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RADIATION-INDUCED STERILITY IN NATURAL POPULATIONS OF LIZARDS (*CROTAPHYTUS WISLIZENII* AND *CNEMIDOPHORUS TIGRIS*)

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ABSTRACT

Leopard lizards (*Crotaphytus wislizenii*) and whip-tail lizards (*Cnemidophorus tigris*) have been exposed to gamma radiation in a fenced 20-acre area since January 1964. Free-air exposure rates over most of the area were around 4–6 R/day. Average annual tissue doses have been estimated with implanted lithium fluoride microdosimeters at 400–500 rads/year for *Crotaphytus* and about half this for *Cnemidophorus*. Demographic data and failure of mature female *Crotaphytus* in the irradiated area to assume typical reproductive coloration indicated absence of reproduction by this species in 1967 and 1968. Three female leopard lizards taken from the irradiated plot in May and June 1969 exhibited complete regression of ovaries, undeveloped oviducal walls, and hypertrophied fat bodies. One of three irradiated males collected at the same time was sterile. All of 20 control individuals taken in 1969 exhibited ovaries, oviducts, testes, and epididymides normal for the season. The first sterile female *Cnemidophorus* was collected in June 1969. Of four more females taken from the irradiated area in June 1970, three lacked ovaries; one had ovaries and had recently laid eggs. Three males from the irradiated area in 1970 did not differ in sexual condition from three control males. Experimental administration of follicle-stimulating hormone to the three apparently sterile *Cnemidophorus* females collected in 1970 had no effect on oviducal growth. The sterility observed in both species is attributed to long-term exposure to gamma radiation. Reproduction by another lizard, *Uta stansburiana*, occupying the irradiated area has apparently been normal since the beginning of the experiment. The difference in response of *Uta* and the other two species is attributed to their markedly different life-spans and demographic regimes.

INTRODUCTION

Possible ecological effects of gamma radiation have been investigated in a desert area at the U.S. Atomic Energy Commission's Nevada Test Site (NTS) for over six years. A fenced 20-acre plot in Rock Valley has been almost continuously exposed to radiation from a centrally located source of ^{137}Cs since January 29, 1964. Tissue doses sustained by animals have been discussed by French, Maza, and Aschwanden (1966) and by Turner and Lannom (1968). Two adjoining, but unirradiated, fenced 20-acre control areas have been studied in conjunction with the irradiated plot (French 1964).

Three species of lizards occupying the study areas have been under continued investigation: the side-blotched uta, *Uta stansburiana* (Turner et al. 1969a, Turner et al. 1970); the whip-tail lizard, *Cnemidophorus tigris* (Turner et al. 1969b); and the leopard lizard, *Crotaphytus wislizenii* (Turner et al. 1969c). Annual tissue doses to *Uta* have been estimated at around 360–1800 rads, depending on the individuals' locations within the irradiated plot (Turner and Lannom 1968, Turner et al. 1969a). Most individuals receive annual doses of around 750 rads. Data acquired between 1964 and 1968 disclosed no deleterious radiation effects on the demographic performance of the irradiated population of *Uta* (Turner et al. 1969a). Both the 59% increase of the irradiated population between 1966 and 1967 and the 43% decline between 1967 and 1968 were matched by corresponding changes in three untreated areas. However, leopard lizards occupying the irradiated area have apparently not reproduced since the summer of 1966. Also, some female whiptails in this area are sterile. Annual tissue doses to these species are generally less than those sustained by *Uta*. Annual doses to *Crotaphytus* were estimated to be about 25% of total free-air exposure; doses to *Cnemidophorus* were about half this. The maximal annual dose to a leopard lizard reported by Turner and Lannom (1968) was 320 rads.

This paper has two objectives. First, we attempt to understand more fully the apparent radiation effects on *Crotaphytus* and *Cnemidophorus*. Second, we consider

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the problem of why reproduction of these two species should be impaired with the population of leopard lizards becoming extinct while the irradiated population of *Uta* continues to reproduce.

METHODS

Dosimetry. The irradiated facility in Rock Valley has been described previously (French 1964; Lucas, Burson, and Lagerquist 1966). A 33,000-Ci ^{137}Cs source atop a 50-ft tower is differentially shielded so as to produce a radiation field ranging in intensity from about 2 R/day (free-air) at the periphery to 11 R/day near the center of a 20-acre circular enclosure. About 14.4 acres (70%) of the enclosure are exposed to 3 to 6 R/day. Only 0.5 acre (2.5%) is exposed to more than 8 R/day. During the past six years the radiation source has decayed to about 28,700 Ci. Because of this decline some of the subsequent dose estimates may be slightly high.

Leopard and whip-tail lizards have been captured in the irradiated area and in two fenced 20-acre control plots since 1964. Methods of estimating the density and age-composition of these populations were described earlier (Turner et al. 1969 b,c). Tissue doses to both species have been estimated from doses registered on lithium fluoride microdosimeters manufactured by Edgerton, Germeshausen, and Grier (E.G.&G.) of Santa Barbara, California. Methods of implantation and removal of dosimeters have been described in earlier papers (Turner and Gist 1965, Turner and Lannom 1968). Recovered dosimeters were read by E.G.&G., cleared and calibrated, and initial readings converted to roentgens. Tissue doses, in rads, were obtained by multiplying R values by 0.96. In an earlier paper (Turner and Lannom 1968) we discussed doses to leopard and whip-tail lizards in terms of the average fraction of free-air exposure sustained as tissue dose during different times of year. For *Crotaphytus* this fraction ranged from around 50% during late spring and early fall to around 80% in midsummer. On an annual basis the fraction was estimated as 25%. For female *Cnemidophorus* the annual fraction of free-air exposure sustained as tissue dose was estimated as 11%. In the present paper we consider tissue doses in terms of rads and estimate mean doses for various times of year.

Leopard lizards. In spring of 1969 the total known adult (≥8 months) population of leopard lizards in the irradiated area was 7 females and 11 males. Between May 15 and June 10, 1969, we collected three adult male and three adult female leopard lizards from the irradiated plot for autopsy. The oldest female was estimated to be 94 months of age, and would have been about 29 months old when irradiation began. Another female and the two oldest males were estimated to be 82 months old, and would have been about 17 months of age in January 1964. The youngest female and male were both born in August 1965, about 19 months after the experiment began. Between April 30 and July 3, 1969, 8 other adult males and 12 adult females were collected in Rock Valley or Mercury Valley at NTS. All male leopard lizards were brought to Los Angeles for examination. Six of the females were brought to Los Angeles, and six were examined at NTS. Males were maintained for one day at 37 C prior to sacrifice and injected with 2 ml of 0.05% colchicine 6 hr before death. All animals were killed by injection with nembutal. All individuals were weighed just before sacrifice, and lengths were measured immediately after death.

Freshly removed livers, kidneys, fat bodies (if present), right testes, and ovaries (if present) were weighed and preserved in Hollande's modification of Bouin's solution (Thrasher 1966). Oviducts of females and sections of gastrointestinal tracts of all individuals were also preserved. A section of the epididymis of each male was removed and the contents suspended in water for examination with a compound microscope. Smears of contents of epididymides were prepared and stained with Giemsa.

The tunic of the left testis of six control and all three irradiated males was removed and the testes placed in hypotonic (0.1%) sodium citrate for about 10 min. In general, processing of the left testes was as described by Patton (1967). After centrifugations, the supernatant was discarded and the centrifugate fixed (three parts methanol to one part acetic acid). Fixed suspensions were transferred to slides and stained with Giemsa. These preparations were examined with a microscope and counts made of macrochromosomes and microchromosomes in 50 diakinetic spermatocytes when the condition of the preparation was suitable.

Slides were prepared of portions of gastrointestinal tracts and livers of both sexes. Slides were also made of sagittal sections of oviducts, transverse sections of right testes and male kidneys, and sagittal sections of epididymides. Sections were embedded in paraffin, cut at 5 μ, stained with iron hematoxylin, and counterstained with eosin. In general, histological procedures were as outlined by Thrasher (1966).

Whip-tail lizards. The first evidence of abnormality in *Cnemidophorus* came in the spring of 1969 when, by palpation, a mature female without follicles (or eggs) was detected in the irradiated population. This female (81 months old) was removed on June 25, 1969. On the same day another mature female was collected elsewhere in Rock Valley. These animals were autopsied in the same manner as the female leopard lizards. In early June 1970, four adult females and three adult males were removed from the irradiated area. The females ranged from 46 to 94 months of age; males from 33 to 58 months. At the same time three adult males and four adult females were collected in nearby areas. All males and the four control females were autopsied in the same manner as the leopard lizards, except that no chromosome preparations were made. Five control females (collected in late June) and the four irradiated females were used in a hormone experiment to examine the possible bases of sterility.

The nine females used in the hormone experiment were kept in a controlled temperature cabinet and body temperatures maintained at 37 C for 12 hr (during the day) and cooled to 30 C at night. A 12-hr photoperiod was provided by fluorescent lamps. Animals were fed mealworms and crickets ad libitum. All individuals were administered follicle-stimulating hormone dissolved in water (NIH-FSH-S6; 1.2 units/mg) every other day. Intraperitoneal injections consisted of either 100 or 250 μg of FSH in a volume of 0.05 ml. Hormone injections began on June 12 for three of the irradiated females; on June 26 for the fourth. Injections began on July 4 for all of the controls. One irradiated female died after six injections of 100 μg of FSH. The other three received 100-μg injections until July 15, and injections of 250 μg thereafter until sacrifice. Four of the five controls received five injections of 100 μg and were then sacrificed. The other control (59 months of age) was given an additional six injections of 250 μg of FSH. All four irradiated individuals were laparotomized when first received in Berkeley. Ovarian growth (if any) in all nine individuals was assessed by periodic laparotomy after hormonal injections began. Lizards were anesthetized with ether and incisions made dorsally. Incisions were both sutured and sealed with tissue adhesive (Ethicon COAPT). At autopsy the ovaries and oviducts were weighed and fixed for histological study. The ovaries of three additional control females were examined shortly after collection in August.

RESULTS

Demography of leopard lizards. The estimated number of leopard lizards in the three Rock Valley enclosures have been summarized, through 1967, in an earlier paper (Turner et al. 1969c). However, these data gave no indication of the lack of reproduction in the irradiated plot since 1966.

Table 1 summarizes the most recent census data from the three areas, showing minimal estimates of the numbers of (1) females old enough to be sexually mature, (2) summer hatchlings (1966–1968), and (3) spring yearlings (1966–1969). As we have explained previously (Turner et al. 1969c), the most recent data (1968–9) are incomplete and subject to modifications based on future work.

Because the three areas have been subjected to the same intensity of sampling, we infer that recruitment in the irradiated area has not occurred since 1966. Since 1967 the known number of mature females in the irradiated plot has exceeded the combined number of such females registered in the two control areas. Further evidence of lack of reproduction in the irradiated area has been the failure of females to assume the reddish coloration characteristic of females with large yolked follicles or eggs (Smith 1946).

During 1966 and 1967 mature females in the irradiated area exhibited approximately the same age distribution as those in the two control plots (Table 1). Since 1967 the irradiated area has had a group of older

Table 1. Female leopard lizards (*Crotaphytus wislizenii*) registered in one irradiated and two nonirradiated (control) 20-acre fenced areas in southern Nevada

Treatment	Year	Spring adults (20–46 months)	Spring adults (56–92 months)	Spring yearlings	Summer hatchlings
Irradiated	1966	3	2	20	2
	1967	4	4	1	0
	1968	4	4	0	0
	1969	3	4	0	
Controls	1966	7	2	30	26
	1967	3	4	15	7
	1968	4	0	5	14
	1969	2	0	5	

females (56 months of age or older) not known to exist in the control plots. However, the number of younger females (20–46 months of age) in the control and experimental areas has been about the same (Table 1). Thus there is no reason to believe that the reduction of fecundity in the irradiated plot is associated with reproductive senescence of resident females.

Radiation doses to leopard lizards. Mean tissue doses to leopard lizards in the irradiated enclosure are summarized in Table 2. These estimates have been combined with data in Appendix A to estimate total cumulative exposures over the course of the experiment. (Appendix A gives the days each month – from beginning of irradiation – during which the experimental area received gamma radiation from the source.) The number of days in March, April–May, June–July–August, and September–October were multiplied by the corresponding mean daily doses given in Table 2 to estimate total doses sustained during the periods of above-ground activity. Overwintering doses were estimated by adding the days in November, December, January, and February and multiplying by 0.62 rad/day. Cumulative doses have been estimated in this manner for two periods of time: (1) January 1964 through September 1969, and (2) January 1964 through March 1967 (just prior to the first season exhibiting no evidence of reproduction by irradiated leopard lizards). These data are given in Table 3.

Between 1965 and 1968 the mean number of days of irradiation per year was 311. Combining the mean yearly dose rate given in Table 2 (0.9 rad/day) with this datum yielded an estimate of 280 rads as the mean annual dose. This is appreciably less than the dose estimated from the short-term dose rates given in Table 2. Between 1965 and 1968 the mean number of days per time period multiplied by mean daily dose rates (from Table 2) yielded an estimated annual dose of about 450 rads. It has been suggested that dosimeters left in animals for long periods of time (e.g., one year or longer) may fail to register total doses received because of decay of luminescence (Lucas in litt.). Dosimeters left in leopard lizards for periods of 300 to 1200 days registered, after calibration, doses lower than would be expected by summation of short-term increments.

Radiation doses to whip-tail lizards. Annual doses to whip-tail lizards were less than those sustained by leopard lizards. This is due to the shorter period of above-ground activity carried on by *Cnemidophorus*. As pointed out earlier, "...probably 90% of the total annual dose of *Cnemidophorus* is received between the first of April and mid-August" (Turner and Lannom 1968). Mean tissue doses to whip-tail lizards are summarized in Table 4. In our opinion, annual doses to *Cnemidophorus* do not often exceed 200–250 rads, but

Table 2. Daily tissue doses to leopard lizards in a 20-acre fenced enclosure in southern Nevada

Season	n	Mean rads/day ± one standard error	Rads/day (observed range)
March	3	1.30 ± 0.11	1.09–1.47
April–May	25	2.06 ± 0.17	0.78–4.23
June–July–August	12	2.41 ± 0.27	1.45–4.14
September–October	13	1.28 ± 0.17	0.46–2.82
Overwinter (e.g., October–April)	14	0.62 ± 0.08	0.13–1.04
One year	3	0.90 ± 0.04	0.82–0.95

Table 3. Estimated tissue doses (rads) to leopard lizards

Time of year	January 1964–September 1969	January 1964–March 1967
March	203	140
April–May	597	309
June–July–August	1099	562
September–October	351	198
November–January	361	215
Totals	2611	1424

Table 4. Tissue doses to whip-tail lizards in a 20-acre enclosure in southern Nevada: observed ranges in parentheses

Time interval	n	Mean rads/day ± one standard error	Total dose (rads) during time interval ± one standard error
April–May	10	1.7 ± 0.4 (0.59 – 4.09)	83 (estimated)
June–July	12	1.0 ± 0.1 (0.22 – 1.95)	51 (estimated)
8–10 months (August to ensuing spring)	9		70 ± 9 (39–118)
11–14 months	20		150 ± 19 (69–459)
21–22 months	2		235 ± 49 (165–304)

individuals occupying areas nearest to the radiation source could receive appreciably larger doses. The largest doses registered on dosimeters were 459 rads over a period of 10 months and 304 rads in 21 months. For those animals alive when the irradiation began in 1964, accumulated doses through the spring of 1969 (5+ years of exposure) would presumably be on the order of 1000–1250 rads. Total doses through the spring of 1970 would be about 1200–1500 rads.

Whereas leopard lizards range over the entire irradiated area, the home ranges of female whip-tail lizards are much smaller. As noted above, the dose sustained by an individual *Cnemidophorus* is partly determined by the location of the lizard's range within the fenced plot. We cannot judge precisely the importance of this factor, but we have examined the 22 short-term dose rates (in Table 4) in terms of the locations of the animals involved. There was an inverse relationship between distance and dose rate, but the correlation coefficient was only –0.331.

Morphology and anatomy ot leopard lizards. Leopard lizards taken from the irradiated plot were normal in behavior and appearance, except that females lacked the reddish coloration typical of reproductively active females. The mean body weight of the irradiated females was similar to that of the 12 controls, and the weights of the livers and kidneys of the two groups were also comparable (Table 5).

Six control females lacked fat bodies, and fat bodies of the other six controls were small (overall mean: 0.03 g). Irradiated females had enormously hypertrophied fat bodies (mean weight: 1.93 g), filling the posterior

Fig. 1. Continuously irradiated female *Crotaphytus wislizenii* from Rock Valley. The hypertrophied fat bodies occupy all of the free space in the posterior portion of the pleuroperitoneal cavity.

Table 5. Mean body and organ weights in grams (± one standard error) of irradiated and nonirradiated lizards; sample sizes in parentheses

	Sex	*Crotaphytus wislizenii*		*Cnemidophorus tigris*	
		Irradiated	Nonirradiated	Irradiated	Nonirradiated
Body	m	26.2 ± 1.91 (3)	26.7 ± 1.40 (8)	18.2 ± 1.3 (3)	17.8 ± 2.4 (3)
	f	46.7 ± 1.32 (3)	47.9 ± 4.29 (12)	27.5 (1)	14.9 ± 1.0 (5)
Liver	m	0.42 ± 0.05 (2)	0.43 ± 0.05 (6)	0.29 ± 0.03 (3)	0.27 ± 0.04 (3)
	f	1.40 ± 0.38 (3)	1.27 ± 0.18 (12)	0.76 (1)	0.23 ± 0.03 (5)
Kidneys	m	0.23 ± 0.02 (3)	0.21 ± 0.02 (8)	0.12 ± 0.01 (3)	0.09 ± 0.01 (3)
	f	0.21 ± 0.03 (3)	0.23 ± 0.02 (12)		0.06 ± 0.01 (5)
Fat bodies	m	0.133 ± 0.051 (3)*	0.024 ± 0.015 (6)	0.18 ± 0.09 (3)	0.24 ± 0.01 (3)
	f	1.93 ± 0.34 (3)**	0.030 ± 0.013 (12)	0.964 (1)	0.016 ± 0.004 (5)
Right testis	m	0.16 ± 0.044 (3)	0.11 ± 0.015 (8)	0.035 ± 0.01 (3)	0.039 ± 0.01 (3)
Ovaries	f	None (3)	1.67 ± 0.80 (12)	None (1)	0.19 ± 0.14 (4)

*t test significant at 5% level.

**t test significant at 1% level.

portions of the pleuroperitoneal cavity unoccupied by other organs (Fig. 1).

All control females showed varying degrees of the reddish suffusion of the body typical of reproductive females. None of the irradiated females was reddish. No identifiable ovarian tissue existed in any of the three irradiated females. Control females had ovaries with follicles of varying sizes, or oviducal eggs, depending on the time of collection. The oviducal walls of control females were highly glandularized (Fig. 2A), but those of irradiated females were thin-walled and nonglandular (Fig. 2B).

Mean body weight of the irradiated males was similar to that of controls, and weights of livers and kidneys of the two groups were also comparable (Table 5). Fat bodies of the irradiated males were significantly heavier than those of controls (four of eight control males lacked visible fat bodies).

In 10 of the 11 males, the two testes were of the same size and shape. In one control male the right testis (0.102 g) was smaller than the left (0.232 g). The epididymides of ten males contained motile sperm (Fig. 2C), and sperm morphology was normal. One irradiated male lacked sperm in epididymis, but the wall of this structure was normal and there was evidence in the lumen of normal secretory function (Fig. 2D). Sexual segments of the kidneys of all males were histologically normal.

Testes of all individuals but one showed spermatogenic activity normal for the time of year during which the animals were collected (Fig. 2E). However, the irradiated male lacking sperm in the epididymis showed no spermatogenic activity whatsoever, and the animal appeared to be sterile (Fig. 2F).

Microscopic observations were made on 5-μ sections of the esophagus, cardiac and pyloric stomach, duodenum, ileum, large intestine, and rectum of each animal. No evidence of loss of functional epithelium, hyperplasia of connective tissues, karyorrhexis, or any other form of tissue pathology was found. The functional integrity of the mucous membrane of the gastrointestinal tract was apparently unimpaired. Livers of the irradiated lizards appeared normal.

Chromosomes of leopard lizards. The diploid number of *Crotaphytus wislizenii* is 36, and is composed of 12 macrochromosomes and 24 microchromosomes (Montanucci 1970). Chromosomes were counted in 50 diakinesis figures in spermatocytes of six animals (Table 6). Two animals, one control and one irradiated, died shortly after the injection of colchicine. Liver damage and bloody ascites were found at necropsy, indicating possible injury to abdominal organs. Examination of testis preparations from these individuals revealed no identifiable chromosomes. However, pachynema primary spermatocytes and spermatids were observed, suggesting normal spermatogenic activity. This was confirmed by histological examination of the right testes of both animals.

Normal chromosome counts and karyotypes were found in control animals that survived the colchicine treatment (Table 6). The observed variation was attributed to an occasional loss or gain of chromosomes during preparation of the spreads. There was a definite tendency to lose microchromosomes (Table 6), probably because these chromosomes are small and sometimes difficult to observe in iguanids (Gorman, Atkins, and Holzinger 1967). The two irradiated animals surviving the colchicine treatment showed different responses to irradiation. One lacked spermatogenic cells in the chromosome preparation. This apparent sterility was confirmed by histological observations of the epididymis (Fig. 2D) and right testis (Fig. 2F). The other irradiated lizard had normal counts of macro- and microchromosomes (Table 6). Further examination of chromosomes of this lizard failed to disclose gross chromosomal damage in the form of translocations or deletions, loss of chromosomes, or presence of chromosomal fragments.

Morphology and anatomy of whip-tail lizards. No microscopic examinations of the nonreproductive organs of these lizards were made, but the gross appearance of these organs was normal in both sexes.

Table 6. Counts of chromosomes in diakinetic spermatocytes of five control and one irradiated (No. 8) male *Crotaphytus wislizenii;* figures in parentheses are observed ranges

Lizard No.	Mean number of synapsed macrochromosomes (± one s.e.)	Mean number of synapsed microchromosomes (± one s.e.)
4	6.04 ± 0.05 (5 – 8)	11.78 ± 0.19 (9 – 18)
5	5.96 ± 0.06 (5 – 8)	10.76 ± 0.21 (7 – 14)
8	6.02 ± 0.02 (6 – 7)	11.36 ± 0.17 (7 – 15)
10	6.00 ± 0.00 (6)	11.34 ± 0.18 (7 – 13)
12	6.00 ± 0.00 (6)	11.40 ± 0.17 (7 – 13)
13	5.94 ± 0.04 (4 – 6)	11.54 ± 0.11 (9 – 17)

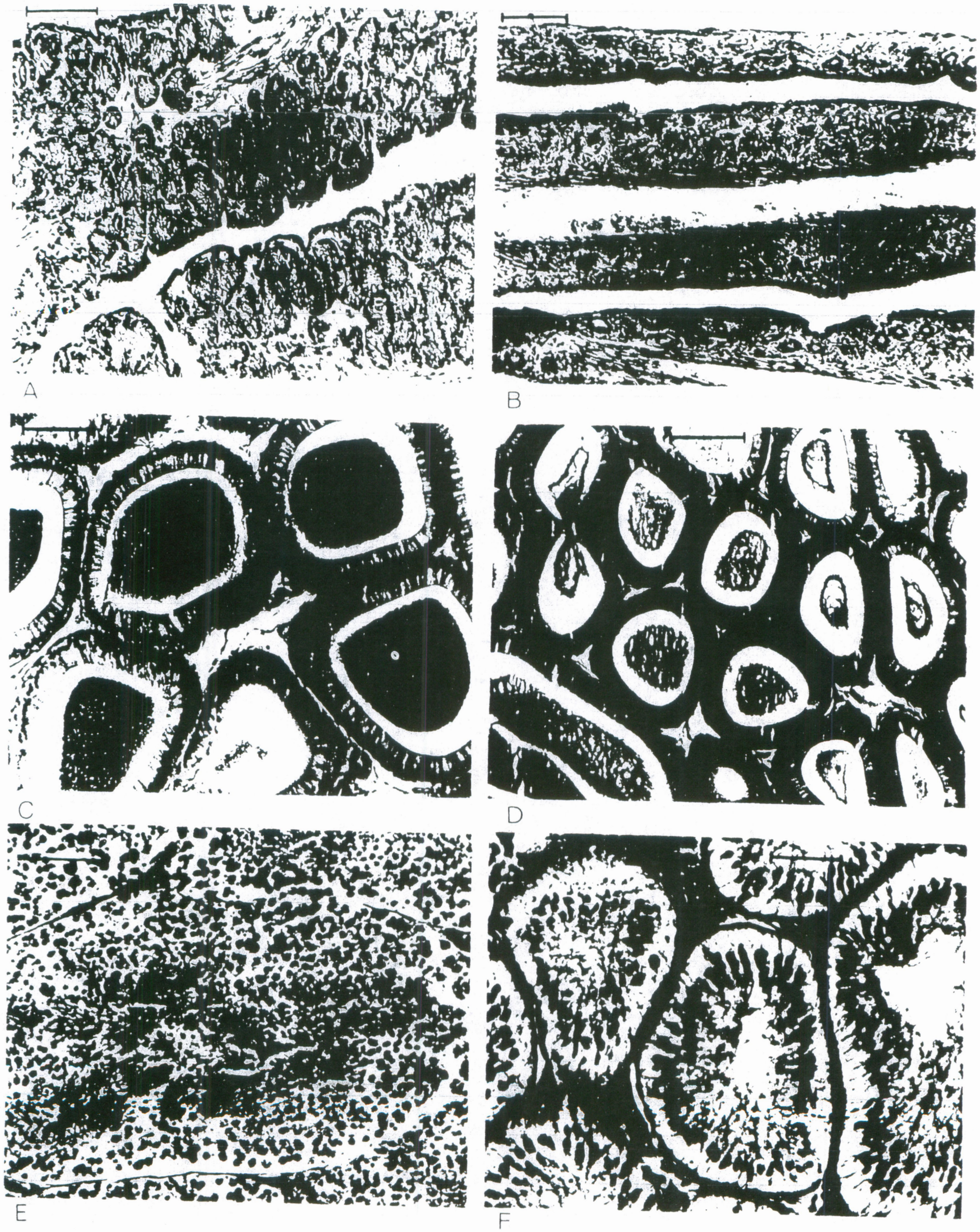

Fig. 2. Reproductive organs of irradiated and nonirradiated leopard lizards. Horizontal bars in A, B, C, and D: 100 μ; in E and F: 50 μ. A. Oviducal walls of a normal adult female. B. Oviducal walls of an irradiated adult female. C. Cross sections of the epididymis of a normal adult male. D. Cross sections of the epididymis of an apparently sterile irradiated adult male. The epididymis lacks spermatozoa but exhibits normal secretory function. E. Testis of a normal adult male with normal spermatogenic activity. F. Testis of an irradiated adult male without spermatogenic activity.

Mean body and organ weights for the six males are given in Table 5. The epididymides of all males contained motile sperm. Mean weights of fat bodies and testes of irradiated and control males did not differ significantly.

Of the five irradiated females, only one (No. 1485) possessed detectable ovarian tissue. In this lizard, both ovaries contained several well-developed corpora lutea, indicating that ovulation had occurred during the spring of 1970. The four irradiated females without ovaries had large fat bodies. All control females possessed ovaries, as revealed by laparotomy or autopsy. The oldest controls (23 and 59 months) had corpora lutea, indicating recent ovulation; the three youngest females (11 months) had small ovaries with but a few undeveloped (nonyolky) follicles.

Hormonal injections to whip-tail lizards. Four irradiated and five control females were used in the gonadotropic hormone experiment. The efficacy of gonadotropic injection was verified by marked ovarian and oviducal growth in the controls. All five controls exhibited regressed ovaries at the start of treatment. Four of these responded rapidly to hormone treatment and developed enlarged yolky follicles and hypertrophied oviducts (cf. Fig. 2A) after five injections of 100 μg of FSH. These females lacked fat bodies (Table 7). The oldest control female (59 months) showed only slight ovarian enlargement after the first five injections. After six additional injections of 250 μg, the ovaries and oviducts enlarged and ovulation occurred (Fig. 3A). It is highly unlikely that any of these females would have shown ovarian development without hormone treatment. Yearling females rarely reproduce at NTS (Turner et al. 1969b), and the three yearlings collected in mid-August had very small ovaries (6–9 mg) with clear follicles and undeveloped oviducts weighing 5–8 mg (Fig. 3C).

The one irradiated female with apparently normal ovaries and corpora lutea (No. 1213) responded rapidly to hormone therapy; ovarian follicles were enlarged and yolky and oviducts hypertrophied after six injections (cf. Fig. 2A). However, the other three irradiated females showed no detectable response to the FSH (Table 7). At autopsy there was still no trace of ovarian tissue (Fig. 3B). Histological examination of the tissues in the region of the interrenal glands (the normal location of the ovaries) revealed no ovarian tissue. The weights of the oviducts were similar to those observed in normal nonreproductive females of approximately the same size (museum specimens). Histological examination showed that the oviducts were atrophied (cf. Fig. 2B). Fat bodies were also enlarged in these irradiated females (Table 7).

DISCUSSION

We conclude that the demographic evidence of reproductive failure among leopard lizards in the irradiated plot has been confirmed by examination of lizards removed from the experimental area, and that the basis of this failure is sterility of the females. Endocrine experiments with whip-tail lizards suggest that the destruction of ovarian tissue rather than some hormonal irregularity is responsible for the sterility.

Although female *Crotaphytus* in the irradiated area apparently reproduced normally in 1965 (after one year of radiation exposure) and produced some young in 1966, we have observed no evidence of recruitment since 1966. A similar effect has been manifested among some female *Cnemidophorus*, but no demographic

Table 7. Results of a hormone (FSH) injection experiment with nine female *Cnemidophorus tigris*

Treatment and animal No.	Age (months)	Dates of hormone injection, 1970	Body weights (g) Initial	Body weights (g) Final	Weight of ovaries (g)	Weight of oviducts (g)	Weight of fat bodies (g)
Irradiated							
1149	94	6/12–7/28	21.6	23.7	None	0.071	1.720
1213	70	6/26–7/28	21.8	19.0	None	0.044	0.800
1485	58	6/12–6/30	13.5	14.9	0.400[a]	0.150[a]	Not weighed
2142	46	6/12–7/28	16.8	17.6	None	0.051	0.880
Control							
1	11	7/4–7/15	11.4	9.6	0.926	0.147	0.00
2	11	7/4–7/15	11.2	10.0	0.521	0.113	0.00
3	23	7/4–7/15	12.4	13.3	0.183	0.073	0.183
4	11	7/4–7/28	11.0	7.8	0.533	0.118	0.00
5	59	7/4–7/28	13.6	19.4	0.843	0.165	0.00

[a]Animal died; approximate value.

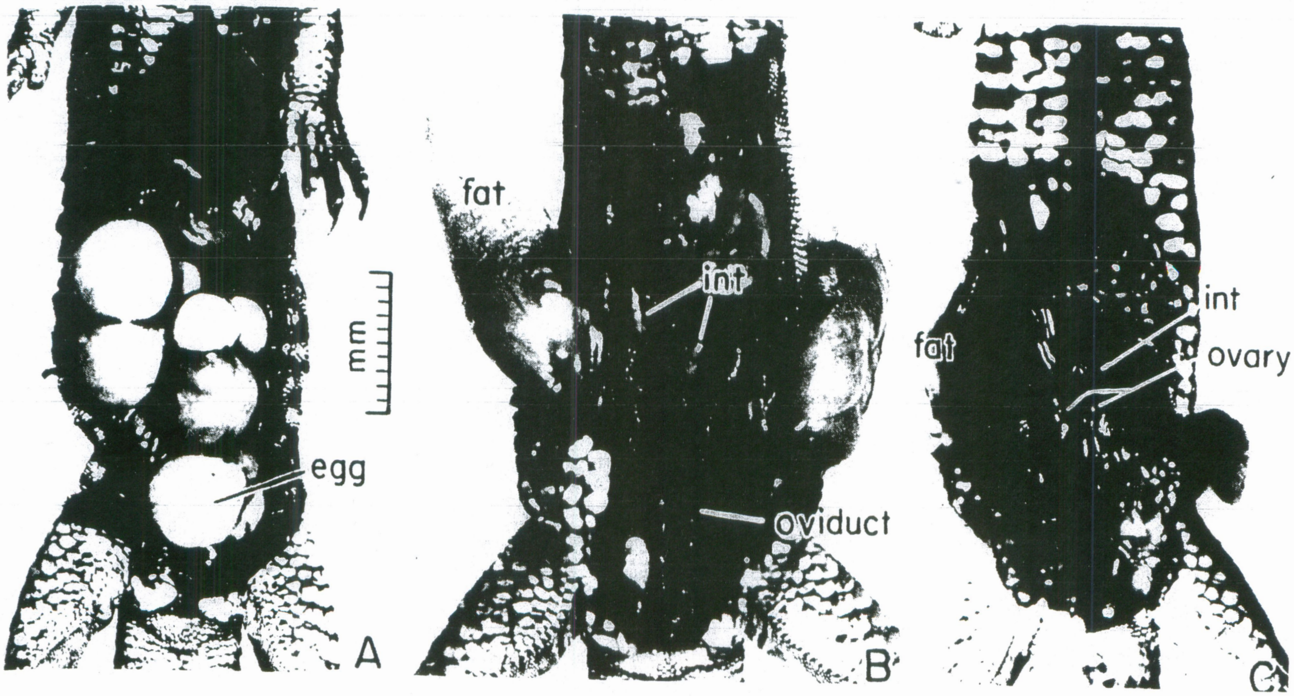

Fig. 3. Reproductive organs of irradiated and nonirradiated female whip-tail lizards. A. Ovulation induced in a nonirradiated adult (No. 5) by the administration of follicle-stimulating hormone for 24 days. Egg is in the oviduct. B. Absence of ovarian tissue in irradiated adult (No. 2142) after 47 days of treatment with FSH. Interrenal gland (int), fat bodies (fat), and oviducts are indicated. C. Nonirradiated immature (12 months) female collected in mid-August 1970 and indicating the appearance of normal regressed ovaries.

consequences of female sterility have yet been established in this species. Although annual doses to both species were modest, the cumulative effect of continued exposure was apparently sufficient to destroy the germinal cells of the ovary. According to Miller (1959), the ovaries of reptiles consist of "a thin stromal wall, a . . . patch of germinal epithelium, and a lymph filled central cavity." Although small amounts of residual connective tissue from the ovaries might have been overlooked, the important conclusion, supported by the hormone experiment with *Cnemidophorus,* is that no competent ovarian tissue persisted in any of these individuals.

Two of the three irradiated male leopard lizards and all three of the irradiated whip-tail lizards exhibited motile sperm, and the situation in males may be analogous to that observed in male mice exposed to low continuous doses. A dose rate of 0.009 rad/min (about 13 rads/day) has been considered marginal for the maintenance of recovery mechanisms. At 0.001 rad/min (1.5 rads/day) an equilibrium was established at approximately 80% of control spermatogonial populations (Oakberg and Clark 1964).

The chronology of the development of female sterility in these lizards, the relationship of the effect to radiation doses, and possible differences in the time response of the two species are difficult to assess. Some of the relevant data are summarized in Table 8. Two of the irradiated leopard lizards were sexually mature when first exposed to irradiation (21 and 33 months of age); the third was born in the summer of 1965 and was exposed to radiation during embryonic development and throughout its posthatching lifetime (roughly four years).

All three females were undoubtedly sterile in the spring of 1969, and demographic data suggest that this condition arose as early as two years before. The figures in Table 3 suggest that sterilizing doses to *Crotaphytus* were apparently on the order of 1500 rads.

Among whip-tail lizards, the first sterile female (seven years old) was detected in the summer of 1969 (although the condition may have existed earlier). Of the two youngest whiptails removed from the irradiated plot, one had ovaries and had apparently reproduced in 1970. The other was sterile. The differences between these two individuals may be related to their general locations within the irradiated plot (Table 8), or possibly to age differences (No. 1485 might have been one year older than No. 2142). In general, it appears that sterility among female whip-tail lizards has de-

Table 8. Ages and durations of exposure of female lizards removed from the irradiated 20-acre area in Rock Valley, Nevada

Species	Number	Age when removed from plot (months)	Years exposed to radiation	Condition	Distance of center of home range from ^{137}Cs source (ft)	Notes on past reproductive condition
Crotaphytus	1112	94	5.4	Sterile		Yolked follicles in 1965
	1174	82	4.4	Sterile		Yolked follicles in 1966
	1392	46	3.8	Sterile		
Cnemidophorus	1137	81	5.4	Sterile	281	
	1149	94	6.4	Sterile	228	Yolked follicles in 1967
	1213	70	5.8	Sterile	154	Small yolked follicles in 1969
	1485	46–58	3.8–4.8	Fertile	409	
	2142	46–58	3.8–4.8	Sterile	118	3 yolked follicles in 1969

veloped later and less uniformly than in female *Crotaphytus*, and this difference is consistent with the lower annual doses sustained by most whiptails.

The response of Rock Valley lizards to continuous gamma radiation is unusual when contrasted with results of other studies of continuously irradiated natural populations. Mosquito fish (*Gambusia affinis*) in White Oak Lake were estimated to receive 10.9 rads/day (about 4000 rads/year) of gamma radiation from bottom sediments (Blaylock 1969). Although the irradiated population exhibited more abnormalities and dead embryos than a control group, the irradiated females produced significantly larger broods of young.

Studies of small mammal populations on White Oak Lake bed have failed to demonstrate radiation-induced changes in exposed populations (Dunaway and Kaye 1961, 1963; Childs and Cosgrove 1966). The free-air gamma exposure rates in the areas studied ranged from 5 to 35 mR/hr (0.12–0.84 R/day) in the lower lake bed (Dunaway and Kaye 1961) to 1.2 R/day in one of the areas studied by Childs and Cosgrove (1966) and 1.92 R/day in the upper lake bed area (Dunaway and Kaye 1961). Childs and Cosgrove (1966) estimated that the accumulated annual dose to *Peromyscus leucopus* was on the order of 175 rads (about 20 millirads/hr or 0.48 rad/day), apparently assuming that all of the free-air exposure was sustained as tissue dose (Childs and Cosgrove 1966). Kaye and Dunaway (1963) measured tissue doses to rodents in one of their areas and estimated external gamma exposures at 2.5 rads/week, with 0.4 rad/week from internal emitters. In these Tennessee studies, tissue doses were presumably lower than those sustained by Rock Valley rodents and lizards. Also, among wild rodent populations of these areas, very few individuals survived more than one year (Dunaway and Kaye 1961, Childs and Cosgrove 1966). Hence total lifetime doses sustained by the majority of individuals were probably less than 200–300 rads.

Heteromyid rodents (*Dipodomys* spp. and *Perognathus* spp.) occupying the study plots in Rock Valley show better survival than the rodents studied in Tennessee (French, Maza, and Aschwanden 1967; French, Maza, and Kaaz 1969). Although mean life expectancy is considerably less than a year, two-year survival rates of around 10% (or better, in *Perognathus longimembris*) have been recorded (French et al. 1967). More recently, French et al. (1969) demonstrated three-year survival rates of 8–12% among *P. formosus* occupying the three fenced areas in Rock Valley. Annual doses to *P. formosus* have been estimated at around 360 rads (French et al. 1966), so some individuals of this species sustain lifetime doses of 900 rads or more. Although French et al. (1969) have suggested a small life-shortening effect in the irradiated population of *P. formosus*, their data did not permit an evaluation of changes in fertility. Trapping during the spring of 1970 indicated that the heteromyid rodents occupying the irradiated area were still capable of reproduction.

Two papers have reported on studies of Russian birds and mammals occupying areas with high concentrations of natural radioactive elements in the soil layer. Background radiation levels are 50–800 times normal, and animals are exposed to both external and internal radiation. Male voles (*Microtus oeconomus*) occupying areas with free-air exposures of 0.10–0.19 R/day were reported to have abnormally small testes and seminiferous tubules of reduced diameter. In areas of highest natural radiation (0.19 R/day) 58.3% of sexually mature young males were sterile by the first breeding season (Verkhovskaya, Maslov, and Maslova 1965). In areas with exposure rates of 0.12–0.19 R/day, mammals living in most intimate contact with the soil (e.g., voles, moles, shrews, etc.) showed morphological and physiological disturbances. Apparently important reductions in litter size and in the proportions of reproductively active female voles were also reported (Maslov, Maslova, and Verkhovskaya 1967).

In the Russian work free-air exposures (R/hr) were measured, but the actual doses to tissues were not estimated. The work of Kaye and Dunaway (1963) and investigations in Nevada (French et al. 1966, Turner and Lannom 1968) indicate that tissue doses are always less than free-air exposures. Hence the reported disturbances among Russian mammals have been caused by remarkably low doses. One reason it is hard to assess this work is that the roles of external and internal emitters have not been distinguished. Whereas the external gamma and beta exposures seem trivial, the possibility of damage to sensitive internal organs from alpha emitters cannot be discounted. Another deficiency in the Russian work is the lack of comment on the life histories of the animals involved. Only indirect indications of the life-span of *Microtus oeconomus* are given. The oldest age group referred to was "greater than four months" old (Verkhovskaya et al. 1965). In addition to effects on the fertility of voles, it was also reported that irradiated animals exhibited decreased amounts of body fat. This finding is contrary to our observations, namely, that sterilized female leopard lizards actually accumulate greater amounts of body fat. Even among male lizards, fat bodies of irradiated leopard lizards were significantly heavier than those of controls. This may be due to a decrease in sexual activity of irradiated males. Among tropical anoles (*Anolis* spp.) there is a strong inverse correlation between male gonadal activity and fat body weights (Licht and Gorman 1970).

The foregoing review emphasizes the difficulty in interpreting the varying responses (or lack of response) reported in different kinds of animals exposed to continuous irradiation. The simplest explanation of these differences would be interspecific differences in radiosensitivity. However, we are dealing with nonlethal doses, and the biological response to be considered is the sensitivity of germinal cells. At this level there is evidence to indicate that "... similar cell stages, in widely diverse organisms and tissues, have similar radiation response. This should be expected, since cytologically similar stages must reflect similar organization at the molecular level, and probably similar metabolic activity also" (Oakberg and Clark 1964). Similarly, Mandl (1964) has pointed out that there is "... a striking uniformity in response between germ cells at homologous developmental stages in widely differing species."

Without ruling out possible interspecific differences in sensitivity, we emphasize another factor which has important bearings on this problem, namely, natural life-span. French et al. (1969) referred to this point with regard to life-shortening effects of radiation, but the overall demographic pattern of an irradiated population has a profound influence on the way in which this population will react to continued radiation exposure. The point can be illustrated by a comparison of three species of lizards occupying the irradiated plot in Rock Valley. One of these *Uta stansburiana* seems unaffected by the radiation exposure (Turner et al. 1969a); another (*Crotaphytus wislizenii*) is being exterminated. In a third *Cnemidophorus tigris* some females are sterile and reproductive capacity may be impaired. To what extent this last may be compensated by enhanced survival of young hatched in the irradiated population is not known.

Female leopard lizards are normally not sexually mature until the age of 22 months, and usually but one clutch of eggs is laid each spring. In unfavorable years (e.g., 1964, 1970) there may be essentially no reproduction. In years of favorable rainfall (e.g., 1965 and 1969) some females lay two clutches of eggs and a few yearling females may breed (Turner et al. 1969c). The pattern is similar in *Cnemidophorus*, but we have never observed total absence of reproduction in this species (Turner et al. 1969b). The limited reproductivity of these two lizards is balanced by long life-spans at least eight years (Turner et al. 1969b,c). Obviously, older year classes (≥22 months) are extremely important in the reproductive dynamics of both populations. In contrast, although the maximum life-span of *Uta stansburiana* is about 44 months, from 94 to 98% of spring populations are composed of individuals less than 24 months of age (Turner et al. 1970). Yearlings (8–10 months old) make up from 70–80% of spring populations, depending on the previous summer's recruitment. Analyses of reproductivity during the breeding seasons of 1966 and 1967 indicated that yearling females were responsible for 64 to 79% of the total egg production of the population (Turner et al. 1970).

The demographic contrast between the three populations is striking. Clearly, any environmental factor affecting only older individuals will have less impact on *Uta* than on *Crotaphytus* and *Cnemidophorus*. In fact, a deleterious stress requiring three years of accumulated exposure for manifestation would be almost impossible to detect in populations of *Uta*. Conversely, such a stress would ultimately have observable effects on populations of the other two species. A somewhat analogous situation has been observed in voles (*Microtus*), which are so short-lived that they accumulate very little DDT from dietary sources. This "life-span protection" is passed along to white-tailed kites subsisting on the voles (Risebrough et al. 1968). A more general statement of the importance of life-span in estimating equilibrium levels of DDT, and hence the likelihood of damage from this substance, has been derived theoretically (Harrison et al. 1970).

If our interpretation of the foregoing data is correct, and if the pattern of response observed in the three species of lizards has bearing on future effects of radiation on other animal populations in Rock Valley, we will witness the gradual disappearance of longer-lived species from the experimental enclosure. The longest-lived species are often predatory types of low density. These forms are not particularly important in terms of energy flow within the community, but they may have important influences upon their respective prey populations. An understanding of such indirect consequences is indispensable for development of predictions of long-term effects of low levels of gamma radiation on ecosystems.

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LITERATURE CITED

Blaylock, B. G. 1969. The fecundity of a *Gambusia affinis affinis* population exposed to chronic environmental radiation. Radiation Res. **37**: 108–17.

Childs, H. E., Jr., and G. E. Cosgrove. 1966. A study of pathological conditions in wild rodents in radioactive areas. Am. Midl. Nat. **76**: 309–24.

Dunaway, P. B., and S. V. Kaye. 1961. Studies of small-mammal populations on the radioactive White Oak Lake bed. Trans. North Am. Wildl. Conf. **26**: 167–85.

Dunaway, P. B., and S. V. Kaye. 1963. Effects of ionizing radiation on mammal populations on the White Oak Lake bed, pp. 333–38 in: Radioecology, V. Schultz and A. W. Klement, Jr. (Eds.). Reinhold, New York, and A.I.B.S., Washington.

French, N. R. 1964. Description of a study of ecological effects on a desert area from chronic exposure to low level ionizing radiation. U.S. Atomic Energy Commission Report UCLA 12-532, University of California, Los Angeles.

French, N. R., B. G. Maza, and A. P. Aschwanden. 1966. Periodicity of desert rodent activity. Science **154**: 1194–95.

French, N. R., B. G. Maza, and A. P. Aschwanden. 1967. Life spans of *Dipodomys* and *Perognathus* in the Mojave Desert. J. Mammal. **48**: 537–48.

French, N. R., B. G. Maza, and H. W. Kaaz. 1969. Mortality rates in irradiated rodent populations, pp. 46–52 in: Symposium on Radioecology, D. J. Nelson and F. C. Evans (Eds). U.S. Atomic Energy Commission Report CONF-670503.

Gorman, G. C., L. Atkins, and T. Holzinger. 1967. New karyotypic data on 15 genera of lizards in the family Iguanidae, with a discussion of taxonomic and cytological implications. Cytogenetics **6**: 286–99.

Harrison, H. L., O. L. Loucks, J. W. Mitchell, D. F. Parkhurst, C. R. Tracy, D. G. Watts, and V. J.

Appendix A

Days of exposure to a 33,000-Ci ^{137}Cs source sustained by a 20-acre enclosure in Rock Valley, Nevada, between January 1964 and January 1969

Year	January	February	March	April	May	June	July	August	September	October	November	December	Total
1964	12	16	28	23	27	26	26	27	26	27	26	27	291
1965	27	24	27	25	27	26	27	27	26	27	26	27	316
1966	28	24	26	23	25	24	25	25	24	25	26	31	306
1967	27	26	27	22	25	25	25	24	24	23	30	31	309
1968	28	26	25	24	25	25	24	25	24	27	30	31	314
1969	31	28	23	22	22	24	24	27	21				

Yannacone. 1970. Systems studies of DDT transport. Science **170**: 503–8.

Kaye, S. V., and P. B. Dunaway. 1963. Estimation of dose rate and equilibrium state from bioaccumulation of radionuclides by mammals, pp. 107–11 in: Radioecology, V. Schultz and A. W. Klement, Jr. (Eds.). Reinhold, New York, and A.I.B.S., Washington.

Licht, P., and G. C. Gorman. 1970. Reproductive and fat cycles in Caribbean *Anolis* lizards. Univ. Calif. Publ. Zool. **95**: 1–52.

Lucas, A. C., Z. C. Burson, and R. E. Lagerquist. 1966. Design of a shielded source for the irradiation of natural animal populations. U.S. Atomic Energy Commission Report CEX 63.10, Edgerton, Germeshausen, and Grier, Santa Barbara.

Mandl, A. 1964. The radiosensitivity of germ cells. Biol. Rev. **39**: 288–371.

Maslov, V. I., K. I. Maslova, and I. N. Verkhovskaya. 1967. Characteristics of the radioecological groups of mammals and birds of biogeocoenoses with high natural radiation, pp. 561–71 in: Radioecological Concentration Processes, B. Åberg and F. P. Hungate (Eds.). Pergamon Press, Oxford.

Miller, M. R. 1959. The endocrine basis for reproductive adaptations in reptiles, pp. 499–516 in: Comparative Endocrinology, A. Gorbman (Ed.). Wiley, New York.

Montanucci, R. R. 1970. Analysis of hybridization between *Crotaphytus wislizenii* and *Crotaphytus silus* (Sauria: Iguanidae) in California. Copeia **1970**: 104–23.

Oakberg, E. F., and E. Clark. 1964. Species comparisons of radiation response of the gonads, pp. 11–24 in: Effects of Ionizing Radiation on the Reproductive System, W. D. Carlson and F. X. Gassner (Eds.). Macmillan, New York.

Patton, J. L. 1967. Chromosome studies of certain pocket mice, genus *Perognathus* (Rodentia: Heteromyidae). J. Mammal. **48**: 27–37.

Risebrough, R. W., P. Rieche, D. B. Peakall, S. G. Herman, and M. N. Kirven. 1968. Polychlorinated biphenyls in the global ecosystem. Nature **220**: 1098–1102.

Smith, H. M. 1946. Handboook of Lizards. Comstock, Ithaca, New York.

Thrasher, J. D. 1966. Analysis of renewing epithelial cell populations, in: Methods in Cell Physiology, D. M. Prescott (Ed.). Academic Press, New York.

Turner, F. B., and C. S. Gist. 1965. Influences of a thermonuclear cratering test on close-in populations of lizards. Ecology **46**: 845–52.

Turner, F. B., G. A. Hoddenbach, P. A. Medica, and J. R. Lannom. 1970. The demography of the lizard, *Uta stansburiana* Baird and Girard, in southern Nevada. J. Anim. Ecol. **39**: 505–19.

Turner, F. B., and J. R. Lannom, Jr. 1968. Radiation doses sustained by lizards in a continuously irradiated natural enclosure. Ecology **49**: 548–51.

Turner, F. B., P. A. Medica, J. R. Lannom, Jr., and G. A. Hoddenbach. 1969a. A demographic analysis of continuously irradiated and nonirradiated populations of the lizard, *Uta stansburiana.* Radiation Res. **38**: 349–56.

Turner, F. B., P. A. Medica, J. R. Lannom, Jr., and G. A. Hoddenbach. 1969b. A demographic analysis of fenced population of the whiptail lizard, *Cnemidophorus tigris*, in southern Nevada. Southwest. Nat. **14**: 189–202.

Turner, F. B., J. R. Lannom, Jr., P. A. Medica, and G. A. Hoddenbach. 1969c. Density and composition of fenced populations of leopard lizards (*Crotaphytus wislizenii*) in southern Nevada. Herpetologica. **25**: 247–57.

Verkhovskaya, I. N., V. I. Maslov, and K. I. Maslova. 1965. Effects of small doses of radiation and incorporated naturally radioactive elements on spermatogenesis of root voles (*Microtus oeconomus*) under natural conditions. Radiobiologiya **5**: 720–29.