UPTAKE AND ELIMINATION OF ^{137}Cs IN AQUATIC INSECTS

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ABSTRACT

by

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Some applications of ^{137}Cs as a radioactive label for entomological investigations in the laboratory and in natural situations are described.

Accumulation and elimination of ^{137}Cs by an aquatic Hemipteran *Lethocerus americanus*, the giant water bug, has been studied. Instar IV and adult water bugs eliminated ^{137}Cs at different rates. The rate of labeled-prey consumption required to maintain the insect's steady-state level of ^{137}Cs was calculated. It is shown that knowledge of an organism's method of feeding is essential to successful use of the radioactive label technique of estimating food consumption.

A study of ^{137}Cs uptake by two species of mosquito larvae (*Aedes aegypti* and *A. atropalpus*) showed accumulation of the radionuclide was suppressed by the concentration of potassium in the larval rearing medium. It is postulated that most of the ^{137}Cs entered the larvae by diffusion across the papillary membrane, and not via the gut. Consequently, ^{137}Cs is not considered to be a suitable radionuclide for use as a label to measure food consumption by mosquito larvae. It readily leaches out of the food into the rearing medium.

A 19 m diameter permanent pond was contaminated with 0.5 Ci of ^{137}Cs, most of which was sorbed onto the pond mud. The integrated dose of ionizing radiation delivered to the pond's benthic organisms was greater than 2 rad/day in some locations. A simple method for measuring the distribution of the radiation dose in the pond employing lithium fluoride dosimeters, was developed. Algal growths concentrated most of the ^{137}Cs taken up by the pond biota. Chironomid larvae had the highest specific activity of all the pond insects which were measured.

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CHAPTER I

INTRODUCTION

Radionuclides are radioactive species of stable elements. A characteristic property of any radionuclide or radioactive isotope, is that it decays. Radioactive decay is the process whereby radionuclides undergo spontaneous disintegration during which energy is liberated, generally resulting in the formation of new nuclides. This process is accompanied by the emission of one or more types of ionizing radiation such as alpha or beta particles, and gamma photons. Ionizing radiation (radiation) is any electromagnetic or particulate radiation capable of producing ions, directly or indirectly, in its passage through matter. The radiation emitted by radionuclides can be detected and measured with a variety of instruments, and it is this property which makes them a unique experimental tool.

The experimental use of radionuclides pre-dates the explosion of the first atomic device in 1945. Shortly after the discovery of X-rays, Malding and Thouvenin (1898) reported their effect on seed germination. By 1904, a biological application of autoradiography, the production of a two- or three-dimensional image on a photographic plate by radiation, had been reported by London (1904). The biological uses of radionuclides has been discussed in historical perspective by Hevesy (1948, 1951), a pioneer in tracer applications. Since 1945 the use of radioactive isotopes and radiation in general biology and entomological studies has become established. These uses are the subject matter of text-books by Kamen (1951), and O'Brien and Wolfe (1964), for example.

For more than 50 years X-rays have been the major source of acute radiation used by workers studying biological effects on cells, tissues and organisms (Bacq and Alexander, 1961). The cost of running X-ray equipment continuously, however, precludes its use as a continuous-radiation source. The study of the ecological effects of chronic exposure to radiation was delayed until radionuclides were obtainable in sufficient quantities to make multi-curie sources. The use of such sources for the irradiation of terrestrial habitats, and the observed effects of these exposures have been the subject of numerous studies in the past ten to fifteen years. Many of these have been part of major gamma irradiation projects such as the Brookhaven Irradiated Forest (Woodwell, 1963; Bower, 1965), the Nevada Test Site Irradiator (French, 1964), and the Puerto Rico Rain Forest Irradiation Project (Odum, 1964). Interest in the ecological effects of nuclear warfare stimulated the development of most of these large dose-rate investigations (Woodwell and Sparrow, 1965). Studies devoted to the ecological effects of radiation in aquatic habitats are not as numerous as those of terrestrial environments, particularly at low dose-rates.

The uptake and elimination of ^{137}Cs by the giant water bug (Lethocerus americanus) and two species of mosquito, the distribution of ^{137}Cs in a pond which was contaminated with this nuclide and the integrated radiation dose which the pond's benthic organisms received, are the subject of this dissertation.

^{137}Cs as an Ecological Tracer

The use of radionuclides to study biogeochemical cycling and trophic level kinetics, originated with concern about the fate of radioactive wastes entering food-chains in which man was a consumer (Odum, 1959). Nuclear weapon debris continues to be the major source of man-made radioactive environmental contamination, while the contribution from discharge of radioactive wastes is small (United Nations, 1962). The biogeochemical cycling of nuclides in aquatic and terrestrial ecosystems is important because the amounts accumulated as a result of physical, chemical and biological concentration processes (Guthrie, 1962; Davis et al., 1963), determines the magnitude of the internal radiation dose received by organisms. Many radionuclides are translocated in ecosystems, thus the ecologist is provided with a unique tool for studying animal dispersion, foodweb relationships, and productivity (Schultz and Klement, 1963; Odum, 1959, and Olson, 1965). Radionuclides may be used to measure feeding and food assimilation rates in two ways: 1) The amount of tracer used may be small relative to the equilibrium body burden of the animal, or 2) the body burden may be increased to achieve a new steady-state level (Southwood, 1966). The latter method was first described by Davis and Foster (1958), and was developed and applied by Crossley and Pryor (1960), Crossley and Howden (1961), and Crossley (1966), who used ^{137}Cs.

Radiocesium (^{137}Cs) is distributed throughout the animal body after ingestion. The physical half-life of ^{137}Cs is about 30 years (Katcoff, 1960). Its biological half-life depends on the animal and the temperature of its environment (Davis, 1963). Radiocesium is suitable for application to food consumption studies (Crossley, 1963), especially since its measurement does not require that the experimental animal be killed. However, with the exception of the work of Pendleton and his co-workers (Pendleton, 1957, 1960, 1962, and Pendleton and Hanson, 1958) who investigated the trophic level relationships in aquatic communities, no use has been made of ^{137}Cs to investigate the feeding habits of aquatic insects.

Radiocesium is of importance for another reason - its presence in fission products. Of all the fission products which are potential long-term contamination hazards to the environment, strontium-90 and cesium-137 constitute the greatest threat. The yield of ^{137}Cs from uranium-235 undergoing fission by thermal neutrons is about 6% of the total fission product inventory (Katcoff, 1960). Consequently ^{137}Cs is abundant in old fission products, and can be expected to comprise a significant percentage of delayed fallout. (Delayed, or global, fallout is that which comes down on an area remote from the site where a nuclear device has been detonated at or near ground level). Of all the sources of radioactive wastes listed by Neal (1960), the solutions from primary separation plants processing nuclear fuel bundles contain the largest amounts of fission products. Cesium-137 constitutes a significant fraction of these fission products. It has been calculated (McCullough, 1957) that irradiated (spent) fuel elements from a nuclear reactor, producing one megawatt of power will contain about 10^5 Curies (Ci) of fission products. This calculation is based on 100 days of irradiation, followed by 100 days cooling. The highly radioactive wastes from fuel processing plants are not discharged to the environment.

The emphasis of the Canadian nuclear power program is on heavy water moderated, natural uranium fuelled, liquid cooled reactors (Nuclear News, 1968). Should the primary coolant escape, the greatest potential threat would be its effect on the aquatic environment. It follows that knowledge of the fate of radiocesium and other fission products in lakes and rivers, is important to those in Canada concerned with the surveillance of radioactivity in the environment (Guthrie, 1963, 1964; Samuels, 1966a and b).

CHAPTER II

MATERIALS AND METHODS

The Experimental Ponds

In October, 1966, two ponds were dug by a small bulldozer in a strip of land along the edge of a stand of young aspen (*Populus tremuloides*) and balsam poplar (*P. balsamifera*). The experimental plot was once cultivated farmland, which was left to return to its native vegetation when purchased by Atomic Energy of Canada Limited. This plot is now one of the study-sites within the controlled, or restricted, area of the Whiteshall Nuclear Research Establishment (W.N.R.E.). The average height of the tree stand is 3.5-4 m, and the ponds are situated 35 m apart on the south side of this stand. A view of the radioactive pond is given in Figure 1, and Figure 2 shows the surrounding area. Each pond was lined with 5 mil black plastic sheeting, to reduce water loss. The sheeting was covered with 15 cms of the excavated soil. The completed ponds measured about 19 m in diameter and sloped uniformly down to the center to a depth of 2 m. After the spring thaw in 1967, both ponds were full of water as a result of melting snow and spring rainfall. The depth contours of the radioactive pond, measured in June 1967, are shown in Appendix 'A'. No plant or animal life was artificially introduced into either pond. The communities developed by invasion from the surrounding terrain, or by chance introduction of renewal buds or other reproductive bodies with the excavated earth used to cover the plastic sheeting.

In May 1967, 0.5 Ci of ^{137}Cs chloride solution was added to one of the ponds, subsequently called the RADIOACTIVE POND. After mixing, replicate samples of pond water were taken from several locations on the radioactive pond for radiochemical analysis of ^{137}Cs, to determine the uniformity of the nuclide's dispersion. The other pond was called the NON-RADIOACTIVE POND. The level of radiocesium in this pond was about one hundred times less than the amount in the radioactive pond and is attributed to fallout.

Sampling

A grid system was established at each pond to orientate and record sampling of the bottom-mud and benthic organisms. A row of wood-stakes, spaced 1 m apart, was placed on opposite sides of each pond. Pre-stretched, 50 mm OD, polyethylene ropes marked off at 1 m intervals provided a simple but effective 1 m^2 grid when stretched across the pond between stakes. To facilitate grid-square identification, the stakes were lettered 'A' to 'Z' and the rope markes numbered 1 to 22 (Appendix 'B'). The rows of stakes were laid out using a transit. A pre-determined mark on each rope was used to gauge the amount of tension applied when fastening it to the stake.

Ten samples were taken from the bottom of each pond at monthly intervals during the period May to October, 1967 and 1968. No grid-square was sampled more than once, and sampling locations were selected by a computer program which generated randomized sets of coordinates. A bottom-sampler, developed by Ireland (1968a), was used to take mud samples measuring 0.029 m^2 and 1 cm in thickness. Free swimming (nektonic) insects were sampled by dip-net. The mud samples were taken to the laboratory for examination on the sampling day. Each sample was spread out on a white enamel tray and the insects removed with forceps. The insects were freed from adhering debris by floating them in a saturated sodium chloride solution. They were then rinsed in distilled water and stored in vials of preservative (70:5:25 ethanol: glycerol: distilled-water). A portion of each mud sample was retained for total-beta counting. This is a gross beta counting procedure, compared to counting a radio-chemically pure nuclide, because all beta particles regardless of origin are counted. The implicit assumptions of the method including the use of ^{40}K as an equivalent standard, have been described by Guthrie and Grummitt (1963). Whenever total-beta counting was employed, ^{137}Cs was the dominant beta-emitting nuclide present.

Study Species

Lethocerus americanus (Leidy), Belostomatidae:Hemiptera (Figure 3), was chosen as the species of aquatic hemipteran to be studied. Hemipterans were of interest for two reasons. First, they commonly feed from more than one trophic level. This is especially true of the giant water bug L. americanus. Secondly, they do not completely ingest their food. The piercing-sucking mouthparts are used to draw out the body contents of the prey. To date, no attempt has been made using the radiotracer method to estimate food consumption of insects which feed in this manner. The measurement of food consumption in the field, by insects which feed by sucking their prey, is difficult. The radionuclide technique appeared to offer a practical solution.

Two mosquito species Aedes aegypti (L.) and A. atropalpus (Coquillett) were chosen as examples of aquatic Diptera to which the tracer method could be applied. An equally important reason for studying Dipterans resulted from the suggestion by Peredel'skii and Bogatyrev (1959a and b) that aquatic insects emerging from habitats contaminated with fission products, could spread them throughout the surrounding terrain.

Principle of Feeding-rate Determination Using an Isotope

The technique of food consumption estimation using radioactive tracers has been described by several workers (Crossley, 1963, Crossley and Howden, 1961, and Kevern, 1966, for example). Southwood (1966) and Reichle (1967), and Schlagbauer (1967), have reviewed the pertinent principles. Insects may consume radionuclide-labelled food at a constant rate, but its elimination occurs at a rate proportional to the amount in the body -- the body burden (Crossley, 1966, and Southwood, 1966). At steady-state, the tracer will be lost at a rate ($\lambda.Q_s$,) where λ is the biological elimination rate constant and Q_s is the steady-state body burden. The steady-state condition is maintained only if the rate at which the tracer is eliminated by the insect is balanced by the amount which is ingested (I.F), where I is the quantity of food ingested and F is the fraction of ingested food that is assimilated. Thus we may write:

$$I.F = \lambda . Q_s$$

The elimination rate is usually written in terms of the biological half-life (T_b). By definition, T_b is the time required for the organism to eliminate one-half of its body burden of a given nuclide.

Let the body burden at time t be Q_t.

The rate of change of the body burden with time is described by the differential equation:

$$\frac{dQ_t}{dt} = -\lambda . Q_t$$

Re-arranging:

$$\frac{dQ_t}{Q_t} = -\lambda . dt$$

$$\therefore \int \frac{dQ_t}{Q_t} = -\lambda \int dt + C$$

$$\ln Q_t = (C - \lambda . t)$$

$$\therefore Q_t = e^{(C - \lambda . t)} = e^{C} . e^{-\lambda . t}, \text{ or } Q_t = Q_o e^{-\lambda . t}$$

The instant that feeding of labelled food is discontinued, $(t = 0)$, $Q_o = Q_s$. At any subsequent time, therefore, $Q_t = Q_s . e^{-\lambda . t}$.

$$\text{When } t = T_b, \; Q_t = \frac{Q_s}{2},$$

$$0.5 = e^{-\lambda . T_b},$$

$$\ln 0.5 = -\lambda . T_b$$

$$\therefore T_b = \frac{\ln 0.5}{\lambda} = \frac{0.693}{\lambda}$$

Determination of ^{137}Cs Uptake and Elimination

A small laboratory colony of *Lethocerus americanus* was established by hatching water bug egg clusters obtained from the non-radioactive pond. The cannibalistic character of the nymphs and adults of this species necessitated individual rearing. The rearing procedure followed was similar to that described by Rankin (1935). Each egg cluster was supported on a block of wood (5cm x 5cm x 0.5cm) floating freely on water contained in refrigerator trays (30x19x10cm). As the eggs hatched the nymphs crawled off the block and entered the water. Soon after hatching, the nymphs were transferred to individual plastic food-freezer boxes, measuring 10x10x8cm, closed with a perforated plastic lid. A small block of wood was placed in each box, to give the nymph an object to cling to. As observed by Rankin (1935), tadpoles were found to be the most satisfactory food source on which to rear L. americanus in captivity. The accumulation of radiocesium by the water bugs was determined by feeding the insects ^{137}Cs labeled tadpoles collected from the radioactive pond. The elimination rate of the nuclide was measured after the water bugs had reached the steady-state, by feeding non-radioactive tadpoles obtained from the non-radioactive pond. The tadpoles collected from the non-radioactive pond also contained some radionuclides as a result of ingesting the fission products entering the pond via fallout. However the level of ^{137}Cs was found to be less 1/1000 of that in the tadpoles taken from the radioactive pond. Radioactive and non-radioactive pond tadpoles were collected daily with a dip-net. The water bugs were fed once per day, immediately after measuring their radiocesium content. Dead tadpoles were removed from the plastic boxes soon after the insects finished feeding. Otherwise, decomposing tadpoles would have polluted the water sufficiently to kill the water bugs (Rankin, 1935).

An experimental colony of Aedes aegypti and A. atropalpus was established by hatching eggs supplied by Dr. R.A. Brust, Department of Entomology, The University of Manitoba, and with the exception of food, his rearing methods were followed throughout (Brust, 1968). The hatching medium was a suspension of 100 mg of nutrient broth[1] in 100 ml

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[1] A product of Difco Laboratories, Detroit, Michigan.

of distilled water. Mosquito eggs were placed in 25 ml vials filled to capacity with hatching medium. A. aegypti eggs were left in the hatching solution for two hours, and A. atropalpus eggs for 24 hours. Groups of 100 first instar larvae were transferred to opaque white, plastic rearing pans (30x19x10cm) fitted with a lid. A. aegypti were fed ^{137}Cs labeled or un-labeled ground Gaine's dog meal. A. atropalpus larvae were fed labeled or un-labeled fish rearing food[1]. The larvae were not allowed to deplete the food. Since there was superfluous food, new media was usually supplied on alternate days to prevent growth of harmful bacteria. About 50 mg of powdered live yeast was added to each tray with the first feeding. The water level in the rearing pans was such that the larvae could breathe and eat at the same time. The larvae used in the radiocesium uptake experiments were given a change of medium, daily.

Measurement of ^{137}Cs

The concentration of radiocesium in the various specimens and samples, including mosquito larvae and water bugs, was measured by low level beta counting or by gamma spectroscopy. Cesium-137 emits 0.52 MeV beta particles and its daughter product emits a 0.662 MeV gamma photon, the result of internal transition to ground state. The principles and applications of beta counting and gamma spectroscopy have been described in several texts, for example those of Cook and Duncan (1952), and Gatrousis and Crouthamel (1960).

Total beta counting was the method used to measure ^{137}Cs in

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[1] Tetra Min baby fish food "E" and "L" mixed in equal amounts. A product of Tetra Kraft Werke Dr. rer. nat. Baensch Melle, Western Germany.