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Effects of Irradiation on the Behavior of Small Mammals under Field Conditions

I. BACKGROUND

The original objective of this study was to determine whether or not a basic aspect of mammalian behavior, home range development and maintenance, is susceptable to change by acute, sublethal exposure to x-radiation. The literature (Furchgott, 1963; Kimeldorf and Hunt, 1965) suggests that irradiation can alter activity levels, change learning abilities of neonatally exposed rodents as adults, and alter learning in exposed adults through motivational changes. Learning is certainly an important factor in home range behavior (Barnett, 1958). On the other hand, changes in home range of individuals would appear to have important implications at the population level (Wynne-Edwards, 1962).

Previous work under this contract has shown that irradiated adults (400r and 800r) retain their home range, but its area is reduced, its position stabilized and the average distance between successive captures decreases (Orr, 1966). Displaced irradiated subjects return to their original home area.

The above work depended on the use of a two-acre field enclosure. Subsequent work, involving introduction of both laboratory born and wild-captured subjects, has shown that the use of enclosures is not as simple as first thought. Details are given later in this report. It became necessary to study the enclosure as a tool and to collect more basic information on the ecology of the subject, Peromyscus maniculatus bairdi, the prairie deer mouse.

Incorporated into the study of the ecology of the subjects was a pilot survey of the total body levels of several trace elements. This work was intended to serve as the basis for a further investigation of the role of this species in nutrient turnover in the enclosure. Also, nutrient levels, which have rarely been studied at the species population level in natural environments, may have a relation to the distribution and abundance of this species, which is found in habitats subjected to manipulation of mineral elements.

In addition, through a Supplemental Fund, work on the eastern chipmunk has been initiated, as part of an overall, cooperative investigation of the basic ecology of the species. Initial phases include sampling of three permanent quadrats, to be used to follow population dynamics over a long-range basis. Later studies will utilize the chipmunk in behavior studies, since its diurnal habits are conductive to very detailed behavior analysis through direct observation.

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Initial study of the chipmunks will include determination of total body mineral levels in specimens from Minnesota, Pennsylvania and Vermont.

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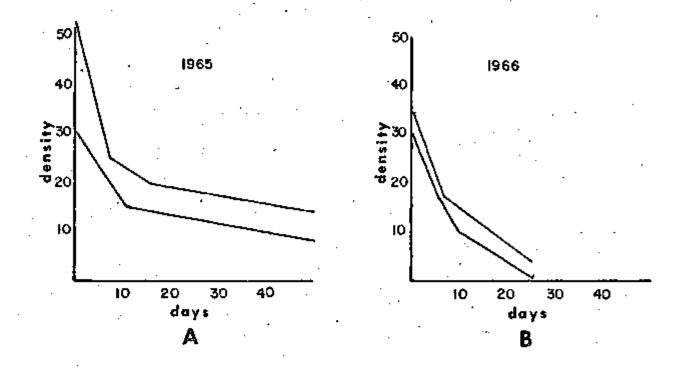
III. Reaction of populations introduced to the 2-acre field enclosure

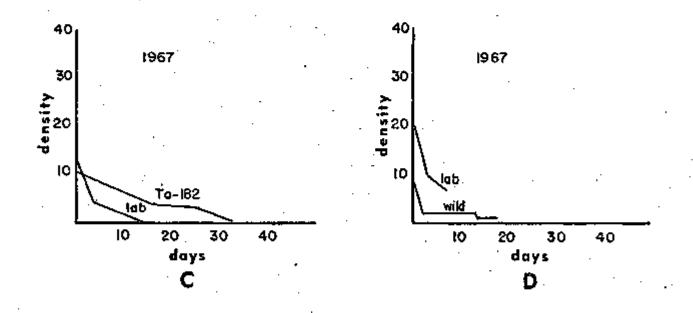
Density changes:

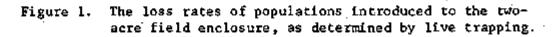
Ten groups of mice have been released into the enclosure during three summer seasons. In general, it has been impossible to maintain propulations once they have been introduced, and no carry over of populations has occurred over the winters. Populations introduced tend to exhibit similar curves, always indicating decline. Populations introduced do not affect those already present. Groups introduced in 1965 showed a tendency to undergo a rapid decline in individuals during a short period following introduction, followed by a slower rate of decline over a more extended period. (Fig. 1). The number of animals remaining was sufficient to study effects of x-radiation on home-range parameters. All animals introduced in 1965 were captured by live-traps from wild populations in the spring and summer. A lab-born group and a wild-captured group introduced in 1966 were similar in size and also exhibited a rapid initial decline (Fig 1, 1966). However, this was followed by a much more rapid secondary loss rate than in 1965 (1/5 days in '65 as against 1/day in 1966.) Two factors indicated that successional changes in the enclosure over the one-year period might be responsible for the differences in population response between 1965 and 1966. First, a mild outbreak of the field mouse, Microtus was noted during the spring of 1966; this species prefers a moist, dense grass habitat. Second, the number of grass stems/unit area was observed to increase by a factor of 5 between 1964 and 1966. Thus succession had seemed to increase vegetation to a <u>Microtus</u> habitat, at the same time creating an unfavorable habitat for <u>Pecorupus</u>. \sim On the basis of this, one half of the enclosure was mown very close, creating a drier, almost coverless situation. A group of 31 subjects introduced following this change showed no difference in loss rate, and no preference for the mown area. This was taken as meaning that overhead cover provided by grasses acts as no particular barrier in itself to deter the prairie deer mouse from grass habitats.

Live trapping for subjects had indicated that the prairie deer mouse prefers bare ground to that found in grassy areas where stems are quite numerous, and a thick mat of undecayed plant material has accumulated. Thus investigation of populations in the fall showed that the species occurs in roadside ditches with tall grasses, but low stem density, in corn fields and in soya bean fields. Where alfalfa has been recently planted in previously cultivated fields, the populations are higher than in older alfalfa fields where grass has moved in.

Thus, the enclosure was plowed before introductions in 1967, with strips of grass left to serve as protective covering during adjustment of introduced subjects. Approximately 85% of the 2-acres was in rough, plowed condition. It was felt that this type of surface would provide many burrows for subjects to use as nest-sites.







Since previous work had failed to indicate whether the subjects were dying in the enclosure or escaping, a group of 10 subjects caught during June 1967 from corn fields near the enclosure were tagged with Ta-182 and introduced into the enclosure. Each subject was subcutaneously injected with a piece of Ta-182 wire 1 cm. in length, having an activity level of approximately 100 microcuries.

Following release of these subjects, live trapping was carried out as usual, and the enclosure was surveyed with a portable scintillation detector in order to locate any animals not trapped. Figure 1C shows the changes which occurred in this group. While no sudden loss occurred, a steady decline occurred in the number of subjects trapped. A careful survey of the entire enclosure with the detector on the 33rd day following release indicated that no mice were present. One subject was trapped alive in the corn field near the enclosure, and another was found dead in a trap within the enclosure three weeks after the group had been introduced. Thus eight mice had either escaped or removed by predators. A complete survey with the detector has been made of all open areas near the enclosure, and no subjects have been located. When the cornfields have been cut, the exact sites where these subjects were originally trapped will be surveyed, and appropriate trapping carried out to recover the radioactive Ta-182 as indicated. The experiment with the Ta-182 wire was inconclusive as to the exact cause of disappearance, but since an animal was recovered alive outside of the enclosure there was a strong indication of escape by subjects.

As a result of this, steps were taken to reduce possible escape routes. The area within a meter of the interior edge of the enclosure fence was carefully examined for escape routes in the form of burrows. Several abandoned and inconspicious ground squirrel burrows were found and closed. In addition: three active burrows were formed, concealed by vegetation. Traps were placed in these, and ground squirrels were captured in them. When it seemed that all holes were closed, a group of 15 lab born individuals were introduced. The interior of the fence was policed every evening before dark for burrows; rarely one was found and closed. In spite of this, the Subjects dissappeared, more rapidly at first, but completely within two weeks (see Fig. 1 C). With all the precautions against escape by use of burrows, the rate of loss was greater during the first 4 days that for the previous group, a fact which casts doubt on escapes as a complete explanation for the losses.

As a further check on escapes as a contributing factor to population loss, an escape route was intentionally created along one side of the enclosure. A burrow with a wide opening was dug under the fence. On the outside of the fence at this point a small enclosure, 6 feet in diameter, was constructed in the same manner as the main fence. Trops were placed in this area to apprehand any escaping subjects. With all other escape routes closed to the best knowledge of the investigator, 10 animals were released into the main enclosure. Trapping within the enclosure was held to a minimum, to provide the least possible interference with escape by subjects. Over a period of 3 weeks, all subjects disappeared while only 2 were ever caught in the small enclosure

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(Fig. 1, 67). Thus, loss by escapes seemed to be at best a partial explanation of the total pattern.

The problem of loss of introduced subjects presents two basic questions: (1) how do animals disappear and, (2) why do they leave. A group of 20 young animals (5-7 wks. old), reared in the laboratory, was released next, to see if age might in some way be related to the tendency of the animals escape, or fail to adapt to the enclosure. Results of this introduction indicated that young animals are not different than adults (Fig. 1,D). It also established, in light of other releases (Fig 1 C and D) that when a number greater than 10 subjects are released, a sharp initial loss occurs, while the smaller groups disappear at a relatively constant rate.

While evidence from work to date indicates that escape has probably been a contributing factor it does not seem to completely account for results. An obvious factor is predation. Evidence is admittedly indirect, but several observations suggest at least some predation. Freshly trapped ground squirrels were usually eaten, a pocket gopher which had been eaten except for the head was found in the enclosure, and an occasional blurred cat or fox track has been seen in fresh pocket gopher mounds. While several direct observation periods were conducted at night, no predators were observed in the enclosure or near it.

Even if predation is a contributing factor, as it might well be when a fairly large group of animals unfamiliar with the enclosure is first introduced, it is highly unlikely that predation could account for the complete disappearance of a population.

Some predation and natural death should be expected in any enclosure of this size. However, escapes would seem to best account for the disappearance of the final few. It may be that subjects which survive predation, and live to adjust to the enclosure, ultimately find a limit to their home range in the form of a fence. This could provide the stimulus to search for a way around the barrier, which in turn could lead eventually to the discovery of an escape route or possibly increased exposure to predation. If this is true, enclosures are much less favorable as a means of working on mammal behavior under natural conditions than first thought.

Until the above basic questions are answered, work on the effects of x-radiation on home range behavior in the enclosure is not feasible.

Further work will be based on the use of smaller enclosures within the present one. These would provide an inexpensive way of studying predation and escape, and would allow the subjects to develop a familar center of security in the area from which a home range in the larger enclosure would be established later.

Individual behavior:

Results from grid trapping over the three summers have shown that animals

introduced to the enclosure will develop a definite home range, even if they disappear later. Figure 2 indicates the type of pattern for nine subjects in 1965 (a like number of individuals were found to have the same tendencies, but ranges overlapped to the extent that confusion resulted when all were included on the same figure).

The 1966 results suggest that the type of habitat is important in the process of home range development. As shown in Figure 2, animals captured 11 times covered more ground than in 1965, at a time when succession had led to a predominence of grass and a habitat more suitable for the field mouse, <u>Micotus</u>. At least one of these animals progressively moved diagonally across the enclosure, failing to orient to a specific place.

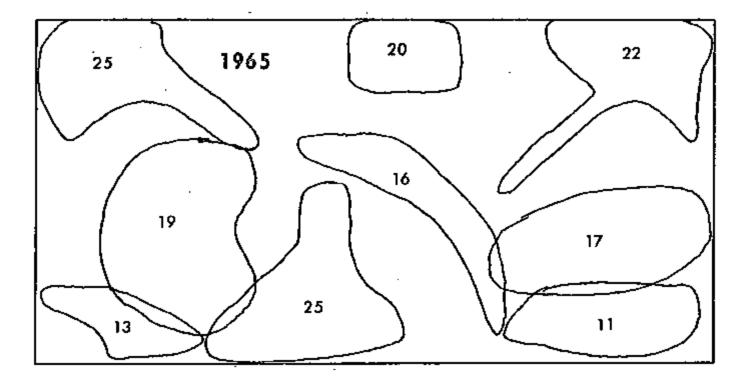
After the habitat was changed by plowing, a greater tendancy to develop home range occurred (Figure 3), although subjects captured from corn fields exhibited more compact movement patterns than did juveniles which had been reared in the laboratory.

The movements of individuals are perhaps best summarized by determination of the average distance between successive captures. This permits inclusion of subjects which were captured but a small number of times in comparison of group reactions to the enclosure. The figures for 1965, 66, and 67 were, in feet, 57, 100, and 90 respectively. Groups within each of the three years did not vary except in 1967, when 4 groups had average values of 41, 78, 101, and 140. However, the two lower values were based on groups which had very few recaptures and these are considered inadequate samples. The other two groups differed in total size (10 vs 20 individuals) and in background (young-labreared vs adults captured from wild populations). Thus it is difficult to account for differences in movements. On the other hand, it is obvious that group size, background and habitat must all be considered potentially important as contributors to the behavior of the individual once it has been introduced to the enclosure.

III. Populations and natural habitats

During the course of the study it has been necessary to capture subjects for use in enclosures and to obtain laboratory reared populations. The results of trapping shed light on the basic ecology of the species, especially in terms of habitats and populations density therein.

Most trapping was done with Sherman livetraps, in four major habitat types: grassy roadside ditches, alfalfa fields, corn fields and soya bean fields. Traps were generally set in single lines of 10 traps spaced 30 feet apart in a given habitat, although at times lines were arranged in grid form where space permitted. Since the prime purpose in most trapping was to obtain live , subjects, simultaneous trapping of all habitats was not common. Good results in grass often led to more concentrated trapping on this habitat, etc. However specific attempts were made to compare habitats during the past year.



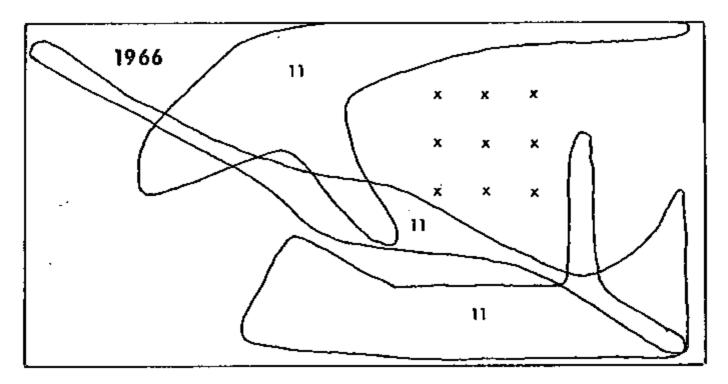
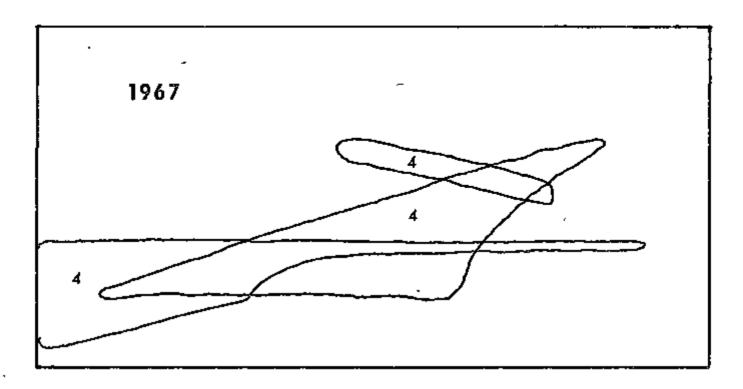


Figure 2. Typical movement patterns of individuals introduced into the field enclosure, drawn by connecting outside points of capture by live traps. Numbers indicate the number of times individuals were recaptured.



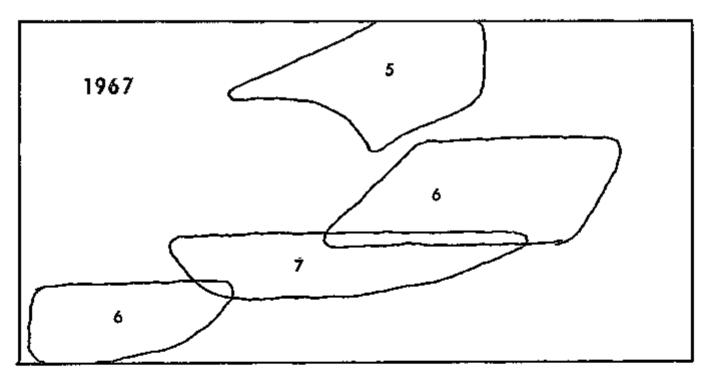


Figure 3. Movement patterns of individuals in the field enclosure following plowing. Upper figure represents animals reared in the laboratory; lower figure shows subjects captured as adults from nearby comfields.

Because of the trapping procedures, particularly the use of single lines, the most appropriate method of comparing results was percent of capture (number of animal/traps set). While this did not give density estimates, it permitted comparison of subject abundance in the various habitats. When all results were pooled, the percent capture in the selected habitats was:

Grass	7.5%	(0, 3-12, 8)
Alfalfa	15.4%	(2.5-35)
. Corn fields	16.2%	(5-20)
Soya Bean Fields	- S.7%	(0-10)

It is obvious that this species occurs in a wide range of habitats. Its distribution is not easily understood because of the variation of capture success within all of the habitats. For example, in the fall of 1965, an alfalfa field near the enclosure produced 35% capture, a figure never attained again at the time of year. In corn fields, the percent capture is near zero after planting, raises steadily over the summer and fall, decreasing to zero if fall plowing occurs. Although cornfields are an example of a habitat with very little variation from one location to another, populations seem to very considerably. Old alfalfa fields are less productive than those recently converted from other crops. Road side ditches, basically grasses, which appear quite similar, also vary widely.

In 1965, when the first animals were introduced into the enclosure, the habitat was much like a primitive alfalfa field. Animals adjusted best to the habitat during this year. Invading grass converted it to what was more like a roadside ditch the next year. Plowing the 1967 set back succession to an early corn field stage. It would appear that the lack of cover, present in all major habitats where the prairie deer mouse normally is found in numbers was a major deterrent from successful creation of an optimal habitat. The small amount of grass not plowed was too dense, as it had been the previous year.

As succession occurs in the plowed areas of the enclosure, the habitat should become more suitable, due in part to the presence of overhead cover. On a long range basis, succession will have to be periodically manipulated.

IV. Effects of x-radiation on the behavior of subjects in the enclosure.

In the summer of 1965, when enclosure subjects remained there for longer it was possible to measure the effects of irradiation- on home range behavior. Adults were allowed to develop a home range, were removed, exposed to a single dose of 100 or 800 r and returned to determine effects. If was found that irradiation decreased average distance between captures, area of home range and change in the center of activity (differences) between irratiated subjects⁻ and controls were significant at the 1% level). In 1966, loss rates were so great when an attempt to determine effects of neonatal exposure to x-radiation was made that no measurements of home range parameters was possible. The small sample of average distances between captures indicated that neonatal exposure had no effect on behavior as measured by live-trap techniques.

Subjects in 1966 were all six to eight months of age. In 1967 a similar group was released and lost so quickly that no adequate sample was possible. However, a group of 10 subjects, 10 of which were exposed to 200 r at birth, and released at approximately 6 weeks of age, remained long enough to obtain some data on average distance between capture. Controls had an average of 196 feet while the neonatally exposured subjects had an average of 103 feet. However, variation within groups (65 to 277 feet for controls vs 30 to 197 feet for x²rayed) indicated that a larger sample was necessary to establish statistically significant differences.

In so far as the effects of exposure to radiation is concerned, no influence on the disappearance of subjects has been found, whether the exposure is given neonatally or to adults before introduction or after introduction. It would thus appear that irradiation (as sublethal, acute exposure) has subjle influence on the exploratory behavior as it relates to home range movements, but does not obviously alter (1) the process of establishment of home range in adults or young or (2) the behavior necessary to maintenence of home range where previously established. If it is possible to extrapolate from the admittedly insufficient date obtained so far, the principle effect of irradiation would be at the population level. By increasing or reducing home range size, or the distance between captures, recruitment into the population might be effected, thus changing density. It should be made clear that this depends largely upon whether the effects of home range noted in 1965 are long range and not restricted to that period during which the effects of radiation sickness act to decrease general activity.

It is obvious that without correction of the causes of subject losses following introduction to the enclosure little progress toward a satisfactory description of individual or population effects of radiation is possible.

V. Effects of x-radiation on behavior of subjects in a linear image

A principle part of establishment of orientation to a home range is exploratory behavior, presumably radiating out from a satisfactory center of security. Trips made from the nest or burrow seem to be, in this species, short at first, (Orr, 1966) gradually increasing to some limit in each of several directions. The individual thus learns the topographic features of an area, to the extent that physiological needs can be satisfied, and escape from predators is highly probable.

If radiation, whether exposure occurs neotally or at a later time in life

history, alters learning directly or through motivational changes, home range behavior should show effects.

Under natural conditions, especially when trapping is the technique relied upon for measurements, behavior changes can be studied only at a gross level. Laboratory studies, with the advantages of controlling movements of subjects, seem expedient in determining radiation effects on exploratory behavior. Information obtained could be used to predict movements under more natural conditions and to suggest better direction in design of field studies.

A linear maze was constructed to aid in the description of exploratory behavior. The image consisted of four equal units. Each unit was a flat box, 20 inches square and 4 inches high. Within the box, a series of upright partitions were placed so that a subject had to travel the width of the box, turn into the next channel, again travel the width of the box and turn once more. 'After 10 such turns, the animal could reach the end of a box, having traveled about 15 feet. No choice existed accept to go on into new channels or return toward the beginning point. The boxes were covered with plate glass, permitting observation of subjects and opportunity for subjects to orient to external cues.

Subjects were placed in a wooden box two feet long, one foot wide and one foot high. The cover was also wood, making the box completely dark. Food and water were present in the box at all times. An opening from the box led into a treadle (similar to one suggested by Dice, 1961) which operated an event recorder. In turn, the subject could enter the first maze box. Treadles also measured passage between maze boxes, as well as passage from the last maze into a trap, which constituted the end of the experiment (total distance 60 ft, total turns, 40).

All subjects tested were placed in the box at approximately 4:00 PM. and left undisturbed until completion of the maze run. Work was conducted in a room where natural light cycles for the time of year were maintained. Subjects were all laboratory born. Controls and irradiated (200 r meonatally, 130 KVP at 300 r/min) were tested.

In all but a few cases, the pattern of behavior was similar. Subjects left the security box within an hour after introduction, spent time getting used to the noise and movements of the first treadle and proceeded into the first maze. Before completion of the first segment of the maze, a relatively high number of return trips were made to the security box. The second maze was treated similarly with fewer returns, and so on, with the remaining mazes. But it was the rule that trips back to the security box were made after the final maze box had been entered. In general, behavior was as predicted in terms of the above concept of home range development, but the time necessary to reach the end of the test was longer than expected.

Thirteen control and 13 meonatally exposed laboratory born subjects were successfully tested. Figure 4 shows the effects of x-radiation in terms

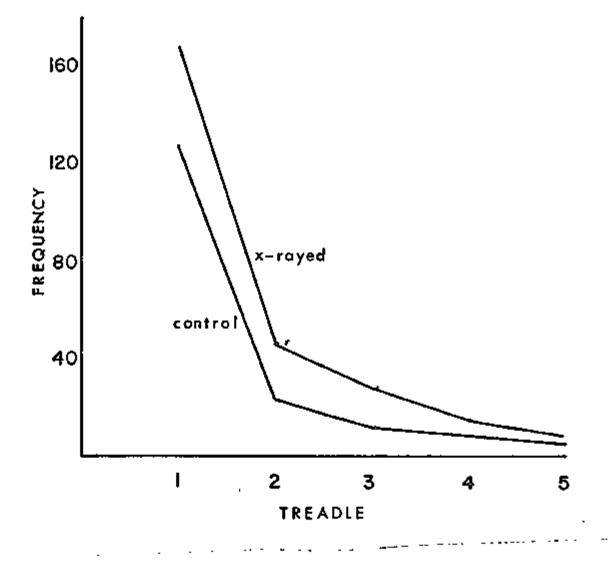


Figure 4. Mean number of treadle crossings at each of the treadle locations by x-rayed and control groups. Treadle "1" was between the introductory box and the first segment of the maze.

of the average number of crossings made at each consecutive treadle. Mean values were consistently higher for the x_{τ} rayed subjects, especially at the first three points. However, there was considerable variation within each group, so that treatment differences were not statistically significant.

Figure 5 compares irradiated subjects with controls in terms of the time required to reach each of the treadles. Means were not greatly different up to the second treadle (just after the first maze) but as a group, x-rayed subjects took increasing periods longer to reach the remaining three points. The total time was therefore much longer for x-rayed subjects. Again, individual variation was great, preventing rejection of the null hypothesis that no differences between groups occurred. Total time in hours for individuals to complete the maze is shown below to provide a first-hand picture of the variation:

Controls	x-rayed neonatally		
124*	112		
34	110		
34	75		
29	55		
9	42		
6	` <u>1</u> 1		
6	8		
5	6		
3	6		
2	2		
2	- 2		
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*hours to complete test, to nearest hour.

The general behavior pattern of subjects, particularly the tendency to return to the introduction box throughout the test, was close to prediction in terms of the hypothesis. In addition, the imposition of a simple change in direction decreased the average distance from the starting point that subjects reached per unit time (this verifies former work, Orr, 1957). Therefore the general nature of the experimental design is assumed to be adequate for measuring exploratory behavior of the species.

The variation between individuals which was found was unexpected. Subjects were all laboratory reared, all raised in sibling groups and fed the same diet. Differences in age at the time of testing did not account for differences in performance, nor did sex. Preliminary data on wild animals, trapped from local populations as adults with previous home ranges, indicate the same level of variability. Since home range movements in the field enclosure and outside do not reflect the same degree of variation, either the laboratory test or trapping procedures are poor. Since much more information is collected

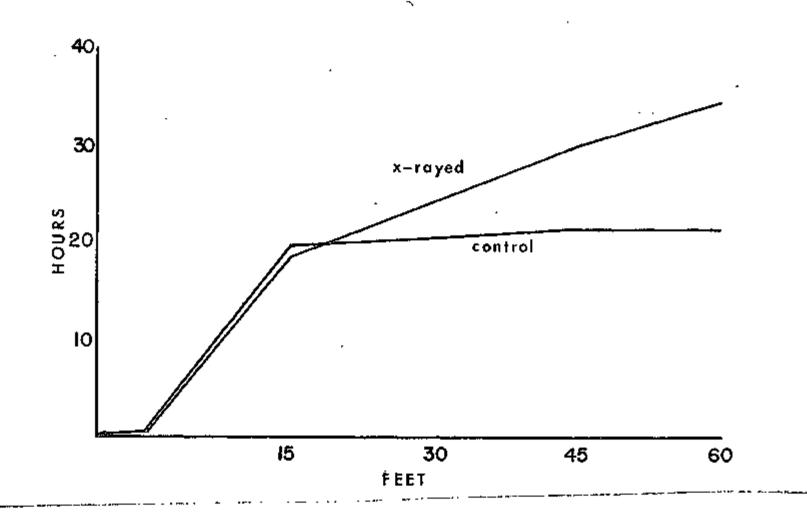


Figure 5. Mean time for experimental and control groups to reach measuring points between successive segments of the linear maze.

by the laboratory test, it is possible that trapping is too gross as a technique for studying development of home range and the individual variations that are present during the process.

Although the amount of time required is great, a series of tests in the maze is planned utilizing direct observation. The objective is to see whether behavior within the maze segments is more constant for individuals than that previously measured by treadles between. It will be possible to study the effects of sudden sounds on performance, a factor which could have influenced previous results. Methods of measuring movements at high number of points (e.g. at each turn) within the maze segments by means of thermal or mechanical sensors, and transistorized logic circuits are also being studied.

VI. Total body levels of selected trace elements in prairie deer mice.

As the dynamics of ecosystems assume more and more importance in environmental biology, it is important to learn more about the distributions of mineral elements in natural systems. Before the rate of turnover of particular elements by a given system can be adequately studied, using radioisotopes the amount of the stable isotopes should be known.

Much of the vast knowledge about mineral metabolism in mammals has been obtained using laboratory animals. Most investigations have had objectives which did not require knowledge of total body levels. Thus, information on the total body content of wild species is extremely limited (Schroeder, 1965). One other reason for their situation is the limitation of traditional analytical techniques. Atomic absorption spectroscopy has eased this problem greatly.

As a preliminary step toward eventual investigation of mineral turnover in the prairie deer mouse under natural conditions, a study was made of the total body level of several essential trace elements in individuals from different habitats. Subjects were obtained by snap-trapping during October and November. Each was frozen until sampling.

Individuals were thawed, their body cavities opened by a median ventral incision, and dried at 95° C for 36 hours. Each was then wet ashed by a perchloric acid technique, and the resultant clear liquid diluted to a convenient volume. Thirty four individuals so treated were then tested for Cu, Fe, Mn, Pb and 2n by use of an atomic absorption spectrometer (Perkin-Elmer 303). Although Pb is not an essential trace element, it was included because the prairie deer mouse lives in agricultural situations where it is subjected to contaminants.

Analysis of variance showed that individuals captured in different habitats differed in amounts of copper and lead (1% level) but not in the other

Table I. Mean total body	level of various trace elements	in PPM for laboratory
reared subjects and those	trapped in different habitats.	Range is also given
for each situation.		

	Lab	Cornfield	Soy abe an	Between	Grass
Cu	6.4	45.8	16.6	7.3	4.7
	(1.39-18.4)	(32.0-91.5)	(8.85-38.8)	(4.73-11.2)	(0.967-12.0)
Fe	281	609	548	584	604
	(216-334)	(417-912)	(339-739)	(393-902)	(483–688)
Min	12	22.2	23.5	20.5	⁻ 20.4
	(6.58-22.1)	(15.3-27.5)	(12.2-26.9)	(10.8-38.6)	(18.5-23.2)
Sn	113	128	122	113	110
	(87.3-137.0)	(101-168)	(104-150)	(93.5-166)	(93.6-133)
РЪ	77.6	40.9	124	120	213
	(28.4-170)	(12.7-96.3)	(86.5~214)	(69.3-175)	(140-274)

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elements measured. Further statistical analysis showed that laboratory reared subjects were significantly different from at least one field group for every element measured.

The latter finding would imply that care must be exercised in mineral metabolism studies where information obtained from laboratory subjects are used to extrapolate to field conditions. The fact that habitat differences occur in some cases suggests that subjects studied in one ecosystem cannot be considered to apply to others.

A striking feature of the results is the amount of individual variation detected in the laboratory group for every element. The subjects used were of approximately the age (all were adults when sacrificed), and differences in total body weight did not account for the variation found.

Further sampling is planned to determine seasonal effects, and a study will be made of the relationship between the amount of stable zinc and the absorption and excretion of Zn-65.

VII. Chipmunk study

Population sampling:

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Three one-acre plots were established for long range study. These plots were located in a forest of about 30 acres. The plant community consisted of a fairly uniform stand of sugar-maple-basswood, as shown a sample of 10 standard quadrats 10 x 10 meters. The topography of the area was fairly level, with very few moist areas.

The sample plots were set out on a grid system of 7 rows of 7 stations, both 10 meters apart. Stations were permanently marked by stakes.

The three plots were sampled in sequence during July and August. A live-trap was placed at each stake, baited with sunflower seeds, while seeds were also scattered around the trap to speed up attraction of subjects. Traps were set each day at 8:00 AM, and checked each hour thru 3:00 PM. Each trapped subject was removed from the area, toe-clipped for identification, sexed, weighed and held until the last check round before release at the siteof capture. For each trap removed with a subject an empty one was substituted; this procedure was assumed to expedite sampling by reducing social hierarchy effects at various stations.

It was assumed that all subjects in a plot had been captured when unmarked individuals ceased to be captured. It required 15 days to complete sampling in each of the first two plots, and 12 days for the third plot. Simultaneous trapping of plots was impossible because of the lack of a sufficient number of traps, and because of the inability of one trapper to operate the large number of traps.

The sample data were as follows:

Area	No. Individuals	Ratio of M:F	Mean Wt. ⦥	Estimated Density/Acre	Ave. Distance Between Captures
I	28	14:14	95.6 gr (61-127)	28	28.8 (ft.)
11	28	21:7	107.2 gr (83-119)	28	33.3
111	22	8:14	107.4 (93~120)	22	31.9

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<u>Population density</u>:

When the actual gradrat area is increased by one-half the distance between stations, the area sampled was approximately one acre for each plot. Thus the number of individuals captured represent the population density perwacre. The average density of the area on this basis was 26 animals. This figure is high incomparison with sampling in results from Vermont, Pennsylvania and West Virginia using similar methods (other investigators). The density estimates need to be verified by sampling from populations in similar communities before a statement regarding density at the western limits of the species distribution can be safely made. However, present work has indicated an adequate density for ease in studying the basic ecological factors as outlined in the overall cooperative program on this species.

Population structure:

The sex ratios in the various plots were quite different, and no complete explanation for this is possible at present. It may be that in this species social behavior tends to cause unequal distribution of the sexes. The overall sex ratios of males to females was 43:35, which agrees with those ratios obtained for populations in Vermont (Snyder, 1966).

Age distribution could not be predicted from this sample. Some indication from weights obtained during early July of the presence of young of the year was noted, but most animals were of adult weights. It will be necessary to trap throughout the annual activity period to obtain estimates of most structural features, such as breeding period, reproductive rates, recruitment, survival, and individual turnover. The condition of subjects was noted, and all seem to be healthy in so far as general appearance was concerned. However, it is possible that animals in poor condition were not trapped because such may avoid competition by utilizing less optimal times of day for aging. (Trapping was conducted in the evenings for several days but no subjects were ever trapped at that time so it was discontinued).

Behavior:

The only behavior characteristic which was measured was the average distance between successive captures. Animals from the three different plots were very similar on this basis. Recaptive data for calculation of a home range was felt to be insufficient, and the fact that subjects are perhaps influenced by the bait, investigator and trapping schedule contributes considerable question regarding validity of home range data. For example, three animals from the first plot were found later in the III plot, making home range values quite wide in value. In my opinion, the average distance between successive captures has less reason to vary than home range. Since what is desired is a behavioral attribute by which enviornmental influences can be detected over time and space, the more simple measurement appears desirable.

Mineral levels in chipmunk populations:

Subjects have been obtained from the sampling area (not from the plots) for study of the total body levels of selected trace elements. These are frozen' awaiting analysis, it has been found that the total dry weight of these subjects requires a modification of the procedure used for the smaller species, <u>Peromyscus</u> (see section VI, above). The wet ash method becomes too dangerous for the amount of mass. Thus several procedures have been studied by means of which dried subjects could be reduced to a homogenious mixture, small sample of which could be analyzed. While no method has proven completely satisfactory, reduction by means of a ball mill and then a Wiley mill is promising. Once the details are worked out, subjects from Minnesota, Pennsylvania and Vermont can be analyzed with dispatch.

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