AN ABSTRACT OF THE THESIS OF

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Title: THE UPTAKE AND RETENTION OF RADIOCESIUM IN THE GRAY-TAILED VOLE MICROTUS CANICAUDUS MILLER

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A study was performed to assess the uptake and retention patterns of cesium-134 in the gray-tailed vole, Microtus canicaudus Miller. To furnish a realistic source of contaminated food for the voles, wheat seedlings were raised in hydroponic solutions to provide radiocesium labeled wheat clippings. The hydroponic system was designed to provide optimal conditions for cesium uptake by the plants. In nutrient solutions containing about 10 and 20 μCi radiocesium/~ 4 liters, eight-day-old wheat clippings accumulated 18% and 12% of the radioactivity, respectively.

The labeled wheat clippings were fed to voles as a supplement to their normal diet. Under a chronic feeding schedule, the average radioactivity of each serving was 0.04 μCi. Under a single feeding schedule, the average activity was about 0.05 μCi/serving. Radiocesium was also administered to voles via a single, intraperitoneal (IP) injection of ~ 0.9 μCi in 4.0 ml normal saline.

In the chronic feeding study, a fluctuating, whole-body equilibrium was achieved. The data give the apparent indication that equilibrium could have been reached as early as the first several feedings.

The retention of radiocesium by M. canicaudus under the three modes of administration, i.e., chronic feeding, single feeding and IP injection was analyzed assuming exponential elimination rates. "Final" components of radiocesium elimination were arbitrarily assessed from retention curves drawn on semi-logarithmic graph paper.

The y-intercepts (a) and biological half-lives (T_b) derived from linear regressions of the respective final components were: $a = 8.0 \pm 8.6\%$, $T_b = 5.8 \pm 2.4$ days in the chronic feeding study; $a = 52.6 \pm 25.5\%$, $T_b = 1.5 \pm 0.5$ days in the single feeding study and $a = 13.9 \pm 7.1\%$, $T_b = 2.2 \pm 0.2$ days in the IP administration study. The T_b for the chronically fed animals was comparatively longer than the T_b for the single administration mode animals. This suggested that the longer period of equilibration resulted in a larger portion of the body burden being deposited in metabolically less active tissues.

From tissue distribution studies, it was found that cesium was fairly uniform in its dispersion throughout the body. Skeletal muscle, due mainly to its large mass, will apparently become the major repository for radiocesium. This confirms an observation widely found in the literature.

In the single feeding study, careful assessment of the radioactivity transfer from nutrient solution to the wheat seedlings and

the wheat clippings to the voles yielded trophic transfers of 8% (after 5 days exposure) and 53.3% (after 0.5 days exposure), respectively. Clearly, even under optimal conditions for cesium uptake by the wheat seedlings, the "bottleneck" of transfer was from substrate to plant. This finding, in conjunction with the relatively rapid turnover of radiocesium in the vole, makes it unlikely that a vole foraging only occasionally in a contaminated region will develop any significant body burden. However, the results of the chronic feeding study indicate that a vole foraging consistently in such an area may indeed achieve a substantial body burden since larger fractions of radiocesium might then appear in metabolically less active tissues.

The Uptake and Retention of Cesium-134 in *Microtus canicaudus* Miller

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INTRODUCTION

With the advent of the nuclear age, radioactive isotopes of cesium from man-made sources have been introduced into the biosphere. Such sources include nuclear detonations, nuclear research facilities, the current generation of nuclear power reactors and allied industrial activity, e.g., nuclear fuel separations facilities (Anderson *et al.*, 1957; Langham and Anderson, 1959; Langham, 1965; Reichle, Dunaway and Nelson, 1970). Of the cesium isotopes that enter the environment, cesium-137 is of particular interest due to its long radiological half-life, high yield of production from the fission process and relatively high energies of emitted radiation (Davis, 1963). In addition, cesium is a physiological analog of potassium in biological systems and is therefore incorporated into both plant and animal tissues (Relman *et al.*, 1957; Graham, 1958; Russell, 1963; Evans and Dekker, 1966; Stara *et al.*, 1971).

Clearly, radiocesium is a pollutant that may eventually be ingested by man and become an internal hazard. Accordingly, much study has been directed to identifying the possible pathways of radiocesium to man, as well as characterizing the subsequent metabolism in man (Hood and Comar, 1953; Ballou and Thompson, 1958; Wasserman *et al.*, 1965; Fredriksson *et al.*, 1966; LeRoy *et al.*, 1966; Lloyd, 1973). As a consequence, valuable data have been garnered as to the mobility of cesium in soils, plants and animals. Of practical

necessity, much of this information has been gathered with the goal of knowing how it relates to man in his ecological setting as the top consumer of his food chain. Moreover, man is the primary organism of concern in terms of potential radiation hazard.

Much of this earlier work concerned radiocesium metabolism in inbred strains of test animals under the artificial conditions of a laboratory environment. More recently, attention has been given to the behavior of radiocesium in more natural settings. This endeavor included generally delineating the pattern of cesium cycling in natural ecosystems and, more specifically, the trophic transfers of radiocesium in wild species of plants and animals (Gamble, 1971; Sharitz et al., 1975).

The need for such ecological information has become evident with the increase of nuclear power utilization in the United States. Indeed, the paucity of such data have led Reichle et al. (1970) to observe ". . . that considerable ignorance still obtains regarding uptake, assimilation, tissue distribution, turnover rates and equilibrium levels for many taxonomic groups." They further emphasized that more effort should be directed at characterizing environmental factors that may affect radionuclide cycling in particular ecosystems. Their specific concerns were for riparian and coastal areas where they felt power-reactor complexes would most likely be located.

It is clear that additional bioenvironmental information concerning the fate of real or potential radionuclide pollutants in natural ecosystems is desirable. Such an attempt was made for

radiocesium in the work to be described, but before this description, it is well to review the present knowledge of the mode of cesium cycling in the environment.

Radiocesium Mobility in Soils and Plant Uptake and Translocation

When cesium is introduced into soil systems it is usually "tightly bound" particularly by the clay fraction and less so by the sandy portion (Schulz et al., 1960; Fredriksson et al., 1966; Francis and Brinkley, 1976). In this bound condition it is generally not readily available for uptake by plants (Nishita et al., 1956; Romney et al., 1957; Squire and Middleton, 1966). However, under wet conditions as under nutrient solution culture, extensive agricultural irrigation or in natural systems such as shallow ponds or wet meadows, cesium appears to be more available for plant uptake via root absorption (Klechkovsky and Gulyakin, 1958; Pendleton and Uhler, 1960; Bourdeau et al., 1965; Cline, 1969; Myttenaere et al., 1969; Shalhevet, 1973).

Besides textural characteristics, the specific chemical inventory of soils may also dictate the amount of cesium that may be taken up by plants. This has been shown by Graham (1958) in his studies of the primary vegetation populating the contaminated (by low level waste effluent from the Oak Ridge National Laboratory) White Oak Lake bed at Oak Ridge, Tennessee. Graham concluded that the extensive uptake of cesium-137 by Polygonum was due to a "moderately" low supply of available potassium in the sediment.

Fowler and Christenson (1959), in a greenhouse study, showed that as exchangeable potassium in four midwest farm soils increased, the concentration of cesium decreased in lettuce, grass and alfalfa. Support for this finding has been offered from laboratory studies of cesium uptake by young wheat seedlings in a nutrient solution culture (Jackson et al., 1966). On the other hand, Cline (1962) has demonstrated that, in young bean plants, there is no consistent discrimination for cesium against potassium. These studies indicate that even though cesium chemically resembles potassium, other factors come into play when investigating the plant uptake of cesium from either potassium deficient or potassium sufficient soils (Fredriksson et al., 1966).

Stable cesium and ammonium (NH_4) have also been shown to affect cesium uptake in plants. Recently, Myttenaere et al. (1974), working with tomato plants in a nutrient solution culture, confirmed the earlier finding of Nishita et al. (1962) that addition of stable cesium to the nutrient medium enhanced the uptake of radiocesium. In Japan, Tensho and co-workers (1961) concluded that high absorption of trace amounts of cesium-137 by lowland rice could be related to the dominant nitrogen source in the soil. More particularly, they found that nitrogen in the form of ammonium increased the uptake of cesium-137 in lowland rice. With respect to this last finding, attempted confirmation in the laboratory has produced conflicting results. Handley and Overstreet (1961) found that ammonium depressed uptake of cesium-137 in excised barley roots, whereas Minotti et al.

(1965) using young wheat seedlings confirmed the observation that ammonium appears to facilitate cesium uptake.

The organic matter content in a soil may also influence the amount of cesium taken up by plants. In general, soils containing fibrous peat or soils that otherwise accumulate organic matter such as pasturage tend to facilitate uptake of radiocesium by the vegetation (Graham and Killion, 1962; Barber, 1964). In an attempt to explain this phenomenon, Barber (1964) suggested that organic matter may reduce the amount of fixation of cesium by clay minerals present in the soil.

Other factors, including the effects of shading, rhizosphere oxygen, erosion and microbial activity, have been studied as possible influences on radiocesium uptake by plants. Pendleton (1959) noted that shading reduced uptake of radiocesium by submerged plants. Birkle et al. (1965) showed that varying concentrations of rhizosphere oxygen could elicit no consistent response as regards plant uptake of radiocesium. Indeed, they concluded that a complex of many factors was involved when considering uptake of radionuclides in general. Rhizosphere oxygen was part of this complex and even this factor varied according to the specific plant and the type of soil utilized. Erosion and soil loss of cesium-137 in vegetation-covered plots have been shown by Rogowski and Tamura (1970) whereas Malone and Reichle (1973) showed that cesium-134 loss is reduced when there is increased microbial activity in the litter accumulation of a fescue meadow.

Although much effort has been expended on uptake studies of stable cesium and radiocesium by plants under varying conditions both in the field and in the laboratory, a mechanism accounting for root absorption has yet to be thoroughly described (Collander, 1941; Menzel, 1954; Cline and Hungate, 1960; Sharitz et al., 1975). There has been speculation, however, that there are two mechanisms at work. The first mechanism is operative under low concentrations of available cesium while the second mechanism becomes operational only at higher concentrations (Bange and Overstreet, 1960). It further appears that the first mechanism is relatively non-selective in that NH_4, K and Rb may also utilize it (Handley and Overstreet, 1961).

Plants may also be contaminated by radiocesium via direct deposition on foliar structures. For example, weathering of deposited fallout particles, subsequent leaching of radiocesium onto foliar surfaces, followed by absorption through leaf cuticles with translocation throughout a plant provide a well documented sequence describing how radiocesium may be metabolically incorporated into plant tissues via foliar entry (Johnson et al., 1966; Levi, 1966; Aarkrog, 1969; Levi, 1969; Handley and Babcock, 1970).

Clearly, incorporation of radiocesium into plant tissues is dependent on the plant species, soil type, various environmental factors and mode of entry. This is true of the initial absorption as well as the subsequent translocation. Handley and Babcock (1970, 1972) offered compelling evidence for this when they showed that

foliarly applied cesium-137 was virtually immobile in xerophytic woody shrubs (Ceanothus and Adenostoma); whereas in mesophytic crop plants, cesium-137 was quite mobile especially into new growth. They suggested that the low mobility of cesium-137 in xerophytic shrubs after foliar application was due to the cesium being bound at the leaf cuticle.

Of the evidence considered thus far, there appear to be no simple generalizations about cesium mobility in soils or in the subsequent uptake and translocation in plants. An attempt has been made in this short review to delimit some of the more important trends discovered to date. In a discussion of real or potential environmental contamination by radiocesium it would perhaps be more prudent to assess each event independently as there are still not enough data available for meaningful comparisons (Sharitz et al., 1975).

Uptake and Retention of Radiocesium in Animals

Whole-body uptake and retention of radiocesium has been investigated in a variety of animals (Davis, 1963; Stara et al., 1971). Early studies involving laboratory strains of white mice and rats, as well as domesticated farm animals, described the basic radiotracer techniques utilized in later studies (Hood and Comar, 1953; Weeks and Oakley, 1955; Ballou and Thompson, 1958; Izawa et al., 1958; Richmond, 1958). These early investigations concentrated on determining the biological turnover of radiocesium in laboratory

animals with the initial purpose of extrapolating these values to man. In this vein, Richmond (1958) found three components of radiocesium retention in mice after both oral administration and intraperitoneal injection. The three components exhibited biological half-lives of 0.5, 2.4 and 6.6 days, respectively. Studies on radiocesium turnover in domestic farm animals were performed for the purpose of identifying the magnitude of contamination that might occur in potential human food sources (e.g., meat, milk and eggs). Later studies, utilizing laboratory animals, described the effects of age, temperature, sex and lethal doses (i.e., large amounts of radiocesium) on radiocesium retention (McPeak et al., 1966; Miller et al., 1968; Thomas et al., 1968; Lengemann, 1970b). For example, the effect of age has been elaborated upon by several investigators. Using mice, Miller et al. (1968) found that very young (21-30 days of age) and very old (22-32 months of age) mice have smaller effective retention periods than mice of intermediate age. In rats one week of age, Stather (1970) discovered the opposite trend, i.e., there was a long retention period with the elimination rate increasing up to four weeks of age and then gradually decreasing. He explains this phenomenon as due to incomplete kidney function at the younger ages and, therefore, inefficient excretion of cesium. The renal system is the primary route of cesium loss in the body. In examining radiocesium retention in rats from one to twenty-one months of age, Lengemann (1970a) noted a definite increase in radiocesium retention with increasing age. In humans, the biological half-life of

radiocesium has also been found to vary, particularly at the juvenile stage of development (Lloyd, 1973).

Diet composition may further affect cesium retention. For example, it has been widely shown in rats where potassium supplemented diets generally increase cesium excretion (Mraz et al., 1957; Richmond and Furchner, 1961; Wasserman et al., 1963; Johnson et al., 1968). Wasserman et al. (1963) have suggested that this effect may be due to increased water consumption in response to the higher salt level. Thus, a diuretic process is implicated in giving increased cesium excretion. It has further been shown that a potassium deficient diet will increase radiocesium retention in the rat (Richmond and Furchner, 1961).

Investigations of radiocesium uptake and retention in small, wild mammals have centered upon bioenvironmental cycling aspects of the nuclide in natural ecosystems. Such investigations have been motivated not only by ecological reasons but also by radiobiological and potential economic (commercial application) interests. They have utilized a variety of radiotracer methods as well as several different species of wild mammals.

Kaye and Dunaway (1962), as part of a radioecological program at the Oak Ridge National Laboratory (Oak Ridge, Tenn.), determined the cesium-137 bioaccumulation in small mammal populations living in contaminated areas on the Oak Ridge reserve.

Kitchings et al. (1969) performed cesium-134 uptake and retention studies using fallout simulant (artificially contaminated

sand) and radiocesium tagged (externally applied) lettuce leaves. Both field trapped and laboratory born cotton rats (Sigmodon hispidus) were used for the studies. It was found that cotton rats receiving a single dose of radioactive simulant displayed shorter retention half-lives than animals chronically fed contaminated lettuce. Their explanation for this trend was that a single dose does not allow sufficient radiocesium equilibration with "long-term" body pools. Long-term pools being those body compartments (e.g., skeletal or muscle tissue) that requires the longest periods of time to turn over the nuclide.

Biological turnover of radiocesium has been utilized as a tool to predict metabolic and food consumption rates in small, wild animals. Baker and Dunaway (1975) found an apparent correlation between radiocesium retention (as indexed by the biological half-life) and the general metabolic rate in Sigmodon hispidus. The chemical similarity of cesium to potassium in physiological action was suggested as the underlying reason for the correlation, even though cesium is generally not a required element in physiological processes. No correlation was found between radiocesium retention and body size in Sigmodon.

Baker and Dunaway (1969) further found a correlation between the amount of cesium retained by the final loss component and the metabolic rate in both Sigmodon hispidus and Peromyscus leucopus (white-footed deer mouse).

Mathies et al. (1971) determined the rates of Pinus strobus (white pine) seed consumption in Peromyscus leucopus and Blarina brevicauda (short-tailed shrew) by using seeds tagged with radiocesium. This investigation was part of a field and laboratory program attempting to estimate the amount of pine seeds consumed by these species in an oak-hickory forest. An important result of this study was the discovery that the short-tailed shrew was a more prominent consumer of white pine seeds than was previously thought. Thus, the rates of pine seed consumption by both species relate directly to the commercial feasibility of promoting white pine regeneration in an oak-hickory forest.

Clearly, these investigations have produced not only economically important information but also have demonstrated the versatility of radiocesium as a tracer in ecological studies. Furthermore, the use of wild (i.e., outbred) mammals (preferably in their natural habitats) in studies of radionuclide cycling lends a greater legitimacy to the results as opposed to the inherent artificiality of studies utilizing inbred laboratory test animals and controlled laboratory conditions.

Purpose of the Study and Experimental Animal

From this short review of cesium movement in soils, plants and animals, it is clear that the element can be transferred through food chains. Thus, in a radiocesium contaminated environment, those populations of animals that are primary (herbivores) and secondary (carnivores) consumers may be expected to accumulate body burdens

through regular food webs. In light of this, a laboratory investigation was undertaken to identify the general pattern of radiocesium uptake and retention in the gray-tailed vole, Microtus canicaudus Miller.

Goertz (1964) and Maser and Storm (1970) have reviewed the pertinent aspects of the life history of Microtus canicaudus. This species is indigenous to the Willamette Valley of Oregon and to Clark County in southernmost Washington. Cropland regions define the microhabitat of the gray-tailed vole, implying that increased agricultural utilization of land by man has benefited this vole. Gray-tailed voles are small (25-45 g as adults) herbivores. Their food includes clovers, grasses, alfalfa, wild onion and false dandelion. Voles are nocturnal by nature and serve as prey for a variety of carnivores, including weasels, snakes, hawks, owls and coyotes.

Microtus species can be propagated under controlled laboratory conditions. The animals used in this study were from a locally maintained, outbred colony. Under colony (i.e., laboratory) conditions, the voles have been found to be induced ovulators with a gestation period of 21-22 days. This is typically followed by post-partum estrus and mating. Litter sizes average 4-6 animals with sexual maturity achieved as early as five to six weeks of age (Forslund et al., 1975).

Since sufficient numbers of wild voles were available for this study, the attempt was made to simulate, as nearly as possible, a concomitantly realistic food chain demonstrating radiocesium transfer

from substrate to plant and from plant to animal. In reality, the trophic hierarchy consisted of a radiocesium contaminated hydroponic system in which young wheat seedlings were raised. Contaminated wheat clippings were then fed to experimental voles. Wheat clippings were chosen since they would be a realistic source of food for the voles in their natural habitat. Thus, three levels of cesium cycling in a natural ecosystem were simulated, as well as two trophic transfers.

Three different modes of radiocesium intake by experimental voles were evaluated for ecological and metabolic significance. A chronic feeding mode was used to simulate voles living in a radioactively contaminated habitat, whereas a single feeding mode simulated a vole foraging only occasionally in a contaminated area. Intraperitoneal (IP) injection was used to assess possible differences in metabolic turnover of radiocesium arising from this "unnatural" means of introduction as opposed to the natural intake of contaminated wheat clippings. The IP injection also provided a means of achieving data that would be more comparable with data found in the literature since the majority of previous studies utilized either IP injection or oral gavage for radiocesium introduction.

Ancillary studies included: 1) Determining the uptake and translocation of radiocesium in young wheat seedlings grown in a contaminated nutrient solution; 2) Assessing the transfer of radiocesium from the contaminated nutrient solution (i.e., substrate) to the wheat and from the wheat to the experimental voles.

By its nature, this study was an investigation into a terrestrial radioecological problem. The attempt was made to trace the fate of radiocesium through a system that was at least analogous to a natural environ. In this case, any riparian or general habitat having a water level at or above field capacity and containing gray-tailed voles or ecologically equivalent species in the food web would serve as appropriate examples. Voles were well suited for controlled laboratory experiments involving radiocesium uptake and retention for the following reasons:

(1) They are strict herbivores (i.e., primary consumers) and therefore readily accept contaminated plant material.

(2) Their small size and excellent reproductive success in the laboratory allow for minimal maintenance space and care.

(3) Adequate numbers of this wild rodent were available from a locally maintained, outbred colony.

MATERIALS AND METHODS

Method of Hydroponic Wheat Seedling Culture

Since it was known that increased cesium uptake by plants could be realized under moist substrate conditions (e.g., Pendleton and Uhler, 1960), a method of culture utilizing a hydroponic substrate was considered optimal. This also avoided problems of differential soil binding of cesium. Accordingly, a hydroponic system was devised to allow wheat seedlings to absorb, via the roots, enough radiocesium to act as a radioactively "contaminated" food source for experimental voles. The growth of wheat seedlings was "forced" (in hydroponic solution) under greenhouse conditions of 24-25°C and constant light for eight days. The hydroponic system consisted of 1) medium-sized (39 x 29 x 9 cm) plastic, cat litter trays; 2) plastic frames (26 x 37 cm), with fiberglass window screening attached by silicone rubber cement, and 3) perforated aquarium tubing which was connected to a filtered air supply (line compressor jet). Plastic materials were used to reduce cesium adsorption to the equipment itself.

The trays contained the hydroponic solution while the framed, window screening held the wheat seeds during germination and also provided a bedding for the eventual seedling growth (see Figures 1 and 2). Silicone rubber cement was used to facilitate quick and easy removal of the screen from the frame. The perforated aquarium tubing was set directly into the tray and served as an aerating device in combination with the air jet (Figure 2).

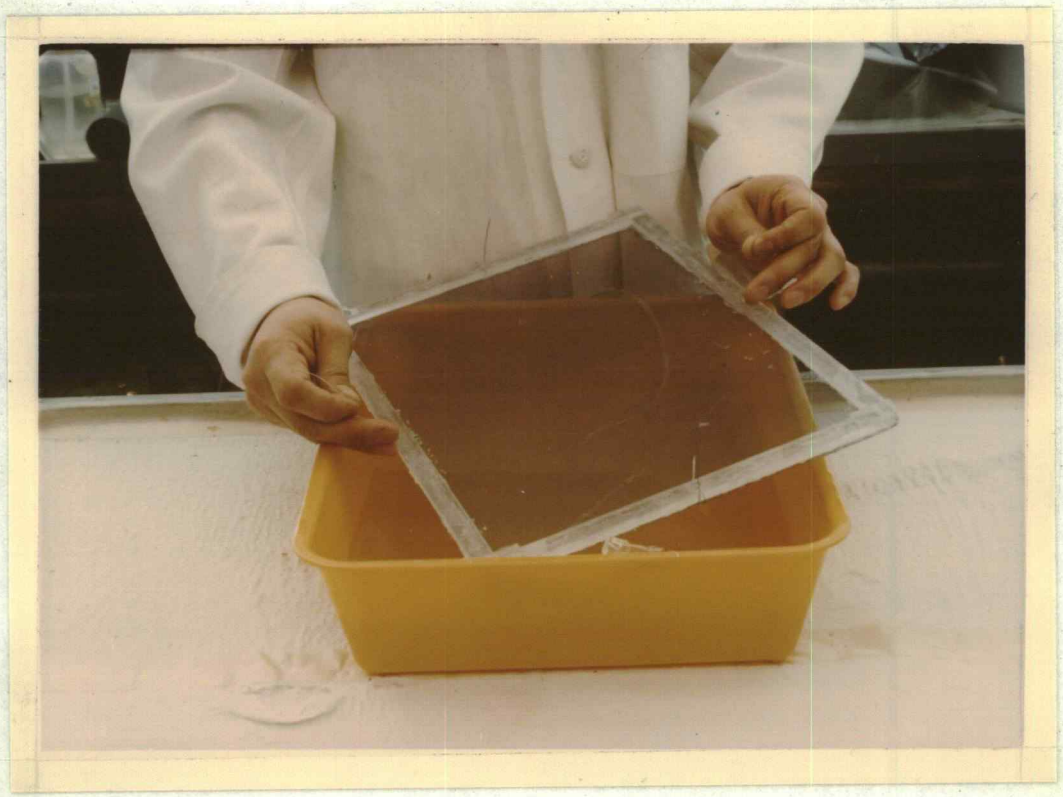

Figure 1. Hydroponic apparatus, single tray.
This picture illustrates the growing tray, framed window screen, aquarium tubing used for aeration and approximate level of water/nutrient solution. Consult the text for details.

Figure 2. Hydroponic apparatus, several trays.
This picture shows how several trays could be aerated simultaneously. The plastic bottle at the top right hand corner filters the air from the air jet (below the bench, not depicted). These young seedlings are the result of four days growth. The plastic sheeting lining the trays was not used during the formal experimentation.

Air from the line compressor was filtered axially through a modified, plastic squirt bottle stuffed with cotton. This makeshift filter adequately removed particles of dust and oil droplets from the line compressor. Several trays could be aerated simultaneously from one air jet by use of two- and three-way aquarium air flow regulators connecting any number of trays in series. Thus, a single crop of eight-day-old wheat seedlings or a series of crops of different ages could be raised according to experimental need.

Hoagland's solution #2 plus supplementary solutions a and b (Hoagland and Arnon, 1938) served as the nutrient media for the wheat seedlings. One liter stock solutions were prepared for the individual macro- and micronutrient salts. Correct proportions of the salts were diluted in a 21 liter Nalgene container which also served as a convenient dispenser.

The growing schedule, as well as the radiocesium application procedure, is described in the following outline:

Day 1

The screened frame was covered with wheat seeds (Triticum sp., Yamhill variety) about 2-3 seeds in depth. The frame was then set in four liters of tap water for 24 hours to allow germination. The tray was covered with a sheet of black polyethylene plastic to facilitate germination.

Day 2

If sufficient germination had occurred, the black plastic sheet was then removed and the frame lifted out of the water. This in turn

was suspended above the surface of the water by hanging wire supports on all four sides of the frame. If insufficient germination occurred, the seeds were soaked for an additional 24 hours.

Day 3

For the chronic feeding study (see Chronic Feeding Study, in this chapter), 3.0 ml of distilled water containing about 10 μCi of cesium-134 was added. In the single feeding study (see Single Feeding Study, in this chapter), 20 μCi was added to the solution in the expectation of achieving a higher activity in the wheat.

After the radiocesium addition, air was bubbled vigorously through the solution for several minutes to assure adequate mixing. Aeration was then terminated and the frame set into the contaminated solution. By this time, the young stems and roots of the majority of the wheat seeds had emerged and measured 0.5 to 1.0 cm in length.

Day 4

Two liters of full strength Hoagland's solution were added. This replaced the amount of water that had been lost by evaporation. With this addition, the level of cesium-134 concentration was approximately 2.5 μCi/liter of final solution. Due to dilution with the water already present, the Hoagland's solution was reduced to about half-strength.

The young sprouts now had rootlets at least one centimeter in length which extended down into the solution. The frame was not set back into the solution but, rather, was suspended as close to the surface as feasible. This was to prevent non-uniform, external

contamination of the young stems. Finally, the aeration regulator was set to give a gentle bubbling and this was maintained until the day of harvest.

Day 5-7

On these days, spot checks were made to assess for adequate seedling growth.

Day 8

The seedlings were 15-20 cm in height and were considered harvestable. They were clipped about 2 cm from the seed and the clippings were thoroughly mixed to nullify any differential uptake that may have occurred. The clippings were subsequently bagged in cellophane and appropriately labeled. Lastly, the entire harvest was weighed to the nearest 0.1 g (wet weight) and three 3.0 g aliquots were assayed for activity in the Packard Armac Liquid Scintillation system (see Radioisotope and Assay Techniques).

In the chronic feeding study, the average count rate of the three 3.0 g aliquots served as a quick determination of the radioactivity of the entire harvest. In the single feeding study, the entire harvest of clippings was apportioned into 3.0 g aliquots and only those aliquots within a uniform range of activity were used for feed purposes.

Because this was an open (i.e., non-sterile) hydroponic system, extensive fungal (mycelium) growths occurred principally at the base of the seedlings. The above growth schedule was empirically determined to give the minimum amount of fungal growth. Extensive

preliminary experiments showed no apparent detrimental effects on the young seedlings from the mycelia.

The pH of the hydroponic solution ranged from 5.0 on Day 1 to 8.5 on Day 8.

To assess the uniformity of radiocesium uptake by individual wheat seedlings, plants were sampled from all four corners and in the center of two trays (Tray #1 and Tray #2). Two plants, one from each tray, were separated into roots, coleoptile, stem, old leaf and new leaf. The roots were thoroughly rinsed with distilled water to eliminate adsorbed radiocesium and blotted "dry." Similar structures from both trays were combined and weighed to the nearest 0.1 mg. Following this, the radioactivity of each anatomical structure per sampling location was then determined. Therefore, differences in uptake per plant structure per sampling location could be simultaneously assessed.

A similar sampling procedure was utilized in an intra-tray study. In this case, the percentage of activity acquired by each anatomical part was compared between Tray #1 and Tray #2. Five plants from each tray were selected and dissected, according to the above method, in each tray. These plants were taken from the corners and the center of the tray. Similar parts from the five plants were subsequently combined, weighed and assayed for radioactivity.

Animal Handling and Maintenance

The experimental animals were from an outbred (siblings and first cousins were not mated) colony maintained by Dr. Larry G. Forslund at Oregon State University (Department of General Science). The original wild stock was trapped in Benton County, Oregon in 1973 (Tyser, 1975). Juvenile voles were weaned at 18 days and group housed (five animals per cage) until sexually mature (see Tyser, 1975, for detailed elaboration of the housing conditions). At this point, males between 10-12 weeks of age were randomly chosen for experimental use. The average initial weight of all the experimental voles was 37.1 g. The weights ranged from 29 to 45 g. Females were not tested as it was felt the estrous cycle might affect cesium metabolism.

Experimental voles were individually housed in standard, hanging, wire-bottomed cages (17 x 18 x 23 cm). Hardware cloth (0.5 cm mesh) was placed over the cage bottoms to provide suitable footing for the voles but still allow urine and feces to pass through. Upholsterer's cotton was provided for cover and nesting purposes.

Food in the form of Oregon State University rat and rabbit chows (from the Small Animals Resources Laboratory, OSU) and water were given ad libitum. Other maintenance conditions included a photoperiod of 16 hours light-8 hours dark and an air temperature of 20-22°C.

Experimental animals were acclimated for at least one week under the above living conditions. During this period, non-radioactive 3.0 g portions of wheat clippings were placed into the cage on at least

two occasions. This allowed the voles to adjust to this new food and permitted the experimenter to determine whether or not any of the voles would reject the clippings. This food was offered in the late afternoon in both the acclimation and experimental time periods, in deference to the nocturnal nature of the voles. Twelve hours later, it was found that few of the proffered clippings had fallen through the wire mesh and that the majority had been devoured by the voles. It was also noted that the voles would eat both fresh and frozen clippings.

For purposes of transport to the radioactive counting facility (see Radioisotope Assay Techniques), the voles were individually contained in black, plastic boxes whose lids were perforated for aeration. False floors constructed of hardware cloth (0.5 cm mesh) were added to these carrying boxes so that feces and urine could be passed without contaminating the animal. Animals were hand carried to lessen the trauma of transport and handling.

At each radioassay session, the weight of each test vole was recorded to the nearest 0.1 g. This was done so that activity measurements could be reduced to a per gram basis and also to monitor the general health of the animals over the experimental time regime. Every whole-body assay session included all experimental animals, even those designated for early sacrifice (see Experimental Protocol, part b). Thus, all voles received the same handling up to the appointed time of sacrifice.

Since the voles would actually handle contaminated wheat clippings in feeding, as well as excrete radioactive urine, the possibility of external contamination was considered. To check on this possibility, the radioactivity levels of the right forepaw and the penis from five of the animals in the chronic study (see Experimental Protocol, part c) were compared to controls. No significant activity of these parts above the background level was detected in the comparison.

Radioisotope Assay Techniques

In radiotracer experiments involving cesium, cesium-134 is generally the isotope of choice. The shorter physical half-life of about two years contrasts sharply with the approximate 30 years half-life of cesium-137. This makes the former nuclide more desirable in light of possible accidental spillage and the consequent long-term contamination of laboratory work areas. In its decay scheme, cesium-134* emits a beta particle with a maximum energy of .662 MeV and prompt gamma rays with principal energies near 0.6 and 0.8 MeV.

A Packard Armac (Model 446) liquid scintillation detector system was used for the in vivo, whole-body radioassays, as well as for radioassays of the wheat clippings. The Armac counting chamber is a horizontal cylinder (with a volume of 1800 cc) fitted with a plastic sleeve and offers ample space for a vole.

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*Cesium-134 used in this study was in the chloride (CsCl) form. Specific activity = 107 mCi/mg. Concentration of this stock solution = 5 mCi/ml in a 3 ml volume. Source: New England Nuclear Corp.

In order to maintain a reproducible counting geometry while in the chamber, each vole was placed in a two ounce, plastic vial closed with a screw cap. The cap and bottom were perforated for ventilation. In general, most of the voles fit snugly in these vials although several could make a 180° turn while inside. Each animal was assigned his own individual vial to eliminate the possibility of cross-contamination.

After a vole was placed in its vial, the latter was supported on a plastic holder that was then inserted into the counting chamber. This holder oriented the vole such that it was both co-axial to the cylinder and at the center of the medial, horizontal plane. This arrangement placed a vole at the center of the counting chamber at each assay. The aliquots of wheat clippings were treated in the same fashion except that the loose clippings were placed in cellophane bags prior to the assay.

Relative counting standards containing defined amounts of the stock cesium-134 solution were prepared to correct for physical decay of the radionuclide over an experimental time course. Thus, at each assay session, the appropriate standard was also counted so that a correction factor for radioisotope decay and any shift in instrumental efficiency could be determined relative to the beginning of the experiment. By empirically assessing the largest value of the ratio of the square of the sample (standard) count rate to the background count rate, optimal counter settings were determined as follows: window = 175 to 650 (arbitrary units); gain = 6%.

Counting efficiencies of 44% and 39% for the wheat and vole geometries, respectively, were determined through the use of an absolute counting standard. This standard was prepared using a highly purified and standardized concentration (± 1.1%) of cesium-134.*

Both the relative and absolute counting standards were made for the voles, by uniformly applying the cesium-134 solution to a piece of nylon sponge that was pre-fitted to a two ounce vial of the same type used to contain the test voles. Analogously, the wheat clippings geometry was approximated by cutting strips of paper towelling about the same length and mass of a typical aliquot. The strips of paper were uniformly labeled with radiocesium and then double-bagged in cellophane.

To compensate for possible increases in detector background due to naturally occurring, radioactive potassium-40 in the living samples, both voles and wheat clippings were assessed for radioactivity. Four control (i.e., non-radiocesium treated) animals of the same age class and weight range as the experimental animals were repeatedly assayed over a five-week period to determine an average organismal background count rate of about 1400 cpm. This correction factor was used to correct all the net activity measurements in the chronic and single feeding studies. In the IP injection study, two assays of each test animal were performed prior to injection. The average count rate of

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*Obtained from The Radiochemical Centre, Amersham, England. Concentration: 13.41 μCi/g of solution in 5.0097 g.

these two assays was used to correct individual animal background due to potassium-40. Radioassays of nonradiocesium treated wheat clippings revealed no significant radioactivity above natural background so that no correction was necessary.

The radioactivity measurements of whole organs and tissue samples from the voles and the major anatomical parts of eight-day-old wheat seedlings were made with the Packard Auto-Gamma (Model 5017) system. The detector in this unit consists of a 3" x 3" NaI (Tℓ) well crystal. Samples were placed at the bottom of plastic tubes (1.3 x 17.0 cm) which were then capped to prevent spillage.

Relative and absolute counting standards were also fashioned for use in the Auto-Gamma system. The standards were made by uniformly applying a known volume cesium-134 on a small piece of nylon sponge. The sponge was placed at the bottom of a counting tube so that it approximated a typical sample rising two centimeters from the bottom of the tube.

Using the relative counting standard, optimal counter settings were found to be: window = 290 to 450 (arbitrary units); gain = 20%. A counting efficiency of 30% for cesium-134 was determined using the absolute counting standard.

Control tissues and organs of the vole (see Experimental Protocol, part b) were dissected out of six nonradiocesium treated animals of the same age class and weight range as experimental animals. These tissues were then assayed in the Auto-Gamma system. No detectable activity above natural background was found, rendering correction for

potassium-40 unnecessary.

Data Reduction

The net (i.e., corrected for natural background) whole-body count rate at any time x was corrected for physical decay of the radionuclide. This was accomplished by concurrently measuring, at each assay session, the count rate of the relative standard. Thus:

$$\text{Decay Correction Factor} = \frac{S_{t_o}}{S_{t_x}}$$

where S_{t_o} = relative standard count rate at time 0

S_{t_x} = relative standard count rate at time x

Whole-body (WB) activity at t_x (corrected for physical decay) was expressed as a percentage of the initial (t_o) whole-body activity given as:

$$\%WB_{t_x} = \frac{WB_{t_x}}{WB_{t_o}} \times 100$$

Radioisotope retention is generally thought to reflect differing rates of exponential loss from various body compartments. In general, semi-logarithmic plots of percent initial whole-body activity vs. time may show from one (i.e., a straight line) to many components. A single component of radiocesium retention in the vole body may be described as an exponential function of the form:

$$A_t = A_o\, e^{-kt}$$

where

A_t = activity at time t

A_o = activity at time 0

e = base of the natural logarithm

t = time

k = elimination constant

If several components are resolved, each component is described by the above relationship and then summed to give A_t.

The biological half-life (T_b) of a retention component is defined as the time required to reduce the body burden of the radionuclide by a factor of one-half purely by biological elimination. To determine the biological half-life of the component, we set:

$$\frac{A_t}{A_o} = e^{-kt} = 0.5$$

thus

$$T_b = t$$

then

$$\ln 0.5 = -kT_b$$

or $$\frac{.693}{k} = T_b$$

The elimination constant k may be determined from a linear regression of a semi-logarithmic plot of percent whole-body retention vs. time (days) for a single retention component. This analysis is based on the method of least squares and results in the equation for a straight line as would be described on semi-logarithmic paper.

From this equation, the slope defines the elimination constant and is given as:

$$k = \frac{\%\ \text{of initial whole-body activity eliminated}}{\text{day}}$$

while, the y-intercept (a) reveals the relative amount (percentage of initial whole-body activity) of activity contained in the component.

A linear regression is performed for each animal in the test group to determine the mean elimination coefficient ($\bar{k}$). The standard deviation (s_k) may then be calculated for the test group. Given $\bar{k}$ and s_k, the standard deviation of the biological half-life (s_{T_b}) may be obtained from the following equation:

$$s_{T_b} = \sqrt{\frac{(.693)^2\ (s_k)^2}{(\bar{k})^4}}$$

The use of the exponential model for describing whole-body retention of radiocesium (or any radionuclide) has some inherent problems. For example, a retention curve that exhibits a continuously changing slope is indicative of a multicomponent system and resolution into linear portions (i.e., separate components) by "eye-fitting" is quite subjective. Further, when more than one retention component is resolved (i.e., in a multicomponent curve) there is sometimes difficulty in associating a specific component with a single physiological compartment. In the specific case of radiocesium retention in small mammals, the body compartments that participate in the

retention curve still lack definite descriptions (Thomas et al., 1968). Furthermore, the resolution of a retention component does not necessarily imply that only one body compartment has been described. Instead, a single component may represent several body compartments emptying at a single rate.

Comar (1955) has succinctly discussed another problem and that is, in multicomponent systems, even careful least squares analyses may not separate closely related components. Thus, one graphic component may mathematically describe several physiological components, particularly if the elimination coefficients are extremely close. Van Liew (1962) has reiterated this and warned against interpreting a multicomponent retention pattern as the sum of only two or three components. Clearly, there is much validity in this warning since a multicomponent curve most likely reflects a continuum of exponential operations.

Experimental Protocol

a. Introduction

The study was divided into three separate investigations: a chronic feeding study, a single feeding study and an intraperitoneal injection study. Each investigation adhered generally to the following protocol:

1. Radiocesium was administered under specific constraints of time and amount.

2. Whole-body in vivo radioassays were made at regular intervals.
3. Whole-body uptake (when applicable) and retention patterns were identified and resolved graphically if possible.
4. The period of radioassay was continued until background levels were reached.
5. In each investigation, serial sacrifices were performed and selected tissues and organs removed.

b. Radiocesium Distribution Studies

Serial sacrifices with attendant organ and tissue excisions were performed to identify cesium distribution in the vole over time. The following whole organs were excised: kidney, spleen, liver, brain, lungs, heart, testes, and eyes. The alimentary tract from the base of the esophagus to the anal region of the rectum was also removed and divided into the stomach, small intestine and large intestine including cecum. Tissue samples in the form of the gastrocnemius muscle and the femur of the left hind leg were also analyzed for activity. A minimum of 30 µl of blood was extracted from each animal either by orbital sinus puncture or by partial ablatement of the heart. The blood was drained into heparinized hematocrit tubes and separated into plasma and cell fractions by centrifugation. The tissue remaining after dissection was termed the residual carcass. The activity of the residual carcass was determined by subtracting the combined activity of the excised organs and tissue samples from

the whole-body activity at the time of sacrifice.

All tissues, except for the blood fractions, were oven dried to a constant weight at 80°C for a minimum of 36 hours. At the end of the drying period, the tissues were immediately weighed to the nearest 0.01 g and assayed individually for radioactivity.

c. Chronic Feeding Study

Chronic feeding of cesium-134 labeled wheat clippings to voles was initiated to achieve a radiocesium equilibrium in the various body compartments. Specifically, 21 voles were fed 3 gram portions of labeled wheat clippings 4 times a week. The feedings were scheduled such that they occurred on two successive days with alternate one and two day lag times throughout the week.

In vivo radioassays were made twice a week. The first assay was performed 12 hours after the initial feeding. Subsequent assays were performed following the second successive feeding and 12-18 hours were allowed for ingestion of the proffered clippings. These assays revealed when the radiocesium levels in the voles reached an equilibrium state.

When an apparent equilibrium was achieved, the diet of radioactive wheat clippings was discontinued and replaced with a commensurate diet of nonradioactive clippings according to the same feeding schedule. The last radioactivity assay of the uptake portion of this study was taken as the initial body burden in the subsequent retention study.

Groups of five animals were sacrificed at 1, 3 and 50 days after the last radiocesium feeding. Of the 21 original test animals, 11 were whole-body assayed until background activity levels were reached. The other 10 voles experienced the same handling and feeding schedule but 5 each were sacrificed on Days 1 and 3.

d. Single Feeding Study

In this study, 30 voles were offered a single, 3 gram meal of labeled wheat clippings. Twelve hours were allowed for ingestion before whole-body assays were initiated to determine the retention pattern. Seven animals were radioassayed to distinguish the whole-body retention pattern under this mode of administration.

Serial sacrifices were performed at 1, 3, 14, and 25 days post feeding utilizing the other 23 voles. On Days 1 and 3, groups of 8 animals were sacrificed with 4 of each group having the stomach, small intestine and large intestine flushed (with physiological saline) to remove the contents. The remaining four animals had their gut contents left intact. On Days 14 and 25, groups of 7 animals were sacrificed with those animals used for the whole-body retention study serving as the last sacrifice group.

e. Intraperitoneal Injection Study

In this study, 18 voles were injected intraperitoneally with 0.4 ml normal saline containing 0.9 μCi of cesium-134. Injection was via a 27 gauge (0.5") needle attached to a 1.0 cc tuberculin syringe.

One hour was allowed for distribution throughout the body before commencing the first whole-body assay. Eight animals were designated for the whole-body retention study.

Five animals (i.e., from the other ten) were used for early sacrifices three and seven days post injection (DPI). Five of the whole-body assayed animals were randomly selected as the final sacrifice group at 19 DPI.

RESULTS

Radiocesium Uptake and Translocation in Eight-Day-Old Wheat Seedlings

Thirty-two trays of wheat seedlings were grown for eight days in the chronic feeding study. The results of cesium-134 uptake from the hydroponic solution by the wheat clippings are found in Table 1.

The average activity of the three-gram aliquots sampled from each harvest was 8.18×10^4 dpm or about 0.04 μCi per aliquot. Thus, each vole received this much activity per feeding. The mean net activity per gram of clippings was 2.72×10^4 dpm. The product of this value and the average harvest weight of 147.2 g gives a mean net activity per harvest of about 1.8 μCi of cesium-134. Therefore, the average uptake per harvest was about 18% since the hydroponic solution in each tray (i.e., substrate) contained approximately 10 μCi of cesium-134.

Table 1 also indicates that the variance encountered can be quite large when using this hydroponic growing system. This is reflected in the sizeable coefficients of variation generated for each calculated value.

In the sole tray of wheat seedlings used for the single feeding study, 137.5 g of clippings were harvested. From this harvest, thirty 3.0 g aliquots were selected in the range of 10.1×10^4 to 12.1×10^4 dpm. The average activity of the thirty aliquots is found in Table 2.

Table 1. Cesium-134 activity* of harvested wheat clippings used in the chronic feeding experiment [N = 32 trays]

	Mean	S. D.	Coefficient of Variation (%)
Net activity of 3.0 g aliquots (10^4 dpm)	8.18	1.71	21
Net activity per gram harvest (10^4 dpm)	2.72	0.57	21
Harvest weight (g)	147.2	51.9	35
Activity per harvest (μCi)	1.8	0.6	33
% uptake per harvest	18	6	33

*Radioactivity per tray was approximately 10 μCi cesium-134 as the chloride. Concentration in the substrate (hydroponic nutrient solution) was about 2.5 μCi/l.

Table 2. Cesium-134 activity* of 30 selected 3.0 g aliquots used in the single feeding experiment. The values describe 90.0 grams of clippings subsequently fed to 30 voles.

	Mean	S. D.	Coefficient of Variation (%)
Net activity of 3.0 g aliquots (10^4 dpm)	11.34	0.60	5.3
Net activity per gram (10^4 dpm)	3.80	0.20	5.0

*Radioactivity was in the form of approximately 20 μCi cesium-134 as the chloride. Concentration in the substrate (hydroponic nutrient solution) was about 5.0 μCi/l.

As was expected, the average activity of 11.34 x 10^4 dpm per aliquot was higher than the average activity of the same in the wheat clippings used during the chronic study (cf. Table 1), but not proportionate to the administered cesium-134 activity. Further, since the aliquots were in a selected range of activity, the coefficient of variation was also smaller (Table 2 cf. Table 1). Therefore, in the single feeding study, each vole was given a fairly uniform dose of about 0.05 μCi of cesium-134 per three-gram feeding.

The results of the sampling procedure (see Materials and Methods for details) used to determine the uniformity of radiocesium uptake and translocation by wheat seedlings are found in Tables 3 and 4. Specifically, the uniformity of radiocesium uptake by individual wheat seedlings per sampling location (i.e., the corners and the center of a tray) is described in Table 3. The activity of all the above-ground structures was fairly uniform regardless of the sampling location. At sampling locations 4 and 5, the roots contained almost two and three times, respectively, the activities found at locations 1-3.

The proportion of total activity acquired by these anatomical structures, in the two test trays, is found in Table 4. The roots in both trays acquired the majority of the activity. The combination of old and new leaves contained between 20%-23%, while the stem and coleoptile acquired 6%-7% and 2% of the total activity, respectively.

Table 3. Activity of plant parts at sampling locations 1-5. Inter-tray study.*

	(x 10^4) dpm/gram wet weight				
	1	2	3	4	5
Roots	34.3	32.3	29.8	51.2	89.3
Coleoptile	2.7	2.4	1.8	1.9	1.2
Stem	2.3	2.6	1.7	1.7	2.5
Old leaf	3.6	2.7	2.6	2.8	3.1
New leaf	3.3	2.6	2.4	2.8	3.7

*2 plants, 1 from Tray #1 and 1 from Tray #2, were used per location.

Table 4. Comparison of eight-day-old wheat seedling translocation of cesium-134. Intra-tray study.*

	% Total activity (Given as $\frac{\text{dpm/part}}{\text{dpm of total plant}}$)	
	Tray #1	Tray #2
Roots	72	68
Coleoptile	2	2
Stem	6	7
Old leaf	9	11
New leaf	11	12

*5 plants at sampling locations 1-5 were used per tray.

Radiocesium Uptake and Retention by Microtus canicaudus Under a Chronic Feeding Schedule

The uptake of radiocesium by eleven voles chronically fed radiocesium labeled wheat clippings is presented in Figure 3. The graph indicates that an equilibrium, oscillating around 2000 dpm/g, was reached quickly in the voles. The feeding of the labeled clippings ceased on Day 53 of the experiment and this level of activity was taken as the initial body burden in the retention study that followed.

Figure 4 is a graphic presentation of the temporal retention pattern in the same eleven voles chronically fed cesium-134. The plotted points represent the mean activity expressed as a percentage of the initial whole-body activity. Selected standard deviations are also presented to illustrate the variance encountered. Whole-body activity fell below the minimum detectable level at 21 days.

The data between 7 through 21 days after the period of radiocesium feeding were assessed to represent the final component of radiocesium loss from the voles. A linear regression was thus performed for this portion of the curve. The assessment was based on a subjective eye-fit as well as on the coefficient of correlation for each animal. The average "goodness of fit" for the eleven animals was $r = .83$ which indicates a good correlation in agreement with the subjective eye-fit. The biological half-life for this component was 5.8 ± 2.4 days. The y-intercept revealed that $8.0 \pm 8.6\%$ of the initial body burden was contained in this component.

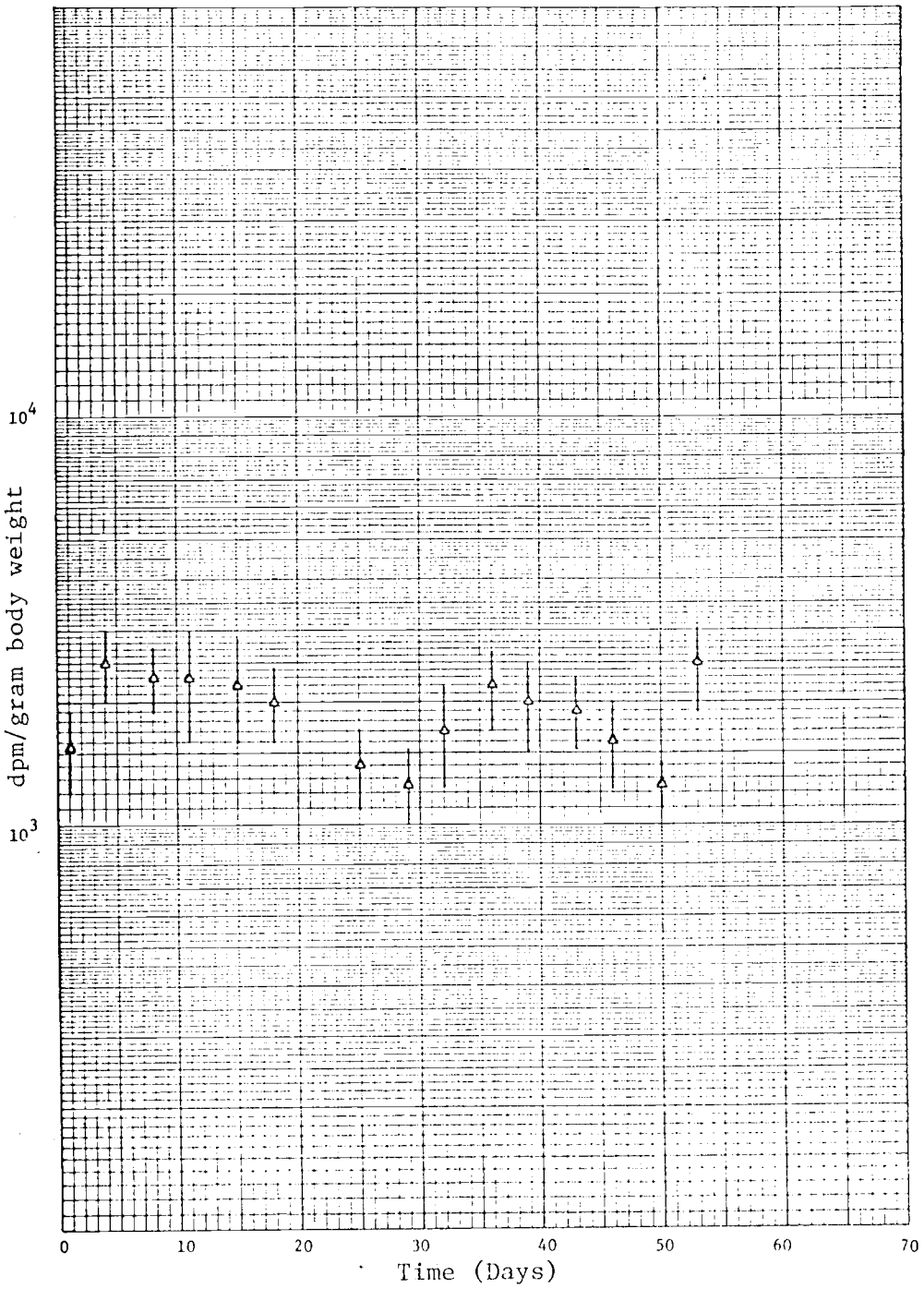

Figure 3. Uptake of cesium-134 in Microtus canicaudus under a chronic feeding schedule. (N = 11).

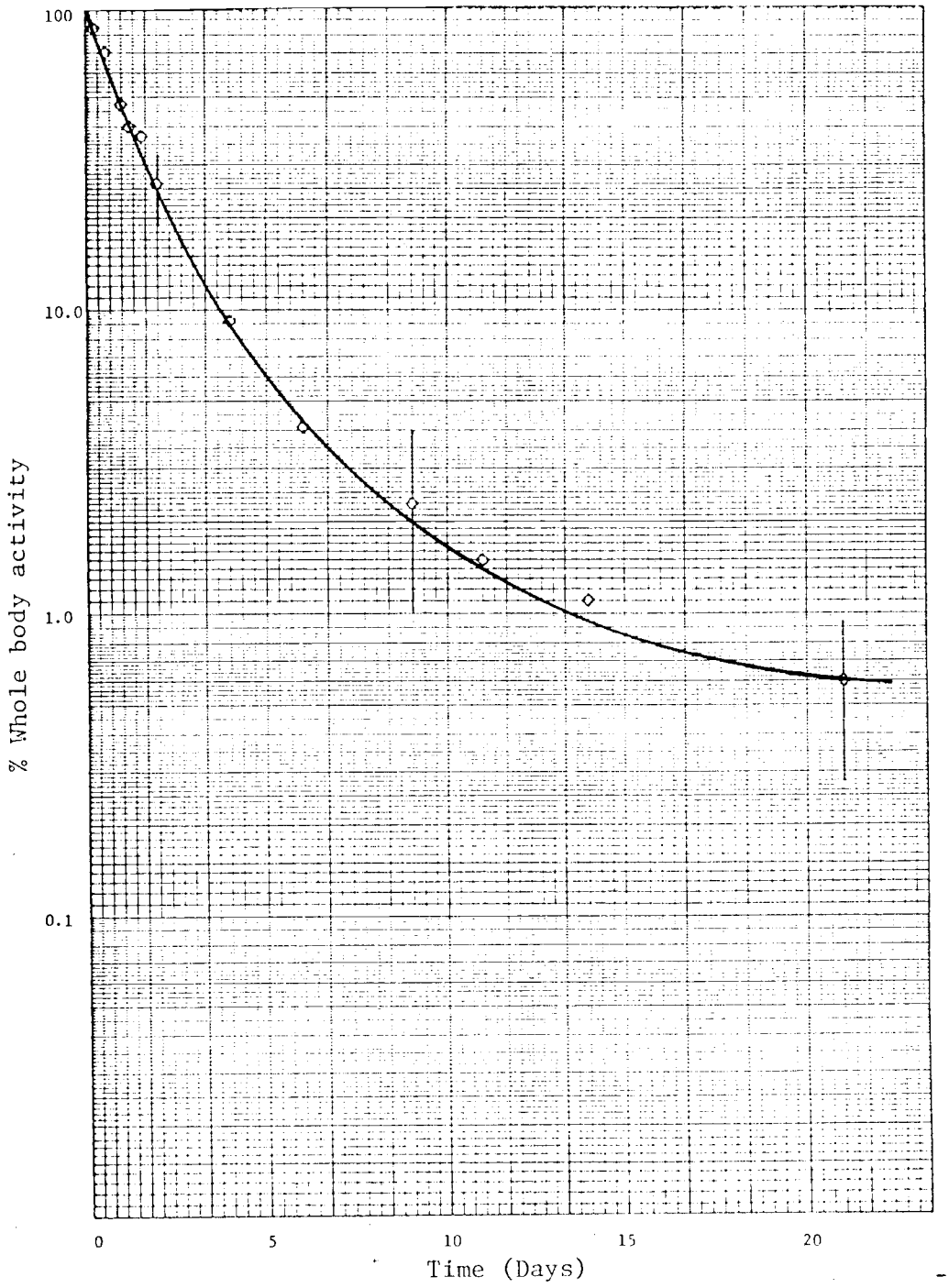

Figure 4. Retention of cesium-134 under a chronic feeding schedule, Microtus canicaudus (males). (N = 11).

Distribution of Radiocesium in Microtus canicaudus After Chronic Feeding

Tables 5 and 6 describe the distribution of radiocesium in selected tissues of five voles on Days 1 and 3 during the whole-body retention portion of the chronic feeding study. Animals sacrificed on Day 50 produced no tissues that were above the minimum detectable activity. In Table 5 the activities found in the excised tissues or tissue samples are expressed as total net activity and as the percentage of the whole-body activity at the time of sacrifice. In Table 6, the distribution of radiocesium is given as the activity per gram dry weight of tissue and also as the activity per gram dry weight divided by the whole-body weight. This last expression was calculated to take into account the variance interjected by differing tissue masses due to larger or smaller animals.

In Table 5, the largest net activities and percentages of whole-body activity on Day 1 were found in the liver, small intestine, large intestine and stomach. On Day 3, these four organs declined in activity while the brain, testes and eyes contained comparable amounts of net activity and acquired a larger percentage of the whole-body activity. The net activity of the small intestine, large intestine and stomach declined appreciably on Day 3.

In Table 6, the activity per gram of dry tissue on Day 1 showed the kidney to be the highest followed by the testes, liver, heart, lungs and muscle. On Day 3, the activity in the kidney was still appreciable but was exceeded by the brain, testes and eyes. The activity per gram of liver, lungs, heart and muscle were drastically

Table 5. Selected tissue and organ distribution of cesium-134 in Microtus canicaudus after chronic feeding. Values represent the mean and standard deviation of five animals.

	Net activity (x 10^3 dpm)		Percent of whole-body activity at the time of sacrifice	
	Day 1	Day 3	Day 1	Day 3
Whole body	78.6 ± 14.1	23.6 ± 7.0	100	100
Kidney	0.7 ± 0.2	0.1 ± 0.1	0.9 ± 0.2	0.5 ± 0.1
Spleen	0.1 ± 0.03	0.03 ± 0.01	0.1 ± 0.03	0.1 ± 0.03
Liver	4.5 ± 1.7	0.5 ± 0.01	5.5 ± 1.4	2.3 ± 0.3
Brain	0.7 ± 0.1	0.6 ± 0.2	0.9 ± 0.1	2.7 ± 1.4
Lungs	0.3 ± 0.1	0.1 ± 0.2	0.4 ± 0.1	0.5 ± 0.9
Heart	0.4 ± 0.1	0.04 ± 0.03	0.5 ± 0.1	0.2 ± 0.1
Testes	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	1.7 ± 0.3
Eyes	0.1 ± 0.02	0.3 ± 0.5	0.1 ± 0.03	1.9 ± 3.4
Small intestine*	2.7 ± 0.8	0.4 ± 0.1	3.4 ± 0.5	1.7 ± 0.5
Large intestine*	4.8 ± 2.2	0.6 ± 0.3	6.2 ± 3.1	3.7 ± 0.7
Stomach*	1.4 ± 0.2	0.2 ± 0.1	1.8 ± 0.4	1.0 ± 0.3
Muscle	0.4 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.5 ± 0.2
Bone	0.1 ± 0.03	0.03 ± 0.01	0.1 ± 0.03	0.1 ± 0.01
Residual carcass†	61.8 ± 11.1	19.3 ± 5.8	78.6 ± 3.2	84.3 ± 4.5

*Contents included.
†Calculated value.

Table 6. Selected tissue and organ distribution of cesium-134 in Microtus canicaudus after chronic feeding. Values represent the mean and standard deviation of five animals.

	dpm/gram dry weight ($\times 10^3$)		dpm/gram dry weight / body weight ($\times 10^3$)	
	Day 1	Day 3	Day 1	Day 3
Kidney	22.6 ± 7.1	3.0 ± 3.3	0.7 ± 0.2	0.1 ± 0.1
Spleen	8.5 ± 3.3	1.8 ± 1.8	0.2 ± 0.1	0.1 ± 0.1
Liver	14.0 ± 4.8	1.4 ± 0.5	0.4 ± 0.2	0.04 ± 0.02
Brain	6.3 ± 2.0	4.0 ± 1.4	0.2 ± 0.1	0.1 ± 0.1
Lungs	11.0 ± 7.9	2.2 ± 3.2	0.3 ± 0.2	0.1 ± 0.1
Heart	11.2 ± 5.4	1.0 ± 0.8	0.3 ± 0.2	0.03 ± 0.03
Testes	18.3 ± 2.2	10.7 ± 5.3	0.6 ± 0.1	0.3 ± 0.2
Eyes	7.2 ± 3.1	19.4 ±25.3	0.2 ± 0.1	0.6 ± 0.7
Small intestine*	19.8 ± 7.8	1.9 ± 0.8	0.6 ± 0.2	0.1 ± 0.03
Large intestine*	13.9 ± 3.3	1.8 ± 0.8	0.4 ± 0.2	0.1 ± 0.03
Stomach*	10.0 ± 4.7	1.0 ± 0.4	0.3 ± 0.2	0.03 ± 0.01
Muscle	10.3 ± 3.8	1.8 ± 1.7	0.3 ± 0.1	0.1 ± 0.04
Bone	3.7 ± 1.7	0.4 ± 0.2	0.1 ± 0.1	0.01 ± 0.01

*Contents included.

reduced while the activity in the eyes doubled. Bone showed very little activity and the blood cells and plasma fractions showed no activity above background either day.

The same trends for Days 1 and 3 were found when the activity per gram of dry tissue was divided by the body weight (Table 6).

The calculated activity left in the residual carcass is found in Table 5. The total activity in the residual carcass declined by about two-thirds from Day 1 to Day 3 although its percentage of the whole-body activity increased from 78.6% to 84.3%, respectively.

Retention of Radiocesium in Microtus canicaudus After a Single Feeding

Figure 5 illustrates the temporal retention pattern found in seven voles after a single feeding of contaminated wheat clippings. The values are expressed as the average percentage of the initial whole-body activity and selected standard deviations indicate the variance. Whole-body activity fell below the minimum detectable level at eleven days.

A final retention component was subjectively assessed to exist between Days 4-11. The average goodness of fit for the linear regression was $r = .97$ and the calculated biological half-life was 1.5 ± 0.5 days. The y-intercept indicated that $52.6 \pm 25.5\%$ of the initial body burden was found in this component.

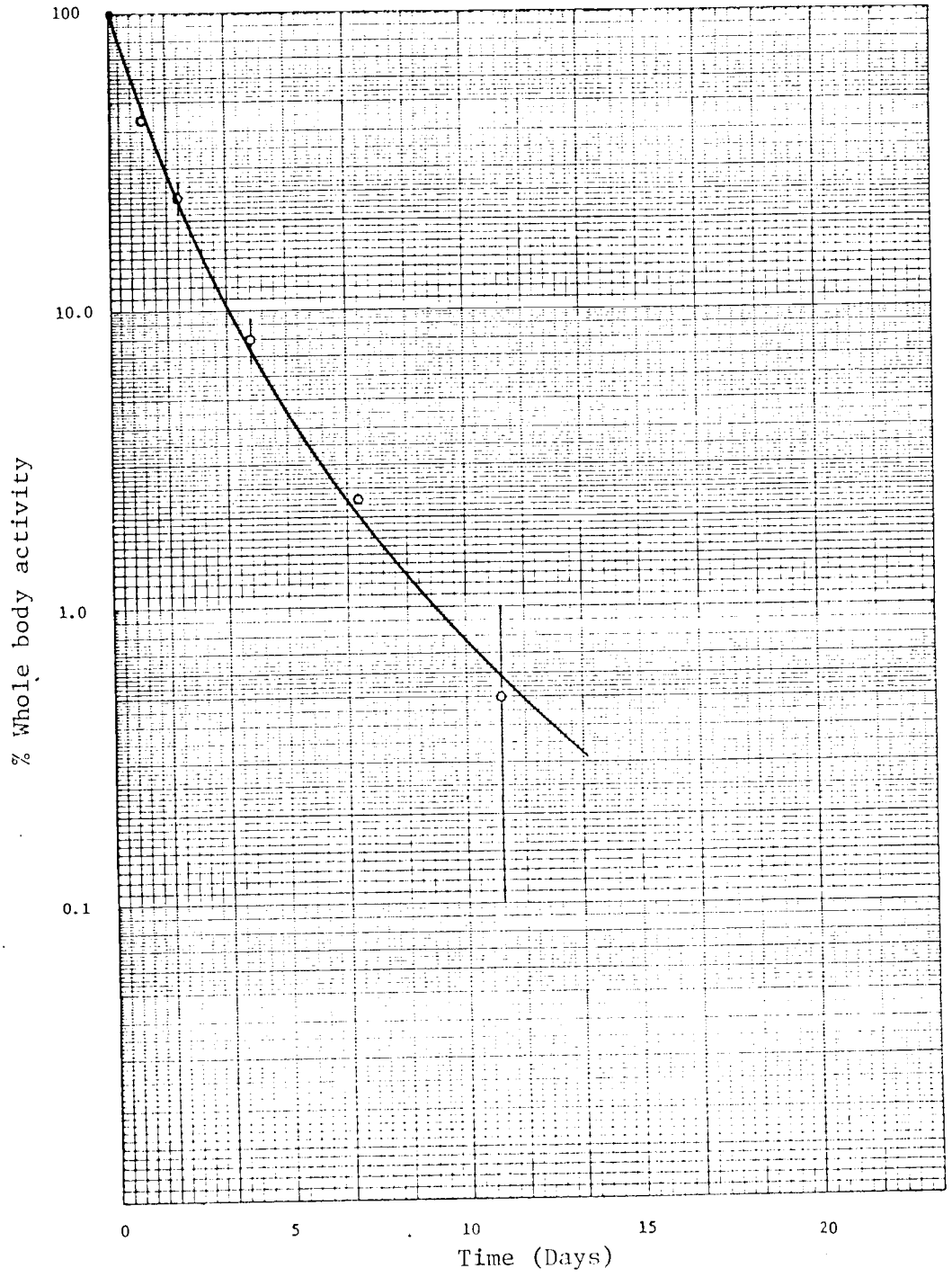

Figure 5. Retention of cesium-134 under single feeding schedule, Microtus canicaudus (males). (N = 7).

Distribution of Radiocesium in Microtus canicaudus After a Single Feeding

The selected tissue and organ distribution of radiocesium in eight voles for Days 1 and 3 after a single feeding of contaminated clippings is found in Tables 7 and 8. The sacrifices on Days 14 and 25 showed no tissues above the minimum detectable level of activity.

The expressions of activity in Tables 7 and 8 are the same as in Tables 5 and 6 of the chronic feeding study.

In Table 7, liver, small intestine, large intestine and stomach again showed the highest net activity on Day 1 (cf. Table 5 of the chronic feeding study). Kidney and spleen contained moderate activity while the remaining tissues were comparatively low. On Day 3, kidney, liver, small intestine, large intestine and stomach all showed a significant decline in activity while the brain and testes remained nearly unchanged. An increase in net activity was seen in the eyes from Days 1 to 3 in Table 7.

The flushed guts declined in net activity to about one-third of the intact guts on both days (Table 7). The percentage of whole-body activity displayed by kidney, liver, lungs, heart, small intestine, large intestine and stomach declined from Day 1 to Day 3. Spleen, brain, testes, eyes and muscle all had higher percentages of total body activity on Day 3.

On Day 1, kidney showed the highest activity per gram dry tissue with the flushed small and large intestines, heart, testes and liver following close behind (Table 8). On Day 3, the testes exceeded the

Table 7. Selected tissue and organ distribution of cesium-134 in Microtus canicaudus after a single feeding. Values represent the mean and standard deviation of eight animals, except where noted. Dashed lines indicate below minimum detectable activity.

	Net activity (x 10^3 dpm)		Percent of whole-body activity at the time of sacrifice	
	Day 1	Day 3	Day 1	Day 3
Whole body	60.0 ± 11.2	14.2 ± 2.3	100	100
Kidney	0.6 ± 0.2	0.1 ± 0.03	0.9 ± 0.2	0.7 ± 0.2
Spleen	0.3 ± 0.3	0.02 ± 0.01	0.1 ± 0.03	0.3 ± 0.4
Liver	3.4 ± 1.0	0.6 ± 0.2	5.7 ± 1.7	4.1 ± 1.3
Brain	0.2 ± 0.03	0.2 ± 0.02	0.3 ± 0.04	1.5 ± 0.3
Lungs	0.2 ± 0.1	0.1 ± 0.02	0.4 ± 0.1	0.3 ± 0.1
Heart	0.3 ± 0.1	0.1 ± 0.02	0.5 ± 0.1	0.4 ± 0.1
Testes	0.3 ± 0.04	0.2 ± 0.1	0.4 ± 0.1	1.2 ± 0.7
Eyes	0.02[c] ± 0.01	0.03 ± 0.03	0.03[c] ± 0.02	0.2 ± 0.2
Small intestine[a]	1.6* ± 0.7 2.1 ± 0.2	0.2* ± 0.1 0.3 ± 0.1	2.6* ± 0.4 3.4 ± 0.2	1.6* ± 0.3 2.5 ± 0.4
Large intestine[a]	2.4* ± 1.1 3.7 ± 0.7	0.3* ± 0.1 0.5 ± 0.1	4.0* ± 0.7 6.0 ± 0.7	1.7* ± 0.3 4.1 ± 0.4
Stomach[a]	0.7* ± 0.2 1.0 ± 0.1	0.1* ± 0.1 0.2 ± 0.04	1.2* ± 0.1 1.6 ± 0.1	0.7* ± 0.2 1.4 ± 0.4
Muscle	0.2 ± 0.1	0.1 ± 0.02	0.4 ± 0.1	0.7 ± 0.1
Bone	0.03[c] ± 0.02	0.02 ± 0.01	0.1[c] ± 0.02	0.1 ± 0.04
Blood cells	----	0.01[b] ± 0.01	----	0.1[b] ± 0.03
Blood plasma	----	0.01[b] ± 0.01	----	0.1[b] ± 0.1
Residual carcass[d]	49.1 ± 9.1	12.1 ± 2.1	81.6 ± 1.8	84.5 ± 2.9

*Contents not included; [a] N = 4; [b] N = 5; [c] N = 7; [d] calculated value.

Table 8. Selected tissue and organ distribution of cesium-134 in Microtus canicaudus after a single feeding. Values represent the mean and standard deviation of eight animals, except where noted. Dashed lines indicate below minimum detectable activity.

	dpm/gram dry weight ($\times 10^3$)		dpm/gram dry weight body weight ($\times 10^3$)	
	Day 1	Day 3	Day 1	Day 3
Kidney	11.7 ± 6.8	2.0 ± 0.6	0.4 ± 0.2	0.1 ± 0.02
Spleen	3.0 ± 1.6	1.7 ± 0.9	0.1 ± 0.1	0.04 ± 0.03
Liver	8.3 ± 2.6	1.1 ± 0.2	0.3 ± 0.1	0.03 ± 0.01
Brain	1.1 ± 0.3	1.3 ± 0.1	0.03 ± 0.01	0.03 ± 0.01
Lungs	5.0 ± 1.9	0.9 ± 0.4	0.2 ± 0.1	0.02 ± 0.01
Heart	10.3 ± 5.8	1.0 ± 0.3	0.3 ± 0.2	0.03 ± 0.01
Testes	8.8 ± 7.6	3.8 ± 1.7	0.3 ± 0.3	0.1 ± 0.1
Eyes	1.6[c] ± 1.4	1.4 ± 1.0	0.1[c] ± 0.04	0.03 ± 0.02
Small intestine[a]	9.5* ± 4.7 10.5 ± 1.6	1.4* ± 0.2 1.3 ± 0.3	0.3* ± 0.1 0.3 ± 0.1	0.04* ± 0.01 0.03 ± 0.02
Large intestine[a]	11.6* ± 5.9 9.1 ± 1.7	1.6* ± 0.2 1.2 ± 0.3	0.4* ± 0.2 0.3 ± 0.1	0.04* ± 0.01 0.03 ± 0.02
Stomach[a]	0.7* ± 0.2 5.4 ± 3.3	1.6* ± 0.5 1.2 ± 0.9	0.3* ± 0.1 0.2 ± 0.1	0.04* ± 0.01 0.03 ± 0.03
Muscle	3.8 ± 1.7	1.6 ± 0.3	0.1 ± 0.1	0.04 ± 0.01
Bone	0.6[c] ± 0.3	0.3 ± 0.1	0.02[c] ± 0.01	0.01 ± 0.01
Blood cells	----	0.3[b] ± 0.2	----	0.01[b] ± 0.01
Blood plasma	----	0.3[b] ± 0.1	----	0.01[b] ± 0.01

*Contents not included; [a] N = 4; [b] N = 5; [c] N = 7

kidney while the heart, liver, small and large intestines declined to a level commensurate with muscle, spleen, lungs, eyes and brain. The brain was the only organ to show an increase in activity per gram of tissue, on Day 3.

Bone had very little activity on Day 1 and Declined on Day 3 (Tables 7 and 8). Blood cells and blood plasma samples were both negligible in activity on Day 1; but on Day 3, activity increased and became comparable to the activity found in the bone sample (Tables 7 and 8).

The flushed small intestine and stomach showed less activity on Day 1 than their non-flushed counterparts on a per gram basis (Table 8). The reverse trend was noted for the large intestine. This result is probably more apparent than real since a quick perusal of the attendant standard deviations indicate a large variation around the mean values. In light of this, the activities of both flushed and non-flushed large intestines are most likely within the error inherent in the experiment and therefore would not constitute different values. By Day 3, the activities of the flushed guts were about the same as the unflushed guts (Table 8).

The activity per gram dry tissue divided by the body weight reflected the distribution trends found when activity was expressed only as activity per gram dry tissue (Table 8).

Table 7 indicates that the calculated residual carcass activity decreased from Day 1 to Day 3. Further, the residual carcass retained a larger percentage of the whole-body activity at the time of

sacrifice on Day 3 than on Day 1.

Retention of Radiocesium in Microtus canicaudus After a Single Intraperitoneal Injection

Figure 6 presents the retention pattern of radiocesium in six voles after a single intraperitoneal injection. Of the original eight animals designated for this portion of the study, two apparently were injected in either organs or fat deposits. These two animals were not included in the whole-body assays due to extreme irregularity of their retention patterns when compared to the other six test voles.

The plotted points are expressed as the average percentage of the initial whole-body activity. Whole-body activity reached background levels at 16 days post injection (DPI).

A final retention component was subjectively assessed to exist between 6-16 DPI. The mean coefficient of correlation was $r = .97$, while the biological half-life for this component was 2.2 ± 0.2 days. The percentage of the initial dose contained in this component was $13.9 \pm 7.1\%$.

Distribution of Radiocesium in Microtus canicaudus After a Single Intraperitoneal Injection

The data for radiocesium distribution in five voles at 3 and 7 DPI are found in Tables 9 and 10. The expressions of radioactivity in Tables 9 and 10 are the same as in Tables 5 and 6, respectively. The sacrifice at 19 DPI showed no tissue activities above the

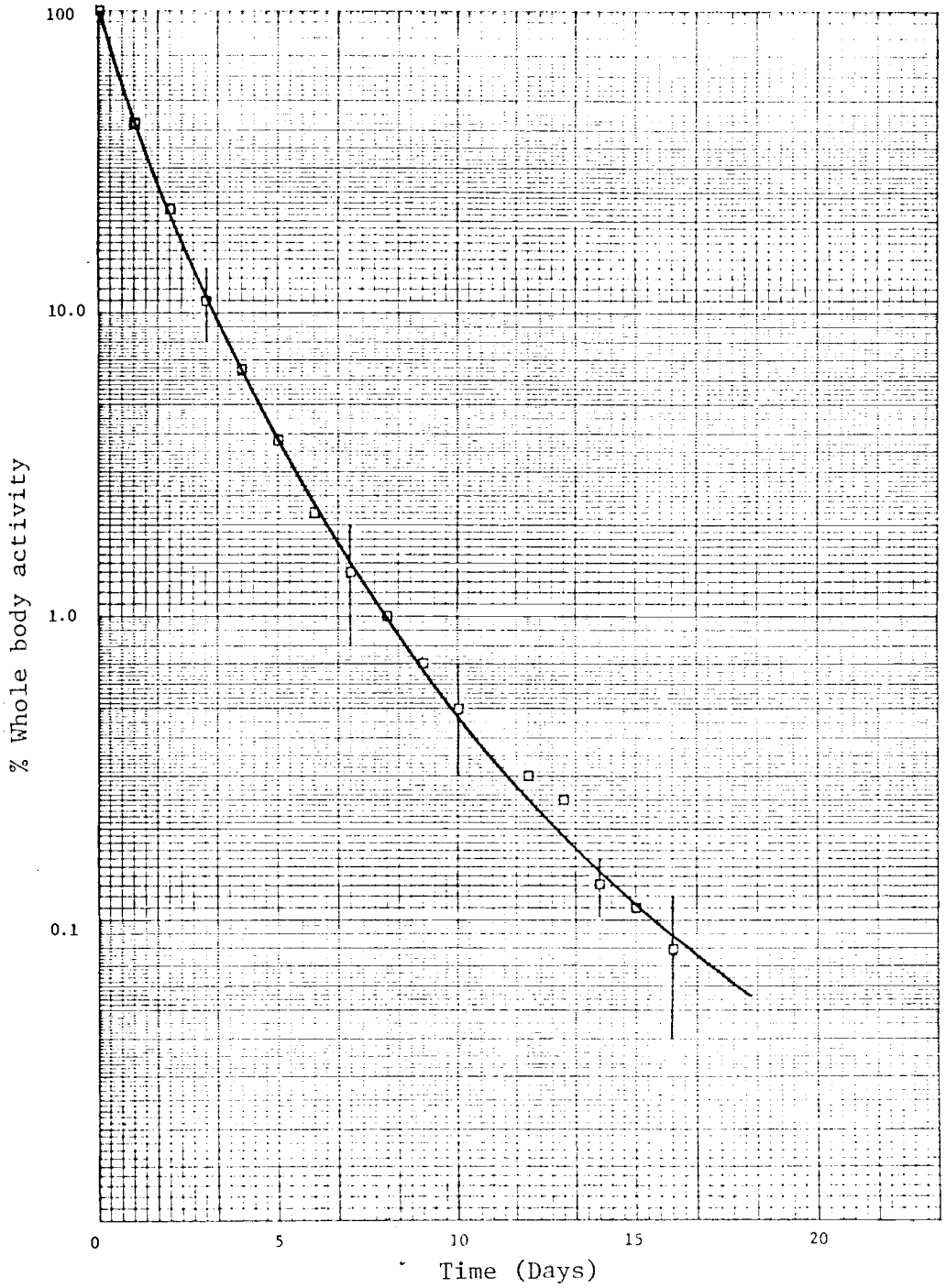

Figure 6. Retention of cesium-134 after intraperitoneal injection, Microtus canicaudus (males). (N = 6).

Table 9. Selected tissue and organ distribution of cesium-134 in Microtus canicaudus after a single intraperitoneal injection. Values represent the mean and standard deviation of five animals, except where noted.

	Net activity (x 10^3 dpm)		Percent of whole-body activity at the time of sacrifice	
	Day 3	Day 7	Day 3	Day 7
Whole body	626.9 ± 385.3	65.5 ± 21.5	100	100
Kidney	5.7 ± 3.4	0.4 ± 0.1	0.9 ± 0.1	0.6 ± 0.2
Spleen	0.5 ± 0.2	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.03
Liver	21.8 ± 12.0	1.9 ± 0.6	3.6 ± 0.6	2.9 ± 0.2
Brain	6.2 ± 2.9	2.5 ± 0.6	1.0 ± 0.2	4.0 ± 1.1
Lungs	2.2 ± 1.5	1.6 ± 0.1	0.4 ± 0.02	0.2 ± 0.1
Heart	2.5 ± 1.2	1.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.04
Testes	6.9 ± 3.4	1.4 ± 0.3	1.2 ± 0.3	2.3 ± 0.9
Eyes	0.6 ± 0.5	0.1 ± 0.03	0.1 ± 0.1	0.2 ± 0.04
Small intestine*	13.8 ± 7.7	1.1 ± 0.7	2.2 ± 0.3	1.8 ± 0.9
Large intestine*	23.1 ± 16.4	2.0 ± 0.9	3.6 ± 0.3	3.0 ± 1.1
Stomach*	7.5 ± 4.2	0.6 ± 0.2	1.2 ± 0.2	0.9 ± 0.2
Muscle	4.1 ± 3.1	0.4 ± 0.3	0.6 ± 0.1	0.6 ± 0.2
Bone	0.7 ± 0.4	0.04 ± 0.03	0.1 ± 0.02	0.1 ± 0.02
Blood cells	0.4 ± 0.2	0.1[a] ± 0.01	0.1 ± 0.01	0.1[a] ± 0.01
Blood plasma	0.2 ± 0.1	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.02
Residual carcass[b]	530.7 ± 329.4	54.5 ± 19.1	84.5 ± 0.6	82.9 ± 2.9

*Contents included; [a] N = 4; [b] calculated value.

Table 10. Selected tissue and organ distribution of cesium-134 in Microtus canicaudus after a single intraperitoneal injection. Values represent the mean and standard deviation of five animals, except where noted.

	dpm/gram dry weight ($\times 10^3$)		dpm/gram dry weight / body weight ($\times 10^3$)	
	Day 3	Day 7	Day 3	Day 7
Kidney	114.8 ± 68.3	8.3 ± 2.5	3.9 ± 3.0	0.2 ± 0.1
Spleen	48.5 ± 15.7	5.0 ± 2.4	1.6 ± 0.8	0.1 ± 0.1
Liver	62.8 ± 45.1	5.0 ± 1.4	2.2 ± 1.9	0.1 ± 0.1
Brain	42.7 ± 18.7	17.1 ± 4.8	1.4 ± 0.9	0.5 ± 0.2
Lungs	56.5 ± 36.7	4.4 ± 1.1	2.0 ± 1.6	0.1 ± 0.03
Heart	74.0 ± 47.3	7.2 ± 4.7	2.6 ± 2.1	0.2 ± 0.1
Testes	188.1 ± 90.1	38.4 ± 8.5	6.4 ± 3.9	1.1 ± 0.2
Eyes	61.7 ± 50.7	14.6 ± 2.7	2.0 ± 2.1	0.4 ± 0.1
Small intestine*	93.4 ± 77.4	6.8 ± 3.1	3.3 ± 3.2	0.2 ± 0.1
Large intestine*	70.2 ± 56.1	4.9 ± 1.7	2.5 ± 2.4	0.1 ± 0.1
Stomach*	79.5 ± 74.9	3.7 ± 1.5	2.8 ± 3.1	0.1 ± 0.04
Muscle	85.3 ± 60.7	7.7 ± 4.1	3.0 ± 2.6	0.2 ± 0.1
Bone	15.5 ± 7.9	1.2 ± 0.5	0.5 ± 0.4	0.04 ± 0.02
Blood cells	7.7 ± 4.3	0.3[a] ± 0.1	0.3 ± 0.2	0.01[a] ± 0.04
Blood plasma	3.3 ± 1.4	0.7 ± 0.2	0.1 ± 0.1	0.02 ± 0.01

*Contents included

[a] N = 4

minimum detectable level.

Even though three days were allowed to pass before the first sacrifice, the activities for each tissue were higher than on Day 1 in the chronic and single feeding studies (Table 9 cf. Tables 5 and 7). Otherwise, the amounts of activity proportioned among the various tissues followed the same pattern as in the two previous studies (Table 9).

Thus, Table 9 indicates that liver, small intestine, large intestine and stomach again contained the highest net activities (cf. Tables 5 and 7). Kidney, brain, testes and muscle showed moderate activity while spleen, lungs, heart, eyes, bone, blood cells and plasma were comparatively low in activity. At 7 DPI, liver, small intestine, large intestine and stomach drastically declined while the brain exceeded these organs in activity. Lungs, heart and testes also contained appreciable amounts of activity at 7 DPI.

The percentage of whole-body activity by the various tissues reflected the trends in the net activity measurements (Table 9). Liver, large intestine, small intestine and stomach contained the majority of the whole-body activity on Day 3, while the brain, large intestine, liver and testes contained the larger percentage by Day 7.

On a per gram basis, kidney and testes exceeded all other tissues by at least a factor of two at 3 DPI (Table 10). Table 10 indicates that moderate activities per gram of tissue were found in small intestine, large intestine, stomach, muscle and heart. Spleen, liver, brain, lungs and eyes followed close behind. Bone, blood

cells and blood plasma were all low in activity per gram of respective tissue.

By 7 DPI, the activity per gram of brain, testes and eyes exceeded all other tissues (Table 10). Activity per gram of kidney was significantly reduced by Day 7 and became comparable with heart, small intestine and muscle. The activity per gram of blood cells also fell below that of the plasma at 7 DPI.

The trends found in the expression of activity on a per gram basis were followed when the expression was divided by the body weight (Table 10).

The calculated average activity remaining in the residual carcass is found in Table 9. The activity remaining at 7 DPI was only one-tenth the activity at 3 DPI. The percentage of whole-body activity decreased from 84.5% to 82.9% in the residual carcass at 3 and 7 DPI, respectively.

Comparison of Linear Regression Parameters and Retention Curves for Three Modes of Cesium-134 Administration

Table 11 compares the linear regression coefficients and calculated biological half-lives for radiocesium retention under chronic feeding, single feeding and single IP injection. The component of interest was subjectively assessed to exist between Days 4-11 in the three retention patterns. This time period conforms to the final component calculated for the single feeding study (Figure 5).

Table 11. Comparison of linear regression* constants and biological half-lives for retention of cesium-134 under chronic feeding, single feeding and a single intraperitoneal injection between days 4-11. Values represent the mean and standard deviation.

	Y-intercept (% retention)	Elimination constant (fractional loss/day)	Biological half-life (days)	N
Chronic feeding	26.6 ± 15.0	0.3 ± 0.1	2.5 ± 0.6	11
Single feeding	52.6 ± 25.5	0.5 ± 0.1	1.5 ± 0.5	7
Intraperitoneal injection	32.2 ± 10.7	0.4 ± 0.03	1.6 ± 0.1	6

*Linear regressions were determined by the method of least squares.

Figure 7 graphically compares the radiocesium retention patterns found in M. canicaudus after chronic feeding, single feeding and IP injection, respectively.

Trophic Transfer of Radiocesium

Figure 8 diagrammatically illustrates the trophic transfer of radiocesium from substrate (i.e., hydroponic solution) to plant (wheat seedlings) and from plant (wheat clippings) to animal (vole). These data were taken from the single feeding study where accurate tabulations of radioactivity were made at all three levels in the simulated food chain.

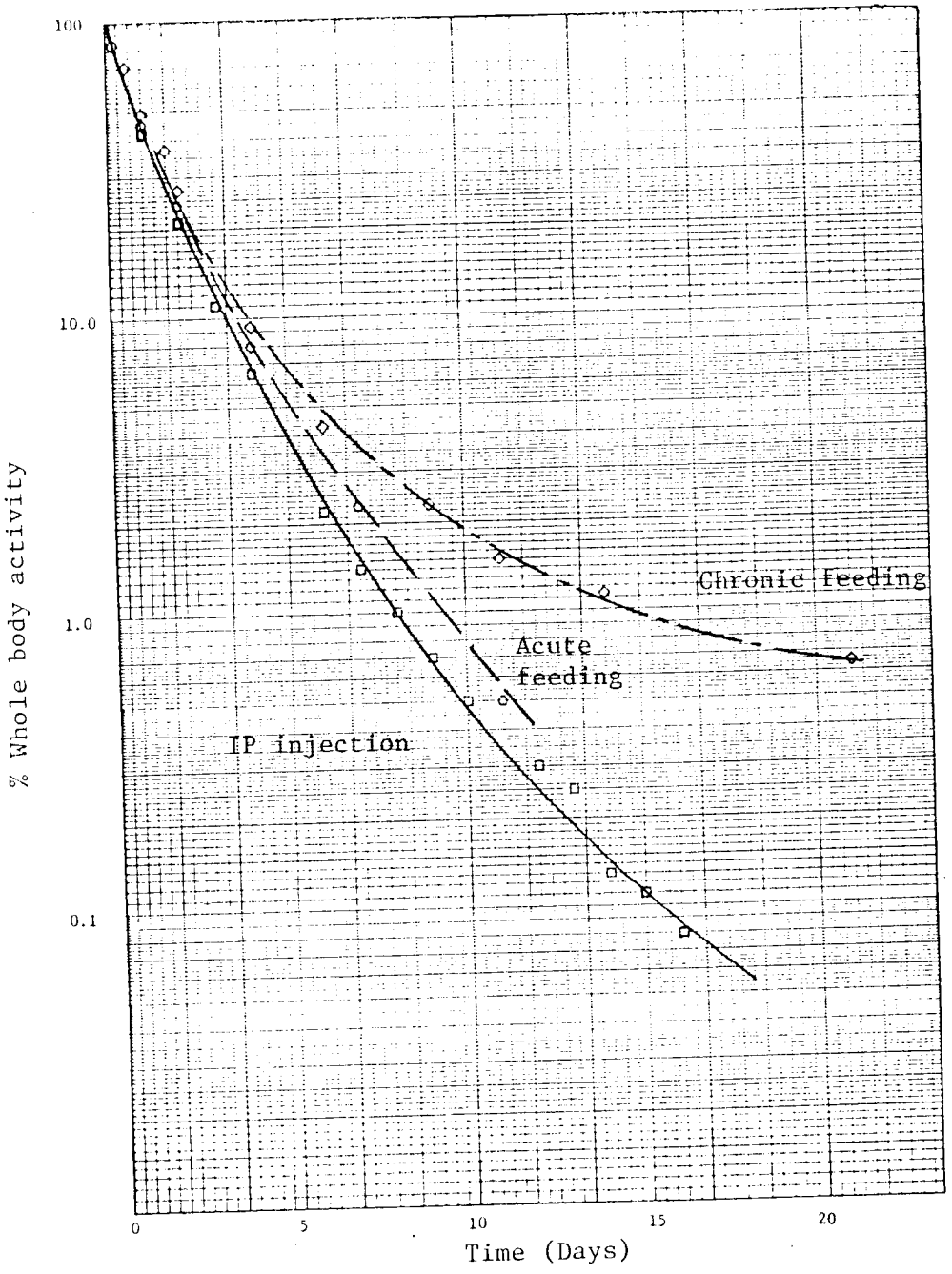

Figure 7. Comparison of cesium-134 whole-body retention patterns.

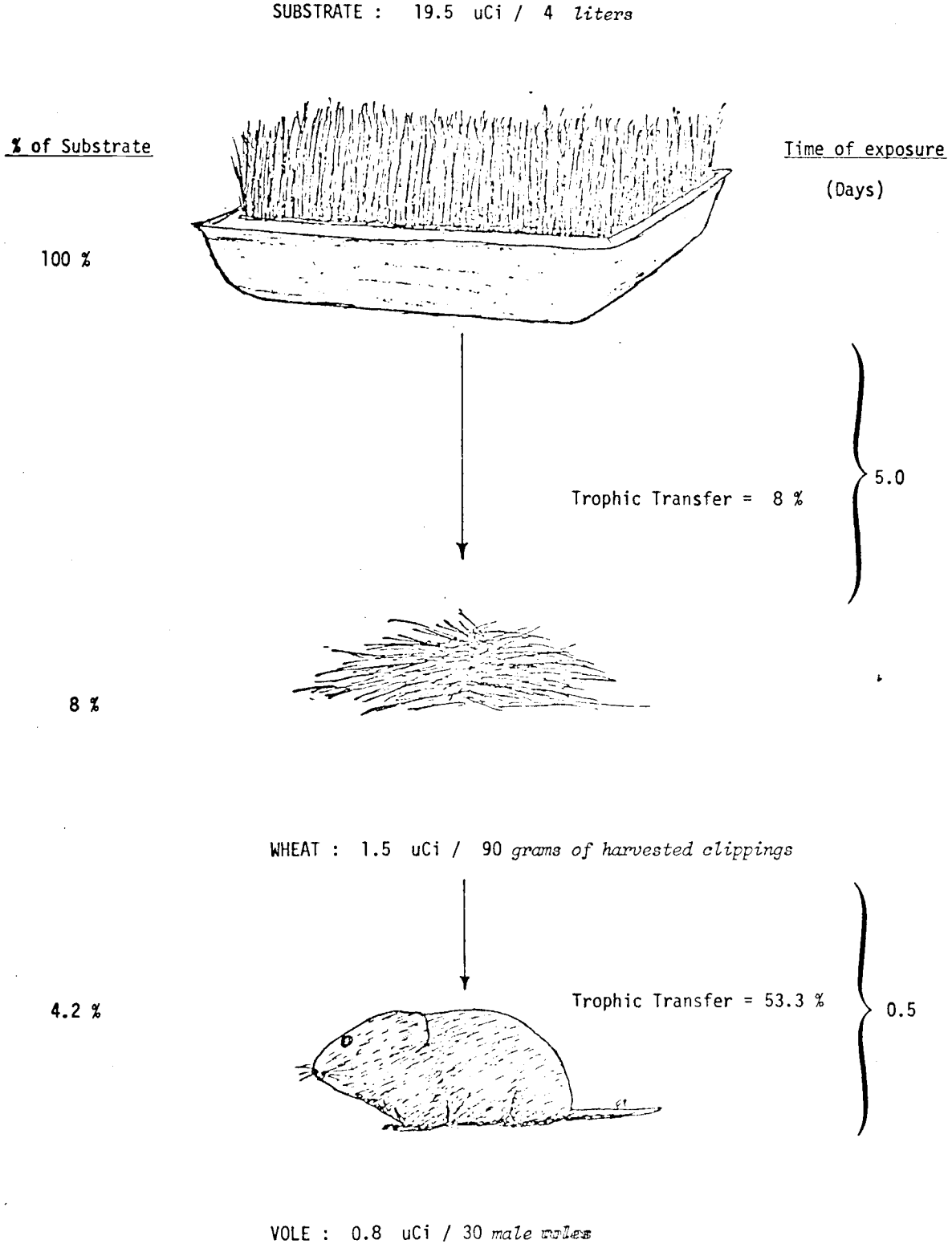

Figure 8. Trophic transfer of cesium-134.

DISCUSSION

Radiocesium Uptake and Translocation in Wheat Seedlings

The uptake and translocation of radiocesium in wheat has previously been studied along with a host of other agriculturally important plants (Menzel and Heald, 1955; Klechkovsky, 1957; Fowler and Christenson, 1959; Handley and Babcock, 1972).

In mature wheat plants grown alternately in a radiocesium solution and then in a nutrient solution (a fractional nutrition method), Guliakin and Yudintseva (1957) found that cesium is absorbed and adequately translocated to aerial structures. On a percentage basis, stems, leaves, chaff and seeds acquired more than 80% of the total plant activity. This was clearly a reflection of the greater bulk of these structures when compared to the roots. The roots, on the other hand, acquired a greater concentration of radiocesium per unit mass of tissue. These investigators also noted that wheat plants absorb proportionately more cesium from less concentrated (i.e., 0.05 mCi/liter) than from more concentrated (i.e., 0.5 mCi/liter) solutions. From a health physics point of view, when comparing the relative uptake and translocation capabilities of several fission products in wheat they found that cesium-137 along with strontium-90 accumulated in the aerial structures while cerium-144, ruthenium-106 and zirconium-95 remain concentrated in the roots. Furthermore, up to 10% of the cesium accumulated in above ground parts was found in the seeds.

Romney et al. (1963) have essentially confirmed these findings by raising wheat, bean and lettuce plants to maturity in both a soil system and a nutrient solution contaminated with mixed fission products. They found that the roots contained the highest amounts of activity in comparison to all other structures, while leaves exceeded other aerial tissues in radioactivity levels. Cesium-137 was absorbed by wheat at higher levels when raised in a contaminated nutrient solution than when grown in a soil system.

As noted earlier (see Introduction), this last finding has been widely confirmed by other investigators and has been recently expanded upon by Cline (1969). Cline described three tenets that allow an increased uptake by plants grown in nutrient solution. First, cesium ions must either be suspended in aqueous solution or be adsorbed to suspended particles. Secondly, the absorptive surfaces of the roots must be in the same liquid phase as the radiocesium. Thirdly, a turbulence in the solution is required to translocate the nuclide to the absorptive surface of the roots.

The hydroponic system devised in the present investigation fulfilled all three of Cline's conditions. Moreover, as moderate amounts of labeled wheat clippings had to be raised within a relatively short period (i.e., eight days), the fractional nutrition method utilized by Guliakin and Yudintseva (1957) was discarded in favor of continuous, direct radiocesium contamination. This technique, in addition to the forced growth conditions, provided adequate harvests of contaminated wheat clippings for experimental purposes.

The average radiocesium uptake for the 32 trays of wheat seedlings used in the chronic feeding study was 18% per tray (Table 1). This was in contrast to the 12% uptake per tray in the single feeding study. Since only 10.0 μCi/tray was used in the chronic feeding study while 20.0 μCi/tray was used in the single feeding study, there was a larger proportional uptake at lower radiocesium concentration when compared to a higher concentration. This is in accordance with the results of Guliakin and Yudintseva (1957).

Although the wheat plants grown in this experiment were not mature, they exhibited much the same type of radiocesium distribution among plant parts. At this young stage, the roots not only accumulated the largest amount of radiocesium but also contained the highest percentage of the total plant activity (Tables 3 and 4). Of the aerial structures, the old and new leaves generally exceeded the stem in containing the larger percentage of the total plant activity, as well as, the higher activity per unit mass of tissue. When compared to the results of Guliakin and Yudintseva (1957), it is apparent that translocation of radiocesium to aerial structures will become more efficient as the seedlings mature. Otherwise, the distribution of radiocesium in eight-day wheat seedlings, exposed only five days to contaminated nutrient solution is in agreement with that found in mature wheat plants.

In the single feeding study, every aliquot of wheat clippings was individually assayed for activity. The 90.0 g eventually chosen represented almost 70% of a typical harvest (see Table 1). Thus,

there was a very good chance of selecting a "uniformly" active group of aliquots simply by random choice alone.

In comparing Tables 1 and 2, there was clearly more variance, in terms of radiocesium uptake, between harvests than within harvests. Manipulative differences such as non-uniform aeration between trays may explain the larger variance in radiocesium uptake.

Radiocesium Uptake in *Microtus canicaudus*

A fluctuating whole-body equilibrium level was maintained in the voles chronically fed radiocesium labeled wheat clippings. Because of this instability, it is not unreasonable to assume that equilibrium may have occurred as early as the first several feedings (Figure 3). These early feedings produced an activity level of over 1,500 dpm/gram. Interpretation of the sinusoidal appearance of the data (Figure 3) presents something of a quandary. It may have been at least somewhat due to the fact that these voles were not fed cesium-labeled food daily but only four days a week. Other similar studies using radiocesium contaminated, solid feed and daily feeding have ultimately achieved a stable level of whole-body activity. For example, Kitchings *et al.* (1969) achieved a stable equilibrium in both laboratory born and wild trapped *Sigmodon hispidus* after 30 consecutive days of feeding radiocesium tagged lettuce. Mathies *et al.* (1971) found that chronic ingestion of radiocesium contaminated pine seeds would result in a whole-body equilibrium by six days in *Peromyscus leucopus*. In this last study, 49 days of consecutive

feeding were allowed for a body burden to accumulate.

The uptake data for the voles follows the trend seen in these previous investigations. Equilibrium was achieved quickly in the voles just as it was in the similar sized Peromyscus. It should also be kept in mind, however, that this comparison is dependent on how much radioactivity was offered per feeding as well as on the feeding schedule. The voles were kept on the radioactive wheat clippings supplemented diet for 53 days. This was done to achieve a higher burden in long-term body pools and also to insure that an equilibrium had occurred.

The initial drop in whole-body activity (between Days 20-30, Figure 3) may be partially accounted for by a weight loss (~7 grams on the average) that occurred several days prior to Day 20. The loss was because only OSU rat chow had been offered to these eleven animals at the beginning of the experiment. Preliminary feeding trials, for up to three months, had shown that the voles could be maintained on this diet alone. This, however, was not the case for these particular test animals. Since they had been raised on a diet of rat and rabbit chows, some had acquired a preference for the smaller rabbit chow pellets. Without the rabbit chow, these voles lost weight. When the rabbit chow was again offered, a rapid weight gain was observed (i.e., within Days 20-30) in these animals. As the data were expressed as activity per unit mass of animal, a dilution effect (due to the weight gain) may explain some of the initial drop in whole-body activity. Furthermore, these animals were handled

according to a fairly regular schedule and may have adapted their metabolic rate to some unknown cycle as a result of handling stresses. This is, of course, speculation and further experiments involving consecutive day feedings would have to be performed to ascertain if a stable equilibrium level can be maintained.

Retention of Radiocesium by Microtus canicaudus

Due to the continually changing slopes of the retention curves (Figures 4, 5 and 6), none of the retention patterns generated after chronic feeding, single feeding or IP injection could be easily resolved into any definite "fast" or "slow" components. All the retention curves were judged as multicomponent representations of radiocesium loss in the vole. This was a reflection of a number of body "pools" emptying radiocesium at different rates. Such pools are thought to represent specific tissues such as extracellular fluid (e.g., blood), skeletal muscle, etc. The pools that lose their radiocesium content relatively quickly are represented by the fast components of a whole-body retention curve. Blood is usually found to be such a pool. Likewise, pools that slowly turn over radiocesium are represented by the slower components of a whole-body retention curve. As an example, Thomas and Thomas (1968) found a final (i.e., the slowest) component for radiocesium elimination in the rat amounting to about 0.005% of the initial dosage (administered intraperitoneally). They suggested that this relatively small amount of deposited cesium was located in the bone. In order to investigate

this component, a large initial body burden (6.7 mCi/kg) of radiocesium was required so that the burden in the final component was still detectable.

Radiocesium elimination rates, as determined by component analysis of a retention curve depend on several factors including: the mode of ingestion (i.e., chronic or acute), species of animal, various physiological and environmental factors (see Introduction), and the minimum detection limit of the radioactivity counting system. The last factor is particularly important in determining a final rate of elimination since the detection limit may not permit discernment of the "true" end component. Because of this, a component designated as "final," including all the parameters that describe it (e.g., biological half-life), is somewhat arbitrary. In this same vein, comparison of so-called final components calculated by different investigators is difficult unless the conditions under which each investigation was performed were fully comparable.

In the present investigation, final components in the three retention curves were somewhat arbitrarily selected to determine the final rate of biological turnover of radiocesium. The y-intercept values generated from the extrapolation of the linear regressions of these components and the calculated radiocesium, biological half-lives will now be discussed.

Y-intercepts of 8.0, 52.6 and 13.9% of the initial body burden for the chronic feeding, single feeding and IP injection studies, respectively, at first indicate that a single feeding will produce a

larger body burden in the final component. This is a misleading impression for one need only recall that the average activity in the single feeding was only about 0.05 μCi. Clearly, even 52.6% of this value is quite low. Thus, what had occurred in this study was that the minimum detectable levels (i.e., when the background levels were achieved) were reached quickly in these animals since the initial (absolute) amount of radiocesium was low. One should be careful when comparing the parameters describing the final components assessed in this study since they do not represent the same period of time. The above example (i.e., comparing the y-intercepts) reveals a false conclusion that may be drawn from an indiscriminate comparison.

The biological half-lives of the final component of 5.8 days for chronically fed and 1.5 days for singly fed voles indicated a rapid whole-body turnover of radiocesium. In laboratory white mice, Richmond (1958) identified a final component T_b of 6.6 days after chronic oral administration and IP injection studies. Mathies et al. (1971) calculated a final component of 20.9 days for Peromyscus leucopus chronically fed cesium-137 tagged pine seeds. In somewhat larger mammals, Richmond (1958) found a final component T_b of about 14 days in laboratory rats given radiocesium IP, while Kitchings et al. (1969) found a 8.12-day T_b for laboratory born and a 8.23-day T_b for wild trapped Sigmodon hispidus chronically fed cesium-134 tagged lettuce.

The final component T_b in M. canicaudus was roughly comparable

to the value found for white mice. It was only one-fourth the value found for Peromyscus. The differences in final component T_b were probably due more to differing modes of administration, dosages and time allowed for a body burden to accumulate than to body size. The larger mammals, however, appear to exhibit longer T_b values and indeed, Stara et al. (1971) have shown that there is a linear relationship between the final component T_b and body weight in interspecific comparisons. However, this relationship is only linear when plotted on log-log paper.

Since the final retention component is representative of long-term body pools, it is dependent on the amount of prior accumulation of cesium-134. This has been confirmed by Mathies et al. (1971) in an intraspecific comparison using Peromyscus leucopus. They found that chronically fed Peromyscus showed a slower excretion rate when compared to IP injected animals. Figure 7 indicates the same trend when a comparison is made of the retention patterns generated after chronic feeding, single feeding and IP injection in M. canicaudus. For the first three days, both the chronically fed and singly fed voles excrete cesium at the same rate. This may indicate a rapid loss of activity through the gut via the feces. This was supported by the organ distribution studies performed in the single feeding investigation. In Table 7, the flushed intestines showed a definite decrease in net activity compared to non-flushed guts on Day 1. By Day 3, the activity in both flushed and non-flushed intestines was virtually the same. This suggests that gut passage, including fast

resorption of radiocesium from the blood occurred in the interim between one and three days post feeding.

After three days, the chronically fed animals exhibited a slower rate of cesium excretion than the singly fed animals (Figure 7). This divergence was most likely due to a larger proportion of the radiocesium body burden in the metabolically less active body pools of the chronically fed animals. The singly fed animals undoubtedly accumulated less cesium in such long-term body pools. The relatively quick loss of radiocesium from the singly fed animals is further supported by the fact that these animals were measurable above the background level for the shortest period of time (Figure 5).

The final component T_b for the IP injected animals was 2.2 days and compares closely with the second component (T_b = 2.4 days) that Richmond (1958) found for white mice (also after IP injection). The third component that Richmond resolved (T_b = 6.6 days) was not evident in the present study. This may be due to the lower sensitivity of the Armac system compared to the detection equipment used by Richmond. Accordingly, he was able to detect less than 0.05% of the whole-body activity (initial activity ~ 0.2 μCi) and so continued his retention study for 60 days. In the present study, using voles and an initial activity of ~ 0.9 μCi, the detection limit was reached at 16 days post injection. The possibility of extending the assay time for the radioactive voles to achieve better counting statistics (i.e., to distinguish more firmly, the background level) was

impracticable. This constraint was largely because the voles appeared to be "stressed" if confined to the counting vials for greater than 10 minutes.

Figure 7 illustrates that the IP retention pattern is divorced from the chronic and single feeding patterns from about 5 DPI. The line is displaced such that the rate of loss is much more rapid on the whole than even the single feeding case. This suggests that IP injection is not only an unnatural means of radionuclide administration but may also give an unrepresentative retention pattern.

Comparison of Biological Half-Lives and Regression Parameters

Table 11 describes the biological half-lives and regression parameters for all three retention patterns during a common period of retention. The period was delimited between Days 4-11 and it coincides with the end component found in the single feeding study.

During this period of radiocesium retention, the chronically fed animals had the smallest percentage of accumulated cesium with the singly fed animals containing the highest percentage of whole-body activity. The elimination constants were virtually the same although that for the chronically fed animals was somewhat slower than that of the singly fed animals. This was probably due to relatively large amounts of radiocesium stored in long-term body pools.

The single administration modes (i.e., single feeding and single IP injection) showed practically the same T_b and elimination constants. The magnitude of these values suggests that smaller amounts

of cesium accumulated in longer term body pools when compared to the chronic feeding study. Specifically, the single entry modes exhibited faster elimination rates and shorter biological half-lives.

Individual differences among the test voles were quite large. The standard deviations of the mean y-intercepts and biological half-lives (Table 11) exemplify this point. This could be expected since the voles were from an outbred stock and therefore were probably genetically dissimilar. Thus, phenotypic differences in rates of metabolism, etc. should be evident.

Tissue Distribution Studies

Izawa and co-workers (1958) have performed a thorough study of radiocesium tissue distribution and accumulation in white mice. In three separate investigations utilizing single intravenous and intra-abdominal injections as well as chronic feeding of cesium-134 via the water supply, they found that cesium tended to accumulate in the soft tissues — particularly in muscle tissue. This essentially confirmed for mice what Hood and Comar (1953) found in rats, cattle, sheep and swine. Izawa *et al.* (1958) also noted that initially high concentrations may be found in the pancreas, kidney and heart. In these tissues, the elimination rate was at first quite fast then slowed within several days. High concentrations were also found in the liver and gut (ingesta included) at 3 hours post injection. Muscle, testes and brain were found to contain low concentrations initially but to peak between 1 and 2 days post-injection. These trends in

cesium distribution were qualitatively the same regardless of mode of administration.

Nelson et al. (1961), using autoradiography, have also studied radiocesium distribution in white mice. Their investigation indicated the same trends found by Izawa et al. (1958). In addition, they found that cesium concentration in the blood decreased rapidly, becoming negligible by four days. Hard tissues including bone and teeth were low in activity; however, cartilage showed a significant uptake. The central nervous system showed the lowest initial activity with the gray matter in the brain having a slightly higher activity than the white matter. High uptake was found in the sclera and lens of the eye with the retina also showing appreciable activity. Contrary to Izawa's et al. (1958) study, Nelson and co-workers (1961) discovered a low activity in the liver at all sacrifice times. Both studies agree that skeletal muscle is eventually the major repository for radiocesium. Hood and Comar (1953) have verified this in white rats and domestic farm animals while Degregorio et al. (1971) have confirmed the same in wild trapped cotton rats.

The tissue distribution of cesium in the vole body after chronic feeding, single feeding and IP injection generally adhered to the results of these studies. Tables 6, 8 and 10 point out that at early sacrifice times, the kidney and intestines possessed a high concentration of radiocesium. The heart and liver were also high which is in agreement with Izawa et al. (1958). The testes showed an initial high uptake and maintained a relatively high concentration over time.

This disagrees with Izawa's et al. (1958) data for white mice. Muscle tissue does not show a build-up of cesium concentration over time, although the eyes and brain showed this effect in the chronic (Table 6) and single (Table 8) feeding studies, respectively. Bone and blood fractions accumulated the smallest amounts of activity confirming the observations of Nelson et al. (1961). Since the blood fractions contained the smallest concentrations at all sacrifice times, the tissues apparently accumulated cesium against a concentration gradient. This had earlier been shown by Hood and Comar (1953) in laboratory rats.

Even though muscle tissue only moderately concentrated cesium, because there is relatively more muscle, it became the major repository for cesium in the vole body. Tables 5, 7 and 9 reveal that about 80% of the whole-body activity was located in the residual carcass at any sacrifice time. Therefore, since the majority of the residual carcass was skeletal muscle and bone, most of the cesium must be located in muscle as it has already been shown that bone accumulates very little of the nuclide. However, it is clear from the tissue distribution studies that cesium dispersal throughout the vole body is fairly uniform which is in accordance with its chemical similarity to potassium, a physiologically ubiquitous element. For this reason and the fact that radiocesium is a gamma emitter, the nuclide is considered a whole-body hazard (U.S. HEW, 1970).

The variance in tissue activity due to individual differences was, with few exceptions, well within the range of normal radiotracer

experiments. Hood and Comar (1953) noted that standard deviations of 0.1 to 0.5 of the mean were not uncommon.

Trophic Transfer of Cesium-134

Since the radiocesium concentration was accurately tabulated at each level of a simulated food chain, the percentage of transfer could be calculated by specifying the exposure time to the contaminated food source. The transfer percentages depicted in Figure 8 were calculated from the single feeding results. After a 5-day exposure, the 90 g of clippings contained 8% of the activity in the contaminated nutrient solution. After a 12-hour period given to ingest the 90 g of clippings, 30 voles contained 53.3% of the activity found in the clippings. The remainder of the activity had, presumably, already passed through the gut. This was quite possible as it was observed that the majority of the voles would devour between half to three-quarters of the 3.0 g offering within one to two hours. This observation coupled with extensive nocturnal activity can cause a rapid turnover through the gut. The presence of ingesta from the rat and rabbit chows could also decrease cesium absorption in the gut. Moore and Comar (1962) in their study of cesium-137 absorption in the gastro-intestinal tract noted that the presence of food in the tract did, indeed, affect cesium-137 absorption. They explained a reduction of cesium-137 absorption in the gut as due possibly to absorption of the nuclide by the ingesta thereby making less cesium available to the gut. McPeak et al. (1966) have

also offered evidence in support of an increased elimination of cesium-137 by a high intake (volume) of food with concomitant increase in defecation.

Given these exposure times, 8% and 4.2% of the substrate activity was transferred to the wheat clippings and voles, respectively. The 4.2% found in the voles resulted from the 53.3% trophic transfer of radioactivity from the single feeding of labeled wheat clippings.

In summary, adequate amounts of radiocesium tagged wheat seedlings were raised in hydroponic solution. The labeled wheat clippings served as a realistic source of food and were used to supplement the normal diet of experimental M. canicaudus. The hydroponic system was devised to provide optimal conditions for increased cesium uptake by the seedlings.

Three separate modes of radiocesium administration were used in the voles. Under a chronic feeding schedule about 0.04 μCi/3.0 g of radiocesium tagged clippings were fed to voles four times a week. In a single feeding experiment, voles received only a single portion of 0.05 μCi/3.0 g wheat clippings. Finally, a single intraperitoneal injection containing about 0.9 μCi in 0.4 ml normal saline was utilized.

A fluctuating whole-body equilibrium level was reached in the chronically fed voles. Although 53 days were allowed for equilibration, this level was probably reached at an earlier date as interpreted by the fluctuations. Further experimentation would be

required for a complete explanation of why the chronically fed voles exhibited an unstable, whole-body equilibrium.

The retention of radiocesium by M. canicaudus under the three modes of administration, i.e., chronic feeding, single feeding and IP injection was analyzed assuming exponential elimination rates. "Final" components of radiocesium elimination were arbitrarily assessed from retention curves drawn on semi-logarithmic graph paper. Arbitrariness was interjected into the assessment for at least two reasons: 1) As all three retention patterns (curves) displayed continuously changing slopes, they were judged as multicomponent systems indicating that a number of physiological compartments exhibited a continuum of elimination constants that were very close in magnitude. Thus, it was difficult to separate specific components. 2) The "true" final components were probably not discerned due in part to initially low radiocesium body burdens and the minimum detection limit of the radioactivity counting system. Biological half-lives of the final components in the chronic feeding, single feeding and IP injection studies were 5.8, 1.5 and 2.2 days, respectively. The longer half-life found in the chronically fed animals may be attributed to a larger proportion of cesium in tissues that have a relatively slow turnover rate for the element.

Muscle tissue was found to be the major repository of accumulated cesium. Thus long-term body burdens will undoubtedly be determined by the rate of radiocesium turnover in the muscle pool.

General Comments

Ecologically speaking, a vole foraging only occasionally in a radioactively contaminated environment (e.g., due to cesium-134 and cesium-137 from nuclear weapons fallout) will probably not be exposed to high internal doses of radiation since the major fraction ingested is rapidly excreted from the body. By contrast, for a vole feeding consistently in a radiocesium contaminated environment, higher body burdens will likely occur and equilibrium with the metabolically less active body pools will be attained. This has radiobiological consequences not only for the vole but for possible higher order consumers; that is, the vole serves as a link in many food chains and thus potential radiation hazards apply not only to it but to its predators. This latter notion is especially salient since muscle, because of its large mass, will be a significant repository for radiocesium in the vole. Clearly, the transfer of radiocesium from vole to predator is enhanced because of this.

Finally, from this study of cesium movement from nutrient solution to plant and from plant to animal, the "bottleneck" of transfer was definitely the transfer from substrate to plant. Translocation of the nuclide to foliar structures (i.e., edible portions), nonetheless, was significant. In view of the poor transfer from substrate to plant, foliar deposition of radiocesium from fallout may take on an added significance. It should also be noted that even when plants accumulate cesium only moderately, they do not tend to

lose it except possibly by guttation and, of course, by the eventual death of the plant. Thus, there is the distinct possibility that long-lived plants may become reservoirs of radiocesium contamination.

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APPENDIX

PERCENT OF WHOLE-BODY RETENTION

Values are the mean and standard deviation

Days post last feeding/injection	N = 11 Chronic feeding	N = 7 Single feeding	N = 6 Intraperitoneal injection
0.25	83.6 ± 3.9	---	---
0.46	70.2 ± 4.6	---	---
1.00	47.8 ± 6.1	44.6 ± 3.1	41.8 ± 4.9
1.25	40.7 ± 6.1	---	---
1.46	36.6 ± 6.6	---	---
2.00	26.5 ± 6.5	24.0 ± 3.0	21.0 ± 4.6
3.00	---	---	11.1 ± 3.0
4.00	9.4 ± 3.4	8.0 ± 1.2	6.5 ± 2.0
5.00	---	---	3.8 ± 1.6
6.00	4.3 ± 3.0	---	2.2 ± 0.9
7.00	---	2.3 ± 2.0	1.4 ± 0.7
8.00	---	---	1.0 ± 0.5
9.00	2.3 ± 1.5	---	0.7 ± 0.3
10.00	---	---	0.5 ± 0.2
11.00	1.5 ± 1.4	0.5 ± 0.6	---
12.00	---	---*	0.3 ± 0.02
13.00	---	---	0.3 ± 0.02
14.00	1.1 ± 1.0	---	0.1 ± 0.1
15.00	---	---	0.1 ± 0.04
16.00	---	---	0.1 ± 0.04
17.00	---	---	---*
18.00	---	---	---
19.00	---	---	---
20.00	---	---	---
21.00	0.7 ± 0.4	---	---
22.00	---*	---	---

*Below minimum detectable activity.