20% died after fluences between 1501 and 2000 kJm^{-2} (Table 4).

Newts in both groups exhibited discoloration, the mid-dorsal pigmented stripe, ulceration, and excessive sloughing.

FIGURE 6

The mortality distributions of the intermediate (I-CH) and the high (H-CH) fluence rate chronic exposure groups.

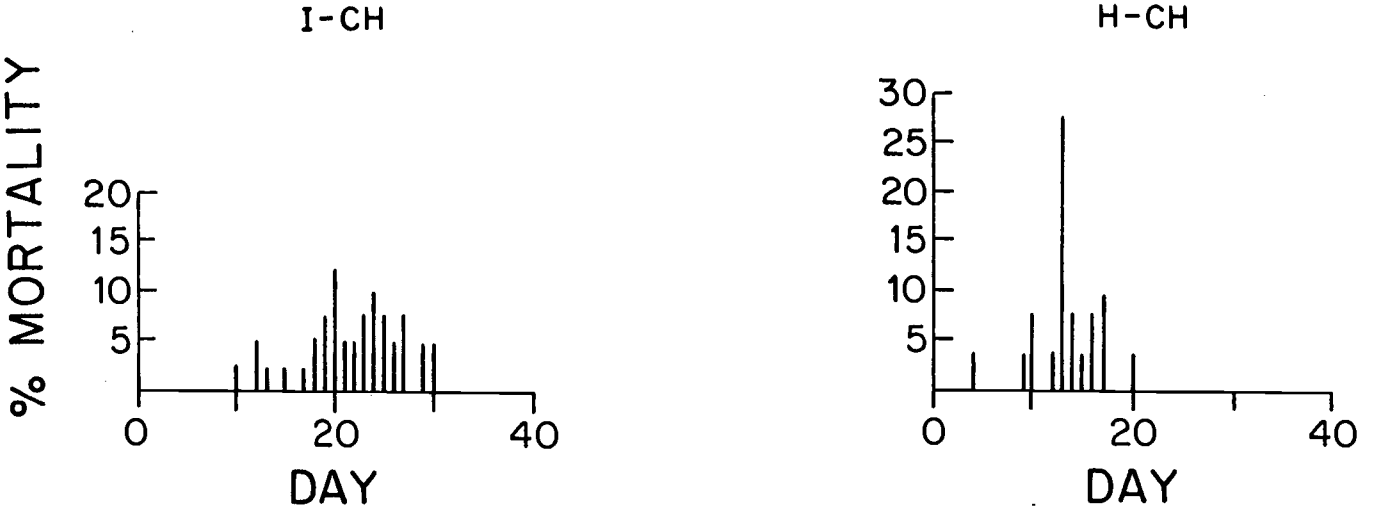

FIGURE 6.

FIGURE 7

Survival curves of T. granulosa irradiated at the intermediate (0.646 Wm^{-2}) and high (1.179 Wm^{-2}) UV-B fluence rates in the chronic exposure study (groups I-CH and H-CH, respectively). The LD50 values were 1280 kJm^{-2} for group I-CH, and 1430 kJm^{-2} for group H-CH.

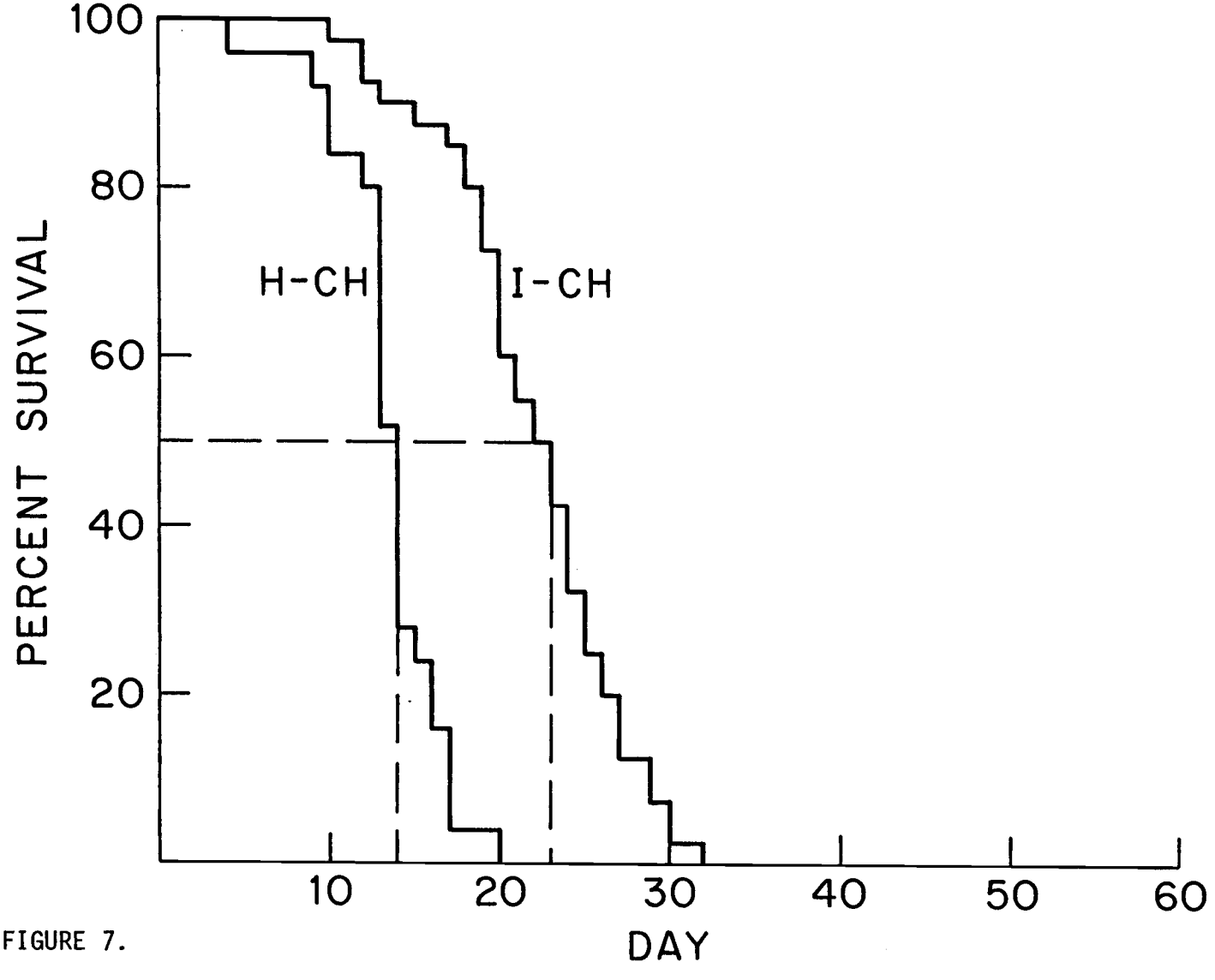

FIGURE 7.

TABLE 4. Mortality Distribution of Chronic Exposure Groups

Distribution of deaths with respect to accumulated fluence for the groups exposed to chronic UV-B irradiation at the intermediate (I-CH) and the high (H-CH) fluence rates.

TABLE 4. MORTALITY DISTRIBUTION OF CHRONIC EXPOSURE GROUPS

ACCUMULATED UV-B FLUENCE (kJm^{-2})	PERCENT MORTALITY I-CH	H-CH
500	0	4
501-1000	15	4
1001-1500	65	64
1501-2000	20	24
2000	0	4

Histological Study

The extent of skin damage in the histological study was rated on a scale of 0 to 3 according to the following scheme: 0 = normal skin, 1 = moderate edema, 2 = marked edema and cytopathology, 3 = marked edema, cytopathology, and desquamation (Table 5). The UV control group, exposed to 15 days of continuous Vita-Lite illumination, showed no signs of skin damage. The UV-B control group, exposed to 15 days of continuous irradiation from four Mylar-filtered sunlamps, exhibited moderate edema of the epidermis. Newts exposed to 150 kJm^{-2} UV-B irradiation also showed moderate edema. The amount of skin damage in the UV-B irradiated animals increased with increasing total fluence. In the animals that were held for five days after the end of the exposure period, some recovery appeared to occur. In both 500 and 650 kJm^{-2} exposure groups, the animals held for five days showed less damage than those sacrificed immediately after exposure.

TABLE 5. Quantification of Skin Damage in the Histological Study

Quantification of skin damage for newts in the histological study: 0 = normal skin, 1 = moderate edema, 2 = marked edema and cytopathology, 3 = marked edema, cytopathology, and desquamation. Values are means $\pm$ S.E., n = 8 skin samples from 3 newts. The first number of the group designation is the number of days under sunlamps, and the second is the number of additional days under Vita-Lites before sacrifice. CA = cellulose acetate, MY = Mylar-D. (Dr. C.L. Sanders, Battelle Northwest Laboratories, Richland, WA, personal communication.)

TABLE 5. QUANTIFICATION OF SKIN DAMAGE IN THE HISTOLOGICAL STUDY

GROUP	DEGREE OF SKIN DAMAGE $\pm$ S.E.
0 + 15	0 ± 0
15(MY) + 0	1.0 ± 0
3(CA) + 0	0.8 ± 0.5
6(CA) + 0	1.5 ± 0.5
6(CA) + 5	1.6 ± 0.5
9(CA) + 0	1.9 ± 0.4
9(CA) + 5	1.6 ± 0.5
12(CA) + 0	2.7 ± 0.5
12(CA) + 5	1.5 ± 0.6
15(CA) + 0	2.6 ± 0.5

DISCUSSION

These studies were carried out in an attempt to characterize the lethal response of the newt, Taricha granulosa, to ultraviolet-B radiation at three fluence rates. The results indicate that irradiation at all three of the fluence rates can be lethal within a 30-day post-irradiation observation period. However, irradiation at the lowest total fluence (approximately 300 kJm^{-2}) did not result in death at any of the three fluence rates. There appears to be a total fluence threshold, below which death does not occur within the 30-day period, and above which the lethal response is dose-dependent. The threshold appears to be similar for the intermediate and high fluence rate groups (between 400 and 500 kJm^{-2}), but it is somewhat higher (between 500 and 650 kJm^{-2}) in animals exposed to irradiation at the low fluence rate. Similarly, the $LD_{50/30}$ values calculated from plots of the intermediate and high fluence rate percent survival values (Figure 5) are approximately the same, while the low fluence rate $LD_{50/30}$ is almost 20% higher. Thus, a dose rate effect appears to be present in the range between the low and intermediate, but not between the intermediate and high fluence rates.

Repair processes seem to be better able to keep up with the accumulating damage at the lowest fluence rate than at the higher ones. However, even at the low fluence rate, repair processes appear to be over-burdened as the total accumulated fluence increases. This suggests that T. granulosa may be near the limit of their repair capabilities when irradiated under the conditions of the low fluence rate study.

This is interesting because the erythemally-weighted daily dose at the low fluence rate was approximately equal to that reaching the earth on a summer day in Western Oregon (Table 6). It should be noted, however, that the DNA-weighted daily dose was approximately 75% higher in this experiment than under natural conditions in June at 45°N latitude. Since the newt is a complex multicellular organism it is unlikely that either weighting factor by itself will be adequate as a predictive factor for the lethal response.

Studies on other whole organisms suggest that many are sensitive to the UV-B radiation intensities now reaching the surface of the earth, and that many may have little capacity to repair or tolerate higher UV-B levels (CIAP, 1974; Barcelo et al., 1978). Although estimates vary somewhat, an eventual decrease of about 10% in atmospheric ozone concentration has been predicted, with half of that decrease occurring in the next 30 years (Damkaer et al., 1981; NAS, 1982). The studies presented here suggest that the resulting increase in biologically effective radiation may have significant consequences for adult amphibians unless they can protect themselves by behavioral mechanisms.

For the newt, avoidance behavior may be an important means of protection against potential damage from ultraviolet exposure. Taricha generally inhabit murky ponds, and in shallow water are often found concealed in the sediment or among the aquatic vegetation. Since these animals have a poisonous skin secretion that discourages predators (Efford and Mathias, 1969), it is possible that the choice of habitat may be influenced by the present UV-B levels. The actual amount of the

TABLE 6. Daily Erythemal and DNA Effective Doses

Daily erythemal and DNA effective doses for the low, intermediate, and high fluence rates, and for June at latitudes similar to that of Oregon. # Low and high fluence rate ERY_{Eff} values were calculated using Green, Mo, and Miller (1974) model. ## From Green, Mo, and Miller (1974). * Computer model based on Green, Cross, and Smith (1980), Dr. R.C. Worrest, personal communication. ** Computer model based on Setlow (1974) DNA action spectrum, Dr. R.C. Worrest, personal communication.

TABLE 6. DAILY ERYTHEMAL AND DNA EFFECTIVE DOSES

IRRADIANCE CONDITIONS	DAILY DOSE (kJm^{-2}) ERY_{Eff}	DNA_{Eff}
LOW FLUENCE RATE	4.960#	0.173**
INTERMEDIATE FLUENCE RATE	---	0.343**
HIGH FLUENCE RATE	17.617#	0.616**
40°N JUNE (ozone = .31 cm)	4.730*	0.106**
50°N JUNE (ozone = .33 cm)	3.680*	0.076**
45°N JUNE (ozone = .32 cm)	4.465##	---
45°N JUNE (ozone = .30 cm)	4.966##	---
45°N JUNE (ozone = .25 cm)	6.482##	---

various UV-B wavelengths reaching submerged newts is highly dependent on the absorbing substances in the water, the depth of the animal, and other factors.

In the three fluence rate studies, no newts died at accumulated fluences of less than 400 kJm^{-2} during exposure or the post-irradiation period. It is possible, however, that lower doses do affect long term survival. Longer observation periods might reveal lower lethal thresholds or life-span shortening. Zavanella and Losa (1981) observed mortality within seven months in European crested newts (Triturus cristatus carnifex) that were exposed to lower fluences at lower fluence rates than were used in these studies. Triturus were irradiated with unfiltered FS40 sunlamps. The lower lethal doses reported by those authors might be a result of the longer observation time, species variation, different experimental conditions, or a combination of these factors.

The mean survival time of the individuals that died before the end of the experiments was found to decline as the fluence rate increased. This is to be expected since potentially lethal damage accumulates more rapidly at the higher fluence rates. In another study conducted in this laboratory (unpublished), newts received 12 hours of irradiation per day until all animals died, and the mean survival time was 20 days. In the present study, newts exposed to 24 hours per day of irradiation at approximately half the fluence rate had a mean survival time of 42 days. This suggests that reduction of the fluence rate is more important in lengthening survival time than is fractionation of the dose.

Both total fluence (dose) and fluence rate (dose rate) are impor-

tant factors to be considered when attempting to predict the effects of UV-B exposure. For example, Damkaer et al. (1981) found that shrimp larvae showed a fluence rate threshold below which no detrimental effects were observed, regardless of the total accumulated fluence. In the present study, on the other hand, even the lowest experimental fluence rate (0.337 Wm^{-2}) exceeded the lethal threshold for T. granulosa. It is interesting to note that the erythemal- and DNA-weighted irradiances in the low fluence rate study (0.057 Wm^{-2}_{Ery} and 0.002 Wm^{-2}_{DNA}) were equivalent to the threshold fluence rate in the Damkaer et al. (1981) study (0.054 Wm^{-2}_{Ery} and 0.002 Wm^{-2}_{DNA}). Thus, when considering fluence rate alone, it appears that newts may be equally or more sensitive than shrimp larvae to UV-B exposure. However, the newts received and were able to tolerate much higher daily and total accumulated fluences than the shrimp larvae. The threshold lethal dose for T. granulosa under the conditions of these studies appears to be around 400 kJm^{-2}.

Ultraviolet-B radiation is almost completely absorbed in the skin, and this is where the primary damage occurs. Skin damage appears to be the major cause of death in the exposed animals, although the precise mechanism involved is not yet clear. The newt has relatively thin skin that is unprotected by integumental appendages such as scales, feathers, or hair. The skin is crucial for protecting the body from physical injury and entry of pathogens. It is involved in osmoregulation (Brown and Brown, 1977), and is also an important respiratory organ in newts (Czopek, 1959). A breakdown in the integrity of the skin could have potentially lethal consequences including: bacterial or fungal

infection, water imbalance, and reduced respiratory capacity. Integumentary function appeared to be altered in many of the newts exposed to UV-B radiation.

A small proportion (three individuals) of the UV-B irradiated newts became severely bloated prior to expiring. Bloating may be indicative of osmoregulatory malfunction. None of the UV-B irradiated animals that survived the observation period, or any of the controls ever exhibited this symptom.

Among the newts that developed skin ulcerations, there were four cases in which the lesions appeared to be infected with some sort of fungal growth. All the infected individuals died, whereas ulcerated non-infected newts sometimes survived to the end of the observation period.

UV-B irradiated newts were often observed with their heads and upper bodies out of the water and mouths open. This behavioral stance may have contributed to the preponderance of head ulcerations. By lifting their heads out of the water, the newts brought themselves out of a UV-B attenuated environment and closer to the radiation source. Thus, they must have exposed themselves to larger doses than they would have received by remaining submerged. At first this behavior seems paradoxical because T. granulosa has been shown to be able to detect UV-B wavelengths (LaTouche and Kimeldorf, 1979) and to actively avoid exposure when given a choice (Kimeldorf and Fontanini, 1974). One possible explanation is that the head-elevation behavior represents an attempt to augment impaired cutaneous respiration with buccal/pharyngeal and/or pulmonary respiration. Newts rely on cutaneous respiration

for up to 50% of their oxygen requirements (Whitford and Hutchinson, 1965). Between 70 and 80% of the newt's respiratory capillaries are found in the skin (Czopek, 1959). In humans and other mammals, UV-B irradiation has been shown to cause dilation of skin capillaries (Geise, 1976), which slows blood flow. UV-B has also been shown to cause changes in the permeability of small blood vessels (Cotran and Pathak, 1968). If such alterations occur in newts, the result would probably be a reduction in cutaneous respiratory efficiency. The direct destruction of skin capillaries that must occur in ulcerated areas would undoubtedly impair cutaneous respiration. If reduced respiratory efficiency in the skin is in fact the basis for the observed head-elevation behavior, it may outweigh the newt's tendency to avoid UV-B exposure.

Other authors have reported UV-B induced ulceration of the skin in shaved mice, hairless mice, and rhino mice (Forbes and Urbach, 1969), in the newt Triturus (Zavanella and Losa, 1981), and in Bufo tadpoles (Worrest and Kimeldorf, 1976). As in this study, Zavanella and Losa report that ulcers occurred mainly on the head in UV-B exposed Triturus, but those authors do not mention the behavioral stance that was observed in Taricha. Ulceration indicates cell death, and/or the lack of epidermal cell replacement due to the inhibition of mitosis in the basal layer of the skin.

The depigmentation that was observed in irradiated newts suggests death, damage, or displacement of melanocytes. In mammals, although UV radiation leads to increased pigmentation in vivo, melanin synthesis is not stimulated in vitro (Kitano and Hu, 1969). Silver and Hu (1968)

found that ultraviolet wavelengths above 295 nm can be lethal to mammalian pigment cells *in vitro*, and that sublethal doses inhibit cell division in direct proportion to the dose received. Hunter et al. (1979) found that melanosomes in the melanocytes of UV-B irradiated anchovy and mackerel larvae were dispersed, whereas in control animals they were aggregated. Fugii et al. (1973) report that UV caused depression of the melanosome response to aggregating substances. Zavanella and Losa (1981) report that the melanocytes of UV irradiated *Triturus* often lack dendritic processes, or have few pigment granules, and at higher total fluences or fluence rates these cells disappear or are displaced. In animals receiving the highest doses (260 kJm^{-2}), they found a general scarcity of pigment cells, but there were areas, especially in the cephalic skin, that had clusters of heavily pigmented melanocytes. It is possible that the general blanching except for a darkly pigmented mid-dorsal stripe, that was observed in many of the UV-B irradiated newts in the present study, is a related phenomenon.

It is not clear whether the mid-dorsal pigmented stripe is a result of a local increase in pigment production, a local increase in the proliferation of melanocytes, or the migration of pigment cells to that area. It is also a mystery why such a stripe should occur. If increased pigmentation is a protective response to UV-B exposure, the whole dorsal surface would be expected to become darker. On the other hand, if the UV-B dose is sufficient to kill the melanocytes or inhibit melanin synthesis the whole dorsal surface would be expected to lose its brown color. Perhaps the areas closest to the source and receiving the most

direct UV-B rays are darkened at the expense of the rest of the dorsal surface.

Blanching of the skin has been observed after exposure of newts to X-rays (Algard et al., 1974). In this case, the effect was attributed to the derangement of pigment granules in epithelial cells, and to the contraction of epidermal melanophores that "unmasked" the underlying iridophores. Reflection of visible light by the iridophores may have caused the silvery appearance reported by Algard et al. and the perhaps comparable light greenish-gray color observed in newts exposed to high levels of UV-B irradiation.

Although sloughing of the skin was not quantified directly, the extent of desquamation appeared to be related to UV-B dose. Excessive sloughing suggests that cells were being killed, and it may be comparable to the peeling that occurs in sunburned humans. The observed discoloration of the newt skin may be correlated to this shedding. If melanocytes are killed or damaged they will not be able to replace the pigment lost as epidermal cells containing melanosomes are sloughed off. Thus, as epidermal layers are shed, skin color may be expected to lighten.

The histological study indicates that UV-B irradiation results in edema of the epidermis, with the development of further cytopathology at moderate to high accumulated fluences. Exposure to Vita-Lite illumination after UV-B exposure appears to allow some recovery to take place. The observation that UV-B control newts exhibited edema, whereas the UV control animals did not, suggests that UV-A radiation may have contributed to the detrimental effects seen in the experimental newts (exposed

to UV-B, UV-A, and visible wavelengths). UV-B control and UV control groups received approximately the same amount of energy in the UV-B region (0.027 and 0.034 Wm^{-2} unweighted, respectively), but the former group was exposed to nearly twice as much energy in the UV-A region (0.802 and 0.439 Wm^{-2} unweighted, respectively). The UV-B controls were exposed to much less energy in the visible region of the spectrum, and much less total energy than the UV controls.

The sub-cellular target for UV-B radiation damage is not known with certainty. UV-B irradiation can result in direct DNA damage in the form of pyrimidine dimers. Such lesions may interfere with DNA replication and/or transcription. If replication is altered or inhibited, mitosis may not occur and cell replacement could be hampered. If the transcription process is altered, important structural or functional proteins may not be made correctly or at all.

It is also thought that UV damage at the cellular level may involve photoxidation of fatty acids present in cell membranes (Spikes, 1977). Lysosome membranes may be particularly sensitive to ultraviolet radiation. The destruction of these membranes and the subsequent release of lysosomal enzymes may be a critical factor in skin damage (Johnson and Daniels, 1969). Since the critical cellular target for skin damage is not known, it is difficult to choose a meaningful weighting factor. The study of skin damage involves the additional complexity of a UV-B attenuating stratum corneum, which should be taken into account when attempting to determine an appropriate weighting factor.

If the magnitude of UV-B induced cellular damage is large, it may lead to systemic effects that are potentially lethal for the organism.

In newts, damage to the skin may reduce the efficiency of cutaneous respiration. Diffusion of oxygen through the skin into the blood may be hindered (functional hypoxia), and tissue hypoxia could result. Tissue hypoxia could lead to death as a result of a positive feedback loop that is known to occur in humans: Tissue hypoxia can lead to cerebral edema and CNS depression, this in turn can result in medullary depression, which can lead to cardiovascular collapse and depression of the breathing response, ischemic and functional hypoxia occur, which magnifies the original tissue hypoxia. Ultimately, the organism will die as a result of respiratory failure. It is not known whether this type of feedback loop occurs in the newt. Even if it does, the hypothesis assumes that pulmonary and buccal/pharyngeal respiration cannot adequately compensate for the reduction in cutaneous respiratory efficiency.

Another possible mechanism of death is the release of toxic products. Taylor (1934) has reported that newts are susceptible to their own toxin when it is introduced under the skin. It is possible that in some cases toxin released from poison glands located in the skin could have penetrated into ulcerated areas, and contributed to the observed mortality. This mechanism is probably not a major one since the majority of newts that died did not develop ulcerations, and some newts that did have lesions survived. The release of harmful enzymes from lysosomes would have a more indirect effect, and would probably only result in death of the organism after large numbers of cells had been killed.

Still another factor that may play a role in UV-B induced mor-

tality is the possibility of systemic infection. As stated previously, skin infection was obvious in only a few cases. However, infection that was not readily observable may have occurred. UV-B exposure has been shown to impair immune processes (DeFabo and Kripke, 1981; O'Dell et al., 1980). It is conceivable that UV-B exposure diminished the newt's immune capabilities so that an infection of some sort was allowed to take hold and ultimately lead to death.

Further research is needed to establish the role of these three hypothetical mechanisms in the UV-B induced lethality of Taricha. In any case, it appears that damage to the skin is involved.

SUMMARY AND CONCLUSIONS

All three fluence rates exceeded the fluence rate threshold for Taricha. Lethality was found to be a dose-dependent phenomenon that occurred when total accumulated UV-B fluence exceeded a certain threshold. This threshold dose was between 500 and 650 kJm^{-2} in the low fluence rate groups, and between 400 and 500 kJm^{-2} in the intermediate and high fluence rate groups. A dose rate effect appears to exist between the low and intermediate fluence rates with respect to threshold lethal dose and LD50.

The experimental newts exhibited the following symptoms: skin blanching, excessive sloughing, ulceration, mid-dorsal pigmented stripe, and "head-elevation" behavior. UV-B induced skin damage may lead to potentially lethal systemic effects such as osmoregulatory dysfunction, reduced immune capacity, and respiratory insufficiency.

These studies suggest that erythemally weighted UV-B doses now reaching the earth's surface in the summer at 45°N latitude can be detrimental to Taricha. Behavioral avoidance may presently be an important means of protection against potential UV-B induced damage. A decrease in global ozone concentration and the resulting increase in the amount of biologically effective UV-B radiation reaching the earth may require further adaptive adjustments.

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