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Effect of Circadian Rhythm on the Radiosensitivity of the Rough-Skinned Newt (*Taricha granulosa*)

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ABSTRACT

The radiosensitivity of the salamander irradiated with 1,000 R and stored at 15°C, was found to fluctuate drastically over a 24 hour period, the greatest change in survival times occurring between 2,100 (9:00PM) and 2,400 (midnight). Radiosensitivity appears to be related to the light-dark cycle and to the circadian rhythm previously noted in these and other animals. This study clearly emphasizes the importance of reporting the exact time of day in which radiation exposures are administered to *Taricha granulosa* and perhaps other animals.

INTRODUCTION

Rhythmical cycles in the mitotic indices of animal cells have been recognized for many years in mammalian cell systems[1-3]. Recently, Chiakulas and Scheving[4] reported that urodele larval epidermal cells of *Amblystoma tigrinum* were governed by a circadian cycle in which the mitotic indices fluctuated more than 200% during a 24 hour period. Consequently, this experiment was designed to test the effect time of exposure over a 24 hour period, on the radiosensitivity of the salamander.

MATERIALS AND METHODS

One hundred male newts, weighing 15–16 grams each, were collected during the summer by net from a small lake in Western Oregon, U. S. A., acclimated for 10 days in individual 12×17 cm polystyrene containers containing 400 ml artificial pond water, held at 15°C±1°C. The water was changed weekly and the newts were maintained under alternating 12 hour light (6 AM–6 PM) and 12 hour dark (6 PM–6

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AM) periods. All newts were maintained in a uniform unfed state throughout this study, not only because this simulates winter conditions, but because previous experience indicated that newts eat inconsistently or not at all while in captivity.

Less than two minutes prior to exposure, ten newts were placed in a rotating (one revolution every 90 seconds) lucite apparatus containing ten compartments, specifically constructed to conform to a predetermined isodose chart to secure uniform exposure. All animals were irradiated at room temperature (22°C) in air. Newts were exposed at 0300, 0600, 2,100, and 2,400 (midnight) in the dark while animals subjected to irradiation at 0900, 1,200 (noon), 1,500 and 1,800 received the same treatment with the exception of light as to not to disrupt their circadian cycle.

All irradiated animals were administered 1,000 R at 35 R/min under the following conditions: 15 mA, 250 kVp, 0.5 mm Cu and 1 mm Al filters, TSD of 70 cm. Twenty controls were sham-irradiated, ten at 1,200 and the remainder at 2,400. Daily observations of survival were recorded.

RESULTS

All of the newts sham-irradiated at 1,200 or 2,400 survived the 15°C storage temperature for the duration of the study (125 days). On the other hand, the mean survival times of the irradiated newts averaged 73.9±6.1 days (Table 1). Animals, exposed at 0300, 0600, 0900, 1500, and 1800, had mean survival times which were not significantly different from the overall mean of all the irradiated newts. However, animals administered 1000 R at 2100 died significantly earlier than expected while newts irradiated at 2400 had an extended mean survival time compared to the overall mean.

Table 1. Modification of the radiosensitivity of newts exposed to an acute exposure of 1,000 R at one of eight different times within a 24 hour period. Sample size was ten animals per treatment.

Time of exposure	Mean survival time±S. D.	Significant difference from overall mean
0300 (d)	71.5± 3.0	No
0600 (d)	72.1± 3.4	No
0900	75.5±11.2	No
1200 (noon)	71.8± 6.8	No
1500	76.7±10.3	No
1800	72.7± 4.7	No
2100 (d)	65.0± 4.6 (1)	P=0.005
2400 (midnight) (d)	86.0± 4.9 (h)	P=0.005
Overall	73.9± 6.1	

(d) Exposures conducted without light
(h) Values signiffcantly higher than overall mean survival time
(1) Values significantly lower than overall mean survival time

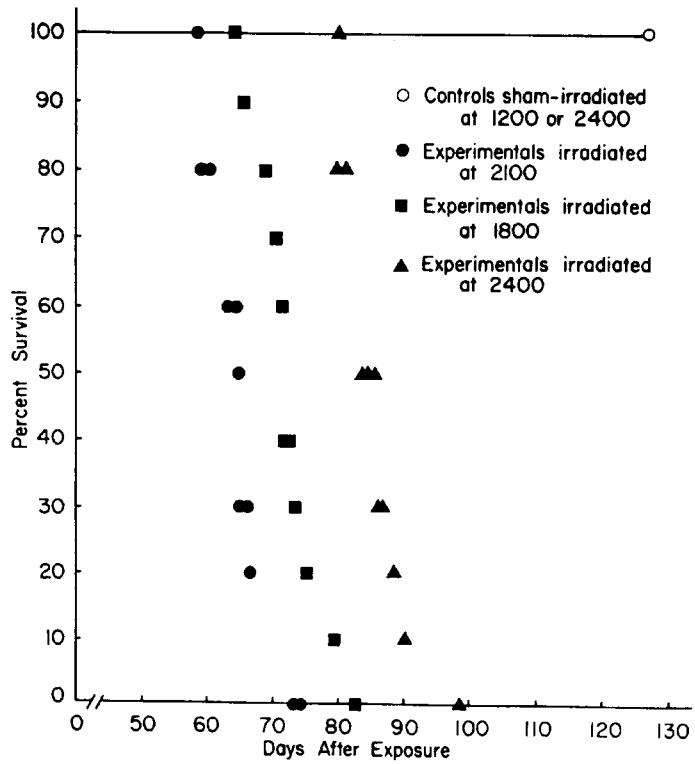

Fig. 1. Survival rates of newts exposed to 1,000 R at 2,100, 1,800, or 2,400, periods of time in which mean survival times were minimal, approximately average for a 24 hour period, and maximal, respectively. Sample size for each treatment was ten animals.

Irradiated animals always exhibited a single mode of death, never bimodal, indicating that the animals were dying predominantly from one syndrome, hematopoietic[5-6]. If a dose reduction factor is calculated by comparing the mean survival times of animals exposed to 1,000 R at 2100 and 2400, a value of 1.32 is obtained (Fig. 1).

DISCUSSION

The mean survival times of irradiated newts stored at 15°C provided quite adequate results, having rather small standard deviations for this type of study (an essential requirement for a good comparative study in radiosensitivity). This temperature is well below the "critical thermal maximum" described by Willis and Prince[7] for this same population of newts and, thus, allowed for extended periods of survival even with unfed animals.

Greatest mean survival times occurred at 2400 (midnight) and coincided exactly with the period of time that Gibbs and Casarett[8] found a dramatic reduction (three

fold drop compared to overall mean) in the mitotic index of stratified squamous cheek pouch epithelium of Chinese hamsters. Furthermore, Chiakulas and Sche ving[4] found a fluctuation in the mitotic indices of urodele epidermis. At 2100 and 2400, the mitotic indices were reported to be approximately 15% above and 25% below the 24 hour mean, respectively. The elevated mitotic index at 2100 and reduced index at 2400 corresponded exactly to the reduced mean survival time of 65 days and the extended mean survival time of 86 days, respectively, found in this study.

Radiosensitivity of the rough-skinnd newt appears to be correlated to the light-dark cycle. The greatest fluctuations in radiosensitivity occurred within six hours after the 12 hour light period was completed. The greatest reduction in survival times was exhibited only three hours into the dark period while an additional three hours provided a maximum mean survival time value.

This study clearly, points out the importance of exposing salamanders, and perhaps other animals, at precisely the same time each day if true comparisons are to be made between species and genera. In radioprotective studies both the irradiated controls and the experimental animals pretreated with some agent should be exposed exactly at the same time and not independently in a series of exposures since a mere three hour delay in the experimentals would provide a dose reduction factor of 1.32 in *Taricha granulosa* if controls were exposed at 2100 and experimentals at 2400, regardless of the action of the chemical.

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