Improved Methods for Measuring Radioactive Tracer Accumulation and Excretion by Microarthropods, with Applications for a Mite Species, Tyrophagus longior (Acarina, Acaridae).

David T. Abbott

D. A. Crossley, Jr.

Department of Entomology

University of Georgia

Athens, Georgia 30602

Date Published - August 1, 1980

Prepared for the
Department of Energy
Under Contract DE-AS09-76EV00641

DISCLAIMER

David Abbott
Entomology Department
University of Georgia
Athens, GA 30602

Improved Methods for Measuring Radioactive Tracer Accumulation and Excretion by Microarthropods, with Applications for a Mite Species, *Tyrophagus longior* (Acarina, Acaridae).

David T. Abbott

D. A. Crossley, Jr.

Department of Entomology

and Institute of Ecology

University of Georgia

Athens, Georgia 30602

Radioisotope techniques continue to be important tools for the analysis of food chain dynamics. By measuring uptake and excretion of radioactive tracers, investigators have estimated such parameters as total food intake, energy or nutrient assimilation, and turnover rates for chemical elements. Estimates usually require the experimental measurement of turnover rates for the pool of chemical elements, using radioactive tracers. Ingestion is then estimated as the amount of intake necessary to balance losses which occur due to turnover. The turnover rates may be described by equations with one, two or three rate constants. Choice of models and applicability for soil arthropods were recently reviewed by Webster and Crossley (1978).

Such applications of radioactive tracer techniques with soil microarthropod (mites and collembolans) systems have been slow to appear, doubtless because of methodological problems induced by the small size of the arthropods. With larger arthropods, usual methods involve experiments in which the radioactivity of individual arthropods is followed through time (Nabholz and Crossley 1978). By the use of gamma emitting radioisotopes, live arthropods can be counted repeatedly. In this procedure, arthropods are given brief access to food containing a radioactive tracer. Those indiivduals which ingest measurable levels of radioactivity are then caged individually with non-radioactive food, and counted through time (usually with a well-type NaI(Tl) detector). Count rates can be normalized to the initial radioactivity, so that the initial differences between individual arthropods can be removed (Webster and Crossley 1978).

To overcome the differences inherent in working with individual microarthropods, previous workers have measured radioactive tracer accumulation through time instead of turnover rate. Kowal (1969) used ^{45}Ca tagged pine needles as a food base in cultures of Cultroribula juncta (Michael) (Acarina: Oribatei). Groups of mites were collected (with Berlese methods) from replicated cultures through time, dried, and counted for radioactivity. Ingestion and excretion rate parameters were estimated from the uptake curves thus generated. Similar methods (serial sacrifice of replicated radioactive cultures) were used in microarthropod experiments by Kowal and Crossley (1971) and McBrayer and Reichle (1971) and in field experiments by Coleman and McGinnis (1970) and Carter and Cragg (1977). The data provided by such experiments are inherently variable, and are not amenable to the detailed analysis possible with data from single-tagging experiments. In particular, separation of rates into two or more components, or the measurement of assimilation, is not feasible. Gist and Crossley (1975) measured turnover of ^{85}Sr by oribatid mites using repeated counting, but only after the mites were maintained for many days on radioactive food.

In this paper we report a simple but successful technique for repeated counting of individual microarthropods. Mites (Tyrophagus longior (Gervais), Family Acaridae) were given brief access to radioactively tagged food and then confined in individual microculture capsules. The entire capsule could be counted for radioactivity, after which the mite was transferred to a fresh microculture capsule. Handling of individual mites was thus reduced to a minimum.

Materials and Methods

Acarid mites were isolated from cultures of collembolans (Sinella curviseta Brook) where they occurred as contaminants. The mites (Tyrophagus longoir) were maintained in pure culture in small (3.5 cm dia) acrylic plastic culture dishes containing a 9:1 mixture of plaster of Paris and activated charcoal as a substrate. Commercially available activated yeast was used as food for the mites. Tagged food was prepared by mixing to a paste the activated yeast with either ^{51}Cr solution (0.7 uCi per mg yeast) or ^{85}Sr solution (3.0 uCi per mg) and allowing excess moisture to evaporate overnight. The mites were then allowed ad lib. access to the tagged food overnight and assayed for radioactivity the next morning.

To count mites for radioactivity and to maintain them during the experiment, individual mites were placed in individual microculture capsules. The microculture capsules consisted of Beem^{R} micro tissue imbedding capsules, to which a small amount of the plaster of Paris-charcoal mixture had been added as a substrate (Figure 1). Small fragments of unlabeled activated yeast were added. Following the initial counting for radioactivity, the microculture capsules containing mites were stored in an environmentally controlled chamber at 30 C. For subsequent counts of radioactivity, the following procedure was followed: First, new microculture capsules were prepared and yeast was added. Then, mites were transferred to the new microculture capsules using a camels-hair brush. Care was taken to insure that none of the old yeast or detritus from the old microculture capsule was transferred, so that

any excreted radioisotope was left behind. The new microculture capsules were then counted for radioactivity, and returned to the environmentally controlled chamber. With this procedure handling and contamination problems were kept to a minimum.

Counting for radioactivity was performed with a NaI(Tl) 7.6 x 7.6 cm well crystal (2.5 cm dia) detector connected to a Packard 410A multichannel analyzer, with a window set to contain the energy range of the principle gamma emission. Each microculture capsule was placed in a 25 mm dia test tube for counting. Separate experiments were performed using ^{85}Sr and ^{51}Cr. For analysis, counts taken at each time interval were corrected for radioactive decay. Then, each count was normalized by expressing it as a percentage of the initial count. Rates of excretion were calculated by using least squares regression analysis for each individual mite.

Results and Discussion

The results of retention experiments with ^{51}Cr and ^{85}Sr in Tyrophagus longior are shown in Figure 2. Mites excreted ^{51}Cr rapidly and ^{85}Sr slowly. Van Hook and Crossley (1969) and Webster and Crossley (1978) believed that ^{51}Cr was virtually unassimilated in crickets (Acheta domesticus L.), and that loss of ^{51}Cr represented gut clearance only. Retention of ^{51}Cr may be described by the equation:

$$R_t = 100\, e^{-kt} \qquad (1)$$

where R_t is percentage retention at any time t, 100 is the initial percentage, e is the base of the natural logarithms, t is time, and k is the elimination coefficient. For the ^{51}Cr data in Figure 2, k is 0.0603 per hr. The half-time for turnover (T_b) that is, the time required to eliminate 50% of the ingested radioactivity, is given by:

$$T_b = \frac{\log 2}{k} \qquad (2)$$

For ^{51}Cr the half-time for turnover was 11.5 hrs.

Retention of ^{85}Sr showed an initial rapid drop followed by a slower phase, suggesting that a two-component curve might be fit to the data (Figure 2). In this procedure a straight line is fit to the tail of the curve and extrapolated back to the origin. The initial data points are then corrected by subtraction of the first component, to yield a second, rapid component (Figure 2). Such a two-component retention curve may be described by an equation of the form:

$$R_t = 100(p_1\, e^{-k_1 t} + p_2\, e^{-k_2 t}) \qquad (3)$$

where R_t is percentage retention at any time t, 100 is the inital percentage, p_1 and p_2 are proportions ($p_1 + p_2 = 1$) of the two components

being eliminated at rates k_1 and k_2, respectively, and e is the base of the natural logarithms. Half-times for turnover (Tb_1 and Tb_2) can be calculated for the rates k_1 and k_2, using equation (2). Table 1 presents values for the parameters for ^{85}Sr retention by T. longior according to the model of equation (3).

The interpretation previously offered for two-component retention curves for ingested radioisotopes in arthropods has been that the shorter component represents loss of nonassimilated tracer from the gut, whereas the longer component represents excretion of tracer which has become assimilated. The fraction p_2, the proportion moving at the longer rate, thus represents the fraction of ingested tracer which has been assimilated (Webster and Crossley 1978). Among the evidence supporting this contention is the similarity between the loss rate (k) for ^{51}Cr and the rate (k_1) for turnover of the more rapid component of other radioisotopes (Van Hook and Crossley 1969). Such a similarity between k for ^{51}Cr and k_1 for ^{85}Sr is evident from data obtained for Tyrophagus longior (0.0603 vs. 0.0700, Table 1). It thus appears that T. longior has a moderately high assimilation rate for ingested ^{85}Sr (62%, Table 1) and that the half-time for gut clearance is rather slow (10-11 hrs., Table 1). However, the data for ^{51}Cr retention are not entirely linear and the intercept of the regression line with the origin is at about 60% rather than 100%. With additional data a two-component retention curve might appear for ^{51}Cr as well. Thus the identification of gut clearance rate for the turnover rate of ^{51}Cr should be tentative. The ^{85}Sr excretion rate for T. longior is slower than the rates reported by Gist and Crossley (1975) for oribatid mites (0.115 per day for T.

longior vs. 0.200 for oribatid mites maintained at 19°C). Slow turnover of Sr might be expected for oribatids, since those mites have a calcareous exoskeleton (Wallwork 1971). The slow turnover of ^{85}Sr by T. longior may be a result of the high reproductive expenditure of this "r specialist" mite. The assimilation value for ^{85}Sr in oribatids (91%) given by Gist and Crossley (1975) is probably unrealistically high, since the mites were fed for several days on radioactive food rather than receiving a brief exposure. The 62% value found for T. longior (Table 1) is probably more realistic and compares favorably with the 63% assimilation reported for Eupterotegaeus rostratus Higgins and Woolley by Carter and Cragg (1977).

The experimental procedures described here seem applicable to other microarthropods in addition to acarid mites. We have been partially successful using the microculture capsules for oribatid mites and collembolans, the latter requiring some adroitness in transferring them between microculture capsules.

Acknowledgements

We thank D. C. Weems for identification of Tyrophagus longior, and Dr. T. R. Seastedt for comments upon the manuscript. Research supported by a contract, number DE-A509-76/EV-00641 between the U. S. Department of Energy and the University of Georgia (D. A. Crossley, Jr.).

Literature Cited

Carter, A. and J. B. Cragg. 1977. Potassium and calcium cycling by Eupterotegaeus rostratus (Acari: Cryptostigmata). Pedobiologia 17: 169-174.

Coleman, D. C. and J. T. McGinnis. 1970. Quantification of fungus-small arthropod food chains in the soil. Oikos 17: 62-70.

Gist, C. S. and D. A. Crossley, Jr. 1975. Feeding rates of some Cryptozoa as determined by isotopic half-life studies. Env. Ent. 4: 625-631.

Kowal, N. E. 1969. Ingestion rate of a pine-mor oribatid mite. Amer. Mid. Nat. 81: 595-598.

Kowal, N. E. and D. A. Crossley, Jr. 1971. The ingestion rates of microarthropods in pine-mor, estimated with radioactive calcium. Ecology 52: 444-452.

McBrayer, J. F. and D. E. Reichle. 1971. Trophic structure and feeding rates of forest soil invertebrates. Oikos 22: 381-388.

Nabholz, J. V. and D. A. Crossley, Jr. 1978. Ingestion and elimination of cesium -134 by a spider, Pardosa lapidicina. Ann. Ent. Soc. Amer. 71: 325-328.

Van Hook, R. I. and D. A. Crossley, Jr. 1969. Assimilation and biological turnover of cesium-134, iodine-131 and chromium-51 in brown crickets. Acheta domesticus (L.). Health Phys. 16: 463-467.

Wallwork, J. A. 1971. Some aspects of the energetics of soil mites. Proc. 3rd Int. Cong. acarology, pp. 129-134.

Webster, J. R. and D. A. Crossley, Jr. 1978. Evaluation of two models for predicting elemental accumulation by arthropods. Env. Ent. 7: 411-417.

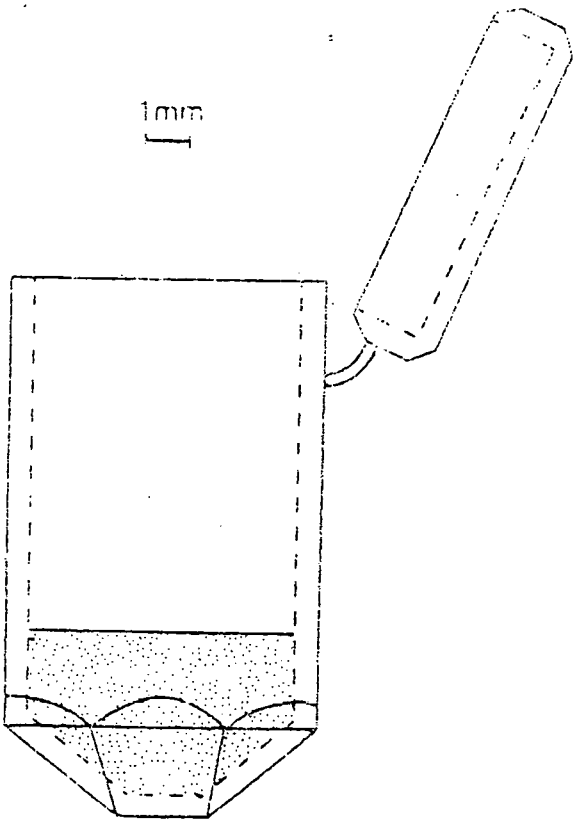

Figure 1. Plastic microculture capsule, showing attached lid and plaster of Paris-charcoal substrate.

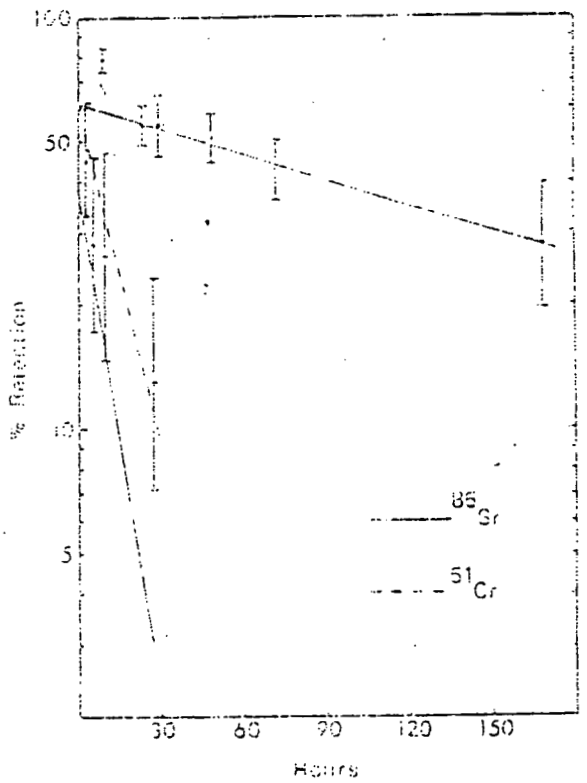

Figure 2. Retention of ^{85}Sr and ^{51}Cr by Tyrophagus longior. Dots represent ^{85}Sr, crosses ^{51}Cr. Shown are means $\pm$ 1 standard error. For ^{85}Sr n = 6, for ^{51}Cr, n = 5.

Table 1. Parameters for models describing retention of radioisotopes by Tyrophagus longior maintained at 30°C. n = number of mites, r^2 = coefficient of determination. Other parameters explained in text.

Radioisotope	p_1	k_1 (hr^{-1})	T_{b_1} (hrs)	p_2	k_2 (hr^{-1})	T_{b_2} (hrs)	r^2	n
^{51}Cr	--*	0.0603	11.5	--	--	--	0.58	5
^{85}Sr	0.38	0.0700	9.9	0.62	0.0048	145	0.64	6

*not applicable