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Radiation Sensitivity of the Hibernating Ground Squirrel, *Citellus tridecemlineatus*.^{*} (31965)

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The sensitivity of hibernating and active ground squirrels, *Citellus tridecemlineatus*, to ionizing radiation (Co^{60} photons) has been the subject of study in our laboratory. In the course of investigations dealing with radiation effects on intestinal absorption in the ground squirrel(1,2,3), we noted that many hibernating animals exposed to lethal levels of radiation survived post-arousal periods of one month and longer, whereas animals irradiated in the active state died much sooner. The increased survival periods of irradiated hibernating squirrels after arousal as compared to the post-irradiation survival periods of active squirrels were not in accord with the findings of others(4,5,6,7). The consensus has been that upon arousal irradiated hibernating animals react the same as irradiated non-hibernating subjects. These apparent differences prompted the present study.

The objectives of the experiment herein described are: Is there a difference in the sensitivity of hibernating and non-hibernating ground squirrels to various high levels of radiation and is there sufficient reason to suspect that radio-protection occurs in a hibernating mammal?

Materials and methods. Active and hibernating ground squirrels. Seventy-two animals for experimentation were taken from a colony of stock animals. The animals were young of the year, *i.e.*, from litters produced during late Spring, 1965. Specimens were selected at random, and both males and females, circa 150 to 200 g, were used. From the same stock colony, a group of 12 animals have been maintained in an identical manner and have served as controls for long term comparisons, *e.g.*, to assess longevity and ageing changes.

Squirrels in the hibernating group were

placed in an environmental chamber at 5° to 6°C for periods averaging about 3 weeks. The individual hibernacula were plastic containers (transparent lucite cylinders 19 cm high and 14 cm in diameter containing about 7 cm of San-i-cel corn cob bedding). During the hibernal period frequent daily observations were made to determine phases of arousal in each animal; in this way only those animals in deep hibernal torpor were chosen for irradiation.

Irradiation of animals. During the irradiation procedure animals were individually housed in plastic containers (described above) and each active and hibernating animal was irradiated singly. In order to minimize possible effects of circadian and/or circanian rhythms all animals were irradiated between 2 PM and 5 PM CST during the months of February and March, *i.e.*, during the usual seasonal period of hibernation.

Representatives from each group were given whole body exposure to radiation doses ranging from 1000 r to 3000 r. The dose rate was 200 r per minute and was considered constant over the entire period of the experiment.

The irradiation was conducted by means of a GR-12 Gamma Cell built by United Nuclear of California. The radiation source consisted of 6 stainless steel "pencils" (20 cm in height, equally spaced around the cylindrical sample chamber 15 cm in diameter) in which the Co^{60} loading was approximately 150 curies. The Co^{60} was arranged in the pencils such that the photon flux over the volume of the sample chamber varied less than 5%. Dosimetry was obtained by the ferrous-ferric sulphate technique(8) and a 250 r Victoreen ionization chamber.

Immediately following the irradiation of a hibernating squirrel, rectal temperature

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and respiration rate were recorded. Each animal was then allowed to arouse completely from hibernation by placing it in a room temperature ($21^{\circ} \pm 2^{\circ}\text{C}$) environment. In this manner two behavioral parameters were established, namely, an upper limit of the state of hibernal torpor during irradiation (rectal temperatures ranged from 6° to 10°C , and respiration rates ranged from 2 to 15 per minute) and a constant post-irradiation animal state, *i.e.*, immediate total arousal to the active state.

Following the irradiation procedures all animals in both groups and the stock controls were housed in transparent plastic cages ($78 \times 28 \times 15$ cm), 2 animals per cage, in the same animal room on the same cage racks, so that all environmental features were identical. All animals were given free access to food and water. For the first 30 days post-irradiation the animals were left undisturbed, except to remove carcasses; thereafter cages were cleaned and changed every 2 weeks.

Frequent daily observations were made on the irradiated squirrels. The symptomatology of radiation sickness and general behavior were noted. Mean survival time (MST) data were recorded in units of 8-hour periods, obtained by dividing the 24-hour day into three equal parts: 3 PM to 11 PM, 11 PM to 7 AM and 7 AM to 3 PM.

Results and discussion. The observations are presented in terms of MST of decedents *vs* dose, cumulative mortality during a 30-day period, and description of long term survivors.

Fig. 1 shows a comparison of MST as a function of radiation dose between the active and the hibernating squirrels. These data clearly show that the entire group of hibernating squirrels are less radio-sensitive than the active animals. The lowest dose at which lethality was observed was 1100 r for an active animal and 1500 r for a hibernating animal. Assuming that the interpretation of the MST curve as given by Bond *et al*(9) can be applied to *Citellus tridecemlineatus*, then the slope of the curves suggests that the range of radiation used was almost entirely with the hematopoietic syndrome for the hibernating squirrels, whereas at the

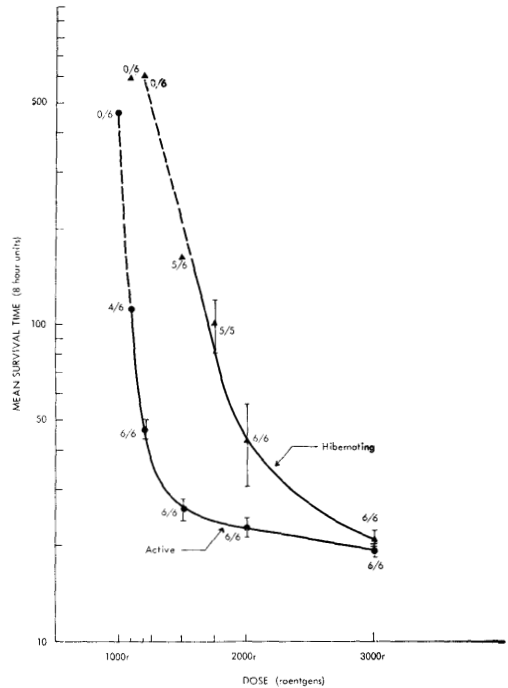


FIG. 1. Mean Survival Time (MST, 1 unit = 8 hrs) following whole body irradiation of the ground squirrel, *Citellus tridecemlineatus*; Co^{60} , 200 r/min. \blacktriangle , Irradiated while hibernating and awakened immediately, \bullet , irradiated while active. Ratios: numerator = number of decedents and denominator = number of irradiated subjects. Broken lines are drawn to data points of survivors (calculated to Sept. 1, 1966).

1500 r dose the active squirrels began to enter the gastro-intestinal syndrome.

Radiation sensitivity of hibernating animals is often reported in terms of percent mortality at 30 days post-awakening time. A composite of data from other investigations is compared with our results presented in Table I. Our data indicate an $\text{LD}_{50(30)}$ for the hibernating squirrels between 1500 r and 1750 r, and an $\text{LD}_{50(30)}$ for the active squirrels as being close to 1100 r.

Künkel(10) irradiated both active and hibernating dormice and found a decrease in percent mortality over a 30-day active period in animals irradiated while hibernating and aroused after 21 days. He found about a 3-fold increase in post-arousal MST of decedents in the hibernating group as compared to the MST decedents in the active group. Thus his data, as do ours, also indicate a decrease in radio-sensitivity in irradi-

TABLE I. Mortality of Irradiated Active and Hibernating Animals.

No. of animals	Radiation dose (roentgens)	Mortality in 30 days at room temperature (%)		Experimental conditions
		Active	Hibernating	
<i>Citellus tridecemlineatus</i>				
6(A)	1000*	0	—	(A) active at 21° ± 2°C
6(A) 6(B)	1100	50	0	(B) hibernating at 5°C, immediately transferred to 21° ± 2°C
6(A) 6(B)	1200	100	0	
6(A) 6(B)	1500	100	17	
— 5(B)	1750	—	60	
6(A) 6(B)	2000	100	83	
6(A) 6(B)	3000	100	100	
<i>Citellus tridecemlineatus</i> (4)				
12(A) 6(B)	800†	100	100	(A) active at room temperature
12(A) 6(B)	1000	100	100	
12(A) 6(B)	1200	100	100	(B) hibernating at 5°C, after 2 to 4 weeks transferred to 20° to 26°C
<i>Citellus tridecemlineatus</i> (5,6,7)				
20(A)	1000†	80		(A) active at 23°C
15(B ₁)			87	(B ₁) hibernating at 5°C, after 21 days transferred to 23°C
16(B ₂)			81	(B ₂) hibernating at 5°C, after 15 to 30 min transferred to 23°C
<i>Glis glis</i> (10)				
18(A) 21(B)	700‡	83	67	(A) active at 20°C
				(B) hibernating at 4°C, after 21 days transferred to 20°C

* Cobalt 60 gamma photons.

† X-ray, 250 kvp.

‡ X-ray, 200 kvp.

ated hibernating animals. By way of contrast, the reports of others(4-7) indicate that hibernation in the ground squirrel does not provide any lasting decrease in radiosensitivity after arousal from hibernation (Table I).

Although there is no immediate concrete explanation for the differences between the results herein reported and those of other workers, variance of results in experimental radiobiology are often relatable to features such as differences in animal species and strains, laboratory conditions, type and energy of radiation, geometry of exposure, and dose measuring techniques(9). Apart from the experimental differences related to the radiation facilities and irradiation procedures, the period of hibernation, before and after, and the depth of hibernation during irradiation are possibly the most significant factors responsible for differences in response.

The difference between the numerator and the denominator of each ratio shown in Fig. 1 gives the survivors to September 1, 1966 for each radiation level in each group

of animals. Initially, all of these animals showed radiation sickness in varying degrees and weight loss. Noticeable weight gain occurred by 1½ to 2 months post-irradiation, and during June and July most of the survivors gained weight to the same extent that the controls did. This weight gain was due to fattening and was assumed to be a preparation for the coming winter's hibernation.

There was a marked loss of characteristic coloration and thinning of the hair at various times following irradiation, and by 2 months all of the survivors showed a distinct greying.

Late effects of radiation became evident at 5 and 6 months post-irradiation and in individual animals they appeared chiefly as a progressive loss in weight, cutaneous ulcerations, and tumorous distentions of the abdominal wall.

The mechanism of radio-protection provided in the hibernating animal is not known and a variety of physiological features is probably involved. Among the most apparent, reduced mitotic activity, hypoxia and endogenous chemical protection might understand-

ably be suggested. Tissue hypoxia can be considered as resulting from circulatory modifications during hibernation. Lyman and O'Brien(11) indicate a uniform vasoconstriction, and Bullard and Funkhauser(12) report marked reduction in blood flow in numerous tissues and organs in hibernating ground squirrels. There is also evidence of increased catecholamine content in tissues of hibernating squirrels(13), and nor-adrenalin and other catecholamines have been implicated as radio-protective agents(14). At this time the question of mechanisms responsible for the decreased radio-sensitivity is still under investigation.

Summary. The basic conclusion of this report is that irradiated hibernating ground squirrels do not respond as "active" animals following arousal as has been hitherto reported, rather, there is a protective effect relatable to hibernation. The hibernators have a longer "mean survival time" and an increased LD₅₀₍₃₀₎.

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The Indigogenic Reaction for Histochemical Demonstration of Sulfatase.* (31966)

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Previous work in this laboratory described the application of the indigogenic principle to the histochemical demonstration of leucine aminopeptidase(1), β -glucosidase (2), β -galactosidase(3), N-acetyl- β -glucosaminidase(4), and alkaline and acid phos-

phatase(5). In the present study we have extended this principle to histochemical localization of sulfatase, utilizing 5-bromo-4-chloro-3-indolyl sulfate. The substrate was synthesized according to methods described recently by Horwitz and coworkers(6,7). The substrate offers the advantage of a precise enzyme localization with no or very slight diffusion. Moreover, the substrate affords a simple and direct method for demonstration of hydrolytic enzymes without the need for a coupling reaction. This compound yields a blue-green deposition of indigo.

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