Radiation Effects in Free-Ranging Pocket Mice, Perognathus parvus, during the Breeding Season

Abstract. *Captive and free-ranging Great Basin pocket mice,* Perognathus parvus, *were exposed to ionizing radiation during the breeding season, April–June, in 1971. The values for the median lethal dose (LD_{50}) at 30 days plus or minus the standard deviation were 880 ± 14 rads and 780 ± 27 rads, respectively, and the slopes of the survivorship curves were significantly different. These differences suggested that there was a synergism between radiation-induced and environmental sources of mortality, since the field data were corrected for natural mortality (5 percent) in the controls.*

Wild mammals have interested radiation biologists ever since published reports indicated that they were less sensitive to ionizing radiation than laboratory strains of rodents. Gerbils, oldfield mice, cotton mice, cotton rats, little pocket mice, and long-tailed pocket mice were all reported to have greater resistance to radiation than laboratory mice (*1–3*). The unusual radiation resistance led some to speculate that there were obvious differences between major taxa, and that members of the family Cricetidae were more resistant than members of the family Muridae (*2*).

Examination of the results led some to conclude that "wildness" as a trait might carry with it some degree of protection from stresses such as radiation insult. They felt that perhaps indigenous species, having adapted to the multitude of environmental stresses, had been preadapted to resist additional stresses such as ionizing radiation. One confusing aspect of the data reported for wild mammals was the tendency of investigators to compare responses of native rodents with published $LD_{50(30)}$ values (median lethal dose at 30 days) for laboratory strains without checking the responses of the latter animals under experimental conditions similar to those experienced by native species.

When native species and laboratory strains of rodents were exposed to ionizing radiation under comparable experimental conditions, including colony care and exposure regimes, the differences between their $LD_{50(30)}$ values were not as apparent (*4*). Two laboratory strains, RF_1 and C57BL × BALB hybrids, had $LD_{50(30)}$ values similar to those of 13 species of native mammals tested.

If we wish to predict the effects of radiation on organisms in the environment, it is important to know how wild species respond to radiation under natural conditions. The task of prediction will be easier if laboratory results can be extrapolated to field conditions. Since the conditions experienced by wild animals in laboratory colonies and fenced enclosures (*5*) are considered by some to be unnatural, predictions extrapolated from data gathered under these experimental conditions would be invalid when applied to unconfined populations if: (i) native species demonstrated a higher resistance in the laboratory, owing to the "ideal" environmental conditions; (ii) native species demonstrated higher resistance in the field, since they were in their home territories unaffected by colony stresses; or (iii) they might exhibit a lowered resistance in the field owing to synergistic effects of radiation and environmental stresses.

In July–August 1967 we irradiated a laboratory colony and a free-ranging population of Great Basin pocket mice, *Perognathus parvus*, to varying doses of ionizing radiation (*6*). We were unable to demonstrate a significant difference between the responses of the captive and free-ranging pocket mice; their $LD_{50(30)}$ ± S.D. values were 858 ± 27 rads and 834 ± 32 rads, respectively, and the slopes of both survival curves were, in essence, parallel. We did observe changes in longevity and reproductive success in irradiated animals.

The unconfined mice were irradiated in late summer as the adults commenced their underground period of torpor. Their metabolism was lowered owing to the onset of torpor and they were not exposed to surface predators as frequently. We wondered if we would obtain the same response from free-ranging pocket mice if they were exposed to radiation at another, perhaps more stressful, time of year. Hence, a second experiment was planned for the breeding season of April–June. This period was chosen because we assumed that breeding would impose additional physiological and behavioral stresses on this solitary species. Then too, seeds of the winter annual grasses, which are their major food item, have not ripened and shattered yet, so that food availability is at its annual low. This increased activity above ground, associated with food-gathering and breeding, could also expose rodents to surface predators more frequently during the spring period.

Beginning in March 1971, a population of pocket mice on a 4-hectare study area was live-trapped regularly until 19 May to establish estimates of density, sex ratios, and range of movements. Individual mice were identified by a series of toe amputations, and records were kept of location, frequency of recapture, reproductive conditions, weight, and overall condition. A computer diary was established and checked at each capture to ensure that individuals were not misidentified.

On 20 May 1971, 136 *Perognathus* were trapped and brought to the laboratory in ventilated cans containing food. Groups of 19 or 20 mice of equal sex ratios were exposed in an annular ^{137}Cs source at a nominal dose rate of 745 rad/min. There were six dose levels at 50-rad intervals between 700 and 950 rads, and 19 animals were sham-irradiated as controls. All mice were released at their point of capture shortly after sunrise.

Live-traps were operated for three consecutive nights beginning 17 June to see which mice had survived until 30 days postirradiation. Animals not captured were assumed to be dead. Susceptibility to repeated live-trapping is a characteristic of the rodent family Heteromyidae, one which prompted the use of *Perognathus* as a test species. Trapping data collected preceding the treatment indicated that most of the trappable population could be captured on any given night. For example, 95 percent of the animals trapped on 19 May, the last preliminary sampling, were recaptured on 20 May, the night of the field exposure; 95 percent of the controls were retrapped 30 days later.

A laboratory colony of 140 pocket mice was established under conditions comparable to those already reported (*6*). Animals were housed singly and acclimated to the laboratory conditions for at least 2 weeks before treatment. On 20 September 1972 groups of 20 mice each were exposed in the same radiation source at the same six dose levels, and 20 animals were sham-irradiated as controls. The colony was checked daily for decedents until 30 days postirradiation.

Data on survival of both groups of mice exposed to ionizing radiation are

presented in Table 1, and curves derived from probit transformations of the log-transformed data are illustrated in Fig. 1. The best-fitting straight lines were obtained for both field and laboratory data in the usual way by using probit analysis and the method of maximum likelihood (*7*). The field data were adjusted for natural mortality (*7*) by using the proportion $1/19 = 0.053$ of field control animals never recaptured. The slopes of the two resulting regression lines were significantly different at the 0.05 level of confidence. This difference in slopes precluded use of the relative potency test (*7*) as a comparison of radiation effects in the field and laboratory.

The $LD_{50(30)} \pm$ S.D. of *Perognathus parvus* exposed to ionizing radiation and then maintained in the colony was 880 ± 14 rads, while that for free-ranging pocket mice was 780 ± 27 rads. The 95 percent fiducial limits about the estimated $LD_{50(30)}$ values were 854 to 915 rads and 694 to 830 rads, respectively. Since the fiducial limits did not overlap, we concluded that the data provide evidence of a statistically significant reduction in the $LD_{50(30)}$ value for the free-ranging mice.

Apparently, free-ranging pocket mice were more sensitive to radiation than those in the laboratory colony, or other environmental mortality factors were operating on the unconfined population which effected lowered survival in irradiated animals. Since 18 of 19 controls were retrapped 1 month later, we must conclude that "natural" mortality and emigration were minimal, at least for the controls. In our analyses we have assumed that irradiated animals were as prone to recapture as the controls. The reduction in recaptures of irradiated free-ranging animals is interpreted as resulting from mortality rather than our inability to retrap irradiated mice still living on the sampling grid. Although we feel it is unlikely, there is always the possibility that radiation insult depressed trapping responses of exposed mice. The population was trapped in September 1971, and April and June 1972. No additional mice were trapped that had not been recaptured during the 3-day sampling period 30 days postirradiation.

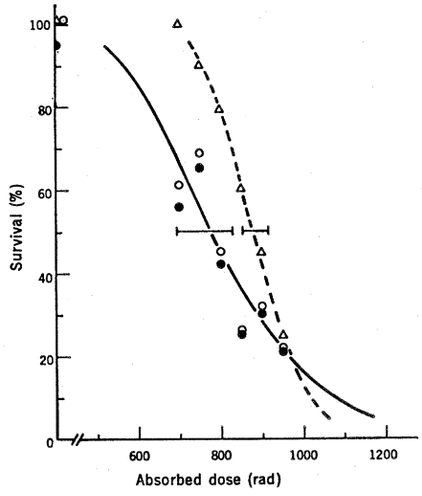

Fig. 1. Survivorship curves of captive and free-ranging *Perognathus parvus* obtained by probit transformations of mortality data following exposures to ionizing radiation. Data for unconfined mice have been corrected for mortality in controls, and the median lethal doses are bracketed by the 95 percent fiducial limits. △ - - - △, Laboratory data and curve, $LD_{50(30)} \pm$ S.D. $= 880 \pm 14$ rads; ○—○, field data and curve corrected for mortality in controls, $LD_{50(30)} \pm$ S.D. $= 780 \pm 27$ rads; ●, uncorrected field data.

Since mortality in the irradiated, free-ranging pocket mice was not additive, that is, it exceeded the combined mortality of radiation insult (laboratory results) and natural mortality (free-ranging controls), we concluded that the stresses were synergistic under our field conditions.

Animals exposed to 700 and 750 rads in the laboratory colony suffered 0 and 10 percent mortality, respectively, indicating that the threshold dose (dose effecting 5 percent mortality) was about 730 rads. In the field, mortality at these two lower exposures was greater than 30 percent when the data were corrected for loss of a control. These two observations, ability to recapture controls, and the higher mortality of unconfined mice exposed to low doses, further support our belief that there was a synergistic effect between radiation insult and environmental stress in free-ranging pocket mice during the breeding season.

This conclusion differs from that of our former study (*6*) in which we were unable to demonstrate any synergism in unconfined *Perognathus* exposed to radiation in the summer. The discrepancy between the conclusions may reflect a seasonal difference in the response of the species to the combined effects of radiation-induced and natural mortality. The availability of seeds, stress of breeding, exposure to surface predators, and differing metabolic requirements may all contribute to the observed differences in mortality.

The results may also be due to improvements in the experimental design. By reducing the interval between absorbed doses to 50 rads, we were able to reduce the variability of the laboratory estimate of the $LD_{50(30)}$ to half that calculated in 1967 by using 100-rad intervals. In 1967 there were only three dose levels for which the mortality data appreciably affected the $LD_{50(30)}$ value. This is owing to the manner of weighting used in constructing the best-fitting straight line. There were five such dose levels in the 1971 laboratory data.

Further field experiments with this and other native species (*8*) will be required to define further the synergistic effects of radiation and environmental sources of mortality. However, the results of this study lead us to suspect that for some indigenous species, such as *Perognathus parvus*, extrapolations of median lethal doses derived in the laboratory may overestimate the resistance of populations of unconfined mice during the breeding season.

Table 1. Survival of *Perognathus parvus* under laboratory and field conditions after exposure to ionizing radiation in 1971.

Dose (rad)	Laboratory exposure			Field exposure		
	Number	Survived 30 days		Number	Survived 28 to 30 days	
		Number	Percent		Number	Percent
0	20	20	100	19	18	95
700	20	20	100	19	11	58
750	20	18	90	20	13	65
800	19*	15	76	19	8	42
850	20	12	60	20	5	25
900	20	9	45	20	6	30
950	20	5	25	19	4	21

* One animal was injured during exposure and removed from the experiment.

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Lambda Phage DNA: Joining of a Chemically Synthesized Cohesive End

Abstract. *The chemically synthesized dodecamer d(pA-G-G-T-C-G-C-C-G-C-C-C) was annealed, and was covalently joined to lambda phage DNA with bacteriophage T_4 ligase. The 5′-end of the dodecamer was joined to a deoxyguanosine residue. Repair with DNA polymerase I established that the position of joining was the left-hand end of lambda DNA. This is the first time that a chemically synthesized oligonucleotide has been covalently joined to a naturally occurring DNA molecule.*

Bacteriophage lambda DNA possesses two mutually complementary single-stranded structures extending from the 5′-termini of the double helix The nucleotide sequence of these cohesive, or "sticky," ends has recently been determined by Wu and Taylor (*1*).

By means of such structures, two molecules of DNA might be joined, or one molecule might be circularized; such phenomena are observed in nature (*2*). A heterologous genetic message might be joined to appropriate phage DNA through cohesive ends, and be carried into bacterial spheroplasts. With this in mind, we recently completed the chemical synthesis of a dodecamer identical with the protruding right-hand end of lambda phage DNA (*3*). We present evidence here that this chemically synthesized deoxyribododecanucleotide (Fig. 1, box) can be physically annealed, and enzymatically attached, to the macromolecular phage DNA in specific covalent linkage.

The enzymes used in this study and the procedure for the ^{32}P labeling of the 5′-terminus of the dodecamer with polynucleotide kinase and [γ-^{32}P]-adenosine triphosphate (ATP) have been described (*4*). After labeling the 5′-terminus of the dodecamer with ^{32}P, we placed the entire reaction mixture on diethylaminoethyl (DEAE)-cellulose paper (Whatman DE-81) and developed it for 6 hours with descending 0.5*N* triethylammonium bicarbonate (TEAB) buffer (*p*H 7.6). The paper was dried and cut into 2-cm lengths. The amount of radioactivity was determined in a Packard scintillation counter with a toluene-based scintillation liquid. The labeled dodecamer moved 2 to 4 cm from the origin and was separated from excess [γ-^{32}P]ATP. Similar strips with ^{32}P-labeled dodecamer were eluted with TEAB buffer (1*M*, *p*H 7.6), and the buffer was removed by repeated evaporation.

The results of binding ^{32}P-labeled dodecamer to lambda DNA is shown in Fig. 2A. A small peak containing ^{32}P (first peak) was found to coincide with the elution position of lambda DNA. A second peak (largest) was shown by a separate experiment to coincide with the elution position of the dodecamer itself; the third peak (found only in this experiment) appeared to be a small amount of contaminating inorganic ^{32}P.

When the annealed lambda DNA and [5′-^{32}P]dodecamer were incubated with bacteriophage T_4 polynucleotide ligase, and then separated by gel chromatography, a peak containing ^{32}P was found to elute with the lambda DNA again (Fig. 2B).

A comparison of resistance to bacterial alkaline phosphatase (BAP) of the two macromolecular peaks from Fig. 2A (annealed only) and Fig. 2B (annealed and incubated with ligase) is illustrated in Fig. 3. (The phosphorus label on the original dodecamer, contained in a phosphomonoester linkage, is labile to the enzyme. Upon joining of the dodecamer to the DNA, it will become an enzyme-resistant phosphodiester.) Dephosphorylation of the first peak of Fig. 2A, and separation on DE-81 paper (Fig. 3A), produced a movement of the ^{32}P label from the origin to a position expected for authentic inorganic phosphate (P_i). Furthermore, the failure of this ^{32}P

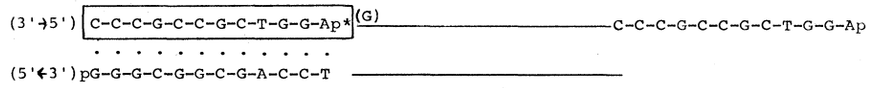

Fig. 1. Structure of the lambda DNA and its cohesive ends. The dodecamer in the box is annealed to the left-hand cohesive end.

Table 1. Joining of a synthetic dodecamer to lambda DNA with T_4 ligase. Lambda repair synthesis was carried out in a total volume of 0.3 ml, containing 28 μmole (83 m*M*) of TES (*10*) buffer (*p*H 7.0), 3.3 μmole (10 m*M*) of $MgCl_2$, 5 μmole (15 m*M*) of dithiothreitol, 27 μmole (80 m*M*) of NaCl, 5 nmole of the deoxynucleoside triphosphates indicated, and 0.5 pmole of lambda DNA (0.35 absorbance units at 260 nm). The mixture was incubated with 1.5 units of *Escherichia coli* DNA polymerase fraction 7 (*11*) for 4 hours at 5°C. The reaction was stopped by heating it for 10 minutes at 75°C. The indicated amount of [5′-^{32}P]dodecamer and 5 nmole of ATP were added, and the solution was cooled to room temperature over 1 hour. After 1 hour incubation at 0°C with 3 units of T_4 ligase, the reaction mixture was incubated for 30 minutes with 5 μg of BAP and was applied to DEAE-cellulose paper. The paper was developed with 0.5*M* potassium phosphate buffer (*p*H 6.9) and the amount of ^{32}P-labeled dodecamer that was joined to the DNA was determined by the amount of radioactivity (counts per minute) remaining at the origin. The number of counts at the origin of a control reaction mixture (without ligase) were subtracted. Joining reactions without repair were carried out with the same reaction mixture minus DNA polymerase and deoxynucleoside triphosphates.

Experiment	Repair with DNA polymerase	Extent of joining (pmole of ^{32}P)	^{32}P-labeled dodecamer (pmole)
1	No	0.06	1.6
2	No	0.21	16.0
3	Yes: dCTP, dGTP	0.20	1.6
4	Yes: dATP, dGTP	0.003	1.6