

THE UNIVERSITY OF MANITOBA

ACCUMULATION AND ELIMINATION OF CESIUM-137 BY FIVE AEDINE
SPECIES OF MOSQUITOES AND BY A COMMON PREDATOR,
NOTONECTA UNDULATA

by

Andrew Zbignef Burzynski

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A dissertation submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

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ABSTRACT

by

Andrew Zbignef Burzynski

ACCUMULATION AND ELIMINATION OF CESIUM-137 BY FIVE AEDINE
SPECIES OF MOSQUITOES AND BY A COMMON PREDATOR,
NOTONECTA UNDULATA

Cesium-137 as a radioactive label for entomological investigations in the laboratory is described.

Accumulation and elimination of ^{137}Cs by five aedine species of mosquitoes (Aedes aegypti, A. atropalpus, A. dorsalis, A. triseriatus, and A. vexans) are studied. Species differences exist in both accumulation and elimination rates for a variety of reasons. The pupal and adult studies are inconclusive.

Accumulation and elimination of cesium-137 by 4th instar normal and degilled larvae in potassium and potassium-free medium is studied. Most of the radiocesium enters the larvae via the anal papillae. Potassium in the medium decreases the amount of radiocesium accumulated but not the rate of uptake. Carbon particles are used to show that degilled larvae increase their gut uptake. Elimination is not affected by either removal of the anal gills or the presence of potassium.

A study of cesium-137 accumulation and elimination by an aquatic Hemipteran, Notonecta undulata, the common backswimmer, shows a decrease in radiocesium uptake by the nymphs prior to molting. Adult backswimmers have a single

accumulation rate and a two phase elimination. The food consumption of adult backswimmers is calculated.

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CHAPTER 1
GENERAL INTRODUCTION

Contamination of the Aquatic Environment

It has been estimated that by the year 2000 more than 115 GW of electricity will be generated in Canada by nuclear power stations (Crawford and Häussermann, 1975). This increase in nuclear generating capacity together with the use of radioisotopes in industry and medicine, has raised public concern about the impact on the environment of the radioactive waste products of the nuclear industry (Citizen Action Group, 1974). Fallout from nuclear weapons tests conducted in the atmosphere and articles which have appeared in the popular press describing the difficulties associated with the handling of radioactive wastes stress the need for accurate assessment of the capacity of the environment to assimilate radioactive materials without incurring an unacceptable risk of biological damage caused by ionizing radiation.

The biosphere has been exposed to radiation since the beginning of geological time. The various life-forms inhabiting the planet have evolved in a natural radiation background, exposed to an annual dose which varies from 90 mrad to 1500 mrad depending on the geographical location (UNSCEAR, 1972). This background radiation results from naturally occurring radioactive minerals in the rocks and soil of the planet, and from cosmic rays. To this natural

background there has now been added a man-made component: fallout from nuclear weapons tests, tailings from uranium mining and ore processing operations, and radioactive wastes from nuclear power stations and fuel reprocessing plants. The man-made radiation component, however, amounts to less than 3% of the natural background radiation (UNSCEAR, 1972). If the Moscow agreement which bans the testing of nuclear weapons in the atmosphere and in water continues, the major source of man-made radionuclides entering the environment, will be the nuclear fuel cycle.

Canadian nuclear power stations use a natural uranium fueled, heavy water moderated reactor, the CANDU reactor (AECL, 1976). In an accident situation it is the aquatic component of the environment that will be exposed to the greatest potential impact from nuclear power generation in Canada.

The Aquatic Insects

The most abundant and diverse group of animals on the Earth are to be found in the class Insecta, many of which spend their juvenile stages in an aquatic habitat and the adult stage on land. Hence, because of the large transfer of biomass to the terrestrial environment which occurs when aquatic forms emerge as adults, there exists the potential for translocation of radionuclides from water to land (Peredel'skii and Bogatrov, 1959). The magnitude of such transfer will depend on environmental

temperature and the extent to which aquatic insects, and other forms, ingest, assimilate and egest radioactive wastes released to the aquatic environment. The aquatic dipterans together with their hemipteran predators constitute a large fraction of the aquatic invertebrates.

Cesium-137

The fission reaction which occurs in the core of a nuclear reactor generates more than 200 radioactive nuclides, the fission products (Katcoff, 1960). However, the majority of these fission products have half-lives of only a few hours, days, or months. It is the few fission products that have half-lives measured in years, and are isotopes of elements which may take part in metabolic processes, such as iodine, or chemically similar to them, such as strontium is to calcium, that are the major concern. These are the biologically important fission products. Such a fission product is cesium-137. It has a high fission yield (6%) and a 30 year half-life (Katcoff, 1960). Radiocesium also mimics the metabolically important element potassium in many physiological processes (Davis, 1963), therefore, it is distributed throughout the animal body following ingestion and assimilation. The amount of radiocesium ingested and the residence time or biological half-life in the animal, determine the radiation dose received and subsequent biological damage (Guthrie, 1962; Davis and Foster, 1958).

The use of radiocesium and other radionuclides to study biogeochemical cycling and trophic level kinetics (Pendleton and Ukler, 1960; Schultz and Klement, 1963; Reichle, 1967; Odum, 1965) originated with the concern about their translocation through food-chains leading to man (Odum, 1965; Cushing, 1970). The significance of the insect component in the cycling of radionuclides in aquatic ecosystems has been discussed by Polikarpov, 1967; Guthrie, 1969; and Reichle et al., 1970.

Radiocesium is translocated in ecosystems, thus the ecologist is provided in certain circumstances with a unique tool with which to study animal dispersion, foodweb relationships and productivity. Its biological half-life depends on the species and its temperature (Davis, 1963). Early application of cesium-137 was as a label for measuring feeding and food assimilation rates. Crossley (1966) estimated the daily rate of plant material consumed by insects living on the plants growing on a former lake bed contaminated with cesium-137 and other fission products. Guthrie and Brust (1969) used the nuclide to measure the assimilation rate of an aquatic hemipteran equipped with piercing-sucking mouthparts, Lethocerus americanus.

Guthrie and Brust (1969) stressed that the application of the radionuclide label to the estimation of the feeding rates of insects rested on the assumption that the rate of label uptake or elimination, required to calculate biological half-life, was reasonably constant

within a insect family (taxonomic family unit). One objective of the work to be reported in this thesis was to test the validity of this assumption. The second objective was to estimate the relative importance of the gut and anal papillae as routes for the uptake of radio-cesium by mosquito larvae. The third objective was to apply steady-state conditions to the calculation of food assimilation by another species of aquatic hemipteran, in order to test if the value reported by Guthrie and Brust (1969) applied to more than one insect family.

CHAPTER 2

MATERIALS AND METHODS

Experimental OrganismsCulicidae (Diptera):

Five aedine species of mosquitoes, Aedes aegypti (L), Aedes atropalpus (Coquillett), Aedes dorsalis (Meiger), Aedes vexans (Meiger), and Aedes triseriatus (Say), were chosen to study the uptake and elimination of cesium-137. These were chosen for several reasons. First, mosquitoes are common around the world (Stone, Knight, & Stark, 1959). Second, aquatic dipterans make up some of the most important pest species in North America. The genus Aedes is one of the most important. Third, it has been suggested that aquatic insects could contaminate the surrounding environment with radionuclides when they emerge from habitats containing these materials (Peredel'skii and Bogatyrev, 1959).

Notonectidae (Hemiptera):

Notonecta undulata, Say, the common backswimmer, was chosen as the species of aquatic hemipteran to be studied for two reasons. First, they are known to be a predator of mosquito larvae (Ellis and Borden, 1970), and are therefore at a higher trophic level than mosquito larvae. Secondly, they have piercing-sucking mouthparts. Only one previous attempt has been made to measure food consumption in a sucking insect by the radiocesium tracer method (Guthrie and Brust, 1969).

General Rearing Methods

The eggs of five aedine species of mosquitoes were supplied by Dr. R. A. Brust, Department of Entomology, University of Manitoba and the rearing methods employed were essentially those described by Brust (1968). The eggs were hatched in a solution of 100 milligrams of nutrient broth in 100 ml of distilled water for two hours. Groups of 100 first instar larvae were transferred to plastic rearing pans with lids (30 x 19 x 10 cm). The rearing medium consisted of 100 ml of distilled water at a temperature of 26°C. The larvae were fed a measured amount of Tetra Min Tube Food 66*. New food was added and the medium changed daily. Pupae were removed and placed in a container of distilled water inside an emergence cage. The relative humidity of 75 - 95% was maintained within the cage with a roll of moistened paper toweling.

The adults were offered honey solution ad libitum. Guinea pigs and/or mice were used as a means of blood feeding the female mosquitoes. Lighting conditions 16L:8D, were provided to stimulate mating. Moist filter paper in a funnel provided the surface for egg laying. The eggs were stored at 10°C for 7 days before hatching.

Rearing of *N. undulata*

Adult backswimmers were collected in the spring

*Tetra Kraft Werke Dr. rev. nat Baensch Melle, Western Germany.

soon after the ponds were free of ice. After identification, they were reared in the laboratory using the method described by Ellis and Borden (1969). The backswimmers were placed in a large aquarium with pond water and were fed tadpoles and mosquito larvae. A wire mesh in the water served as a site for egg attachment. After the eggs hatched, the nymphs were removed and placed in separate 100 ml beakers. Thus the feeding and growth could be observed individually. Each nymph was fed 10 larvae daily.

Cesium-137 Accumulation and Elimination

Mosquito larvae

The same rearing procedure as already described was used except that the rearing medium contained carrier free $^{137}\text{CsCl}$ resulting in an activity of $10,000 \pm 5\%$ counts per minute (cpm)/ml of medium. The radioactivities of the rearing media will be included in the data for individual experiments.

To study cesium-137 elimination, larvae that had attained a cesium-137 body-burden were transferred to non-radioactive medium and food. The larvae were sampled at regular intervals to determine the decrease in cesium-137 activity.

Mosquito Pupae

To measure the uptake of cesium-137 by pupae, late 4th instar non-radioactive larvae were placed in distilled water to pupate and minimize cross contamination. The

newly moulted pupae were placed in cesium-137 solution of approximately 1000 cpm/ml. Pupae of both sexes were sampled, washed, and total-beta counted (beta counted) every 24 hours. To reduce the likelihood that the radioactivity measured was due to surface contamination, the pupae sampled were washed and placed in a container of distilled water to allow the adults to emerge. These newly emerged adults as well as the pupal exuviae were beta counted.

Elimination was measured by transferring late 4th instar larvae reared in radiocesium to a container of distilled water where they were kept until pupation was completed. Again pupae were sampled, washed, and beta counted at 24 hour intervals. Samples of the emergence water were also beta counted.

Notonecta undulata (backswimmers)

Soon after the eggs hatched, individual backswimmer nymphs were transferred to 100 ml beakers. The accumulation of radiocesium was determined by feeding each backswimmer nymph ten cesium-137 labelled mosquito larvae. After feeding, the remains of the mosquito larvae, consisting mainly of the cuticle, were beta counted, and the amount of food consumed estimated. The radioactivity of each individual backswimmer was measured daily by gamma spectrometry prior to feeding.

The elimination rate was measured after the backswimmers had achieved a body-burden of ^{137}Cs , by feeding

non-labeled mosquito larvae. The backswimmers were fed once a day, immediately after measuring their radiocesium content.

The same procedures were used for the adults.

Measurement of Cesium-137

Cesium-137 emits 0.52 MeV beta particles, and its metastable barium daughter product emits a 0.662 MeV gamma photon as the result of internal transition to ground state (Heath, 1964). Preliminary experiments using mosquito larvae, pupae and adults, and backswimmer nymphs and adults reared in the laboratory in local pond water, showed that the amounts of background cesium-137 were below the detection limits of the counting techniques employed. Since the change in counting rate attributable to an increase, or decrease, in cesium-137 was the parameter of interest, absolute counting was not required. Care was taken to ensure that all samples were counted under comparable geometry, hence relative counting was applicable.

The levels of radiocesium in the various samples including mosquito larvae, pupae, and adults, and the water in which they were reared, were measured by low level beta counting. Radiocesium levels in N. undulata nymphs and adults were measured by gamma spectroscopy. The principles and applications of these counting methods are described in reference texts and reports (Gatrousis and Crouthamel, 1960; Guthrie and Grummitt, 1963; Heath, 1964).

Individual mosquito larvae, pupae, and adults, were fastened at the center of stainless steel counting trays with a 1:10 collodion-acetone mixture for beta counting. The trays were dried under heat lamps prior to counting. A Sharpe's Low Background Beta Counter, equipped with an automatic sample changer and a gas-flow end window detector, was used for all beta counting. The background of this equipment with a counting tray under the detector was 0.4 ± 0.05 (s.d.) counts per minute (cpm). The cesium-137 counting efficiency of the detector, determined by counting standard sources of comparable self-absorption and geometry was 42%.

The radiocesium level in N. undulata nymphs and adults was measured by a gamma spectrometer. The spectrometer system consisted of a 7.6 x 7.6 cm NaI (Tl) scintillation crystal coupled with a photomultiplier, housed in a Heath-type shield made of 15 cm thick steel and lined with OFC-grade copper sheeting (Heath, 1964), and a Nuclear Data 130 pulse height analyser. Individual backswimmers were counted by placing them in a small sealed plastic vial with one drop of water. The vial containing the insect was placed on top of the scintillation crystal, and counted for 4 minutes. The net counting rate was determined by subtracting the background count rate from the gross counting rate. Background counting rate was determined by counting an empty vial containing one drop of water. As the change in count rate was the parameter of

interest, it was not necessary to determine the counting efficiency of the detector. The radiocesium activity of each backswimmer in cpm was obtained after subtracting the background and integrating the area under the 0.662 MeV photopeak.

Removal of Anal Gills (Papillae)

Several methods have been described in the literature for the removal of the anal gills (Wigglesworth, 1938; Stobbart, 1964). However, the description of these methods is not very precise, so that some experimentation was required to determine the most effective method.

The anal gills were removed chemically with solutions of NaOH or AgNO_3 . In this process, fourth instar larvae were exposed to different concentrations of each chemical for varying lengths of time. The NaOH and AgNO_3 solutions ranged from 0.1M to 0.5M and exposure times varied from 0.5 to 5.0 minutes.

After the treatments, the larvae were immediately washed with distilled water and placed in non-labeled rearing medium. The larvae were examined after 48 hours to determine which treatment most successfully removed the anal gills and caused the least mortality. Ninety per cent survival after 48 hours was considered acceptable. The larvae were examined under a dissecting microscope to determine the success of degilling. Only completely degilled larvae, those which had four healed scars in place of the gills, were used.

After two experiments with chemically degilled larvae, a surgical method was used. This method proved to be the most successful in the survival of larvae and complete removal of the anal gills. The gills were removed under a dissecting microscope using minuten nadeln. The larvae were held for 24 hours to recover from the stress of the procedure, before being placed in a cesium-137 medium. This allowed for the complete healing of the surgical wounds.

Carbon Particle Uptake

An experiment was carried out to determine if larval feeding was affected by the degilling procedure. Inert substances, such as latex and carbon particles, that are visible in the gut have been used to measure feeding rates (Dadd, 1968). In experiments reported here, carbon particles were used to compare the feeding rates of normal and degilled larvae. The system used for quantifying the amount of carbon in the gut is illustrated in Figure 4.

Statistical Analysis

All radioactivities and insect weights are reported as means \pm one standard deviation (s.d.). The number of observations (n) in each mean (\bar{x}) is also shown. In the graphs the logarithm of ^{137}Cs activity (dependent variable) is linearly regressed on time (the independent variable) and a line is fitted to the points by the least-squares method. Pertinent statistics of these regressions

are given in the appropriate Appendix. Students 't' test was used to determine the significance of any differences between regression coefficients or means, $p = 0.05$.

CHAPTER 3
ACCUMULATION AND ELIMINATION OF CESIUM-137
BY MOSQUITOES

Introduction

Extensive use of radioisotopes has been made in entomology. Crossley (1966) and Reichle (1967) used radioisotope uptake and elimination rates to estimate material and energy flow in terrestrial food chains. Pendleton (1957, 1962) has included insects in his studies of cesium-137 accumulation by various components of aquatic communities. Crossley and Schnell (1961) measured the elimination of cesium-137 and strontium-90 by grasshoppers. Getsova and Volkova (1962) studied uptake of radioisotopes by insects reared in contaminated ponds.

Radioisotope studies with mosquitoes have also been quite extensive. Several radionuclides have been used to 'tag' mosquitoes in order to study their dispersal patterns (O'Brien and Wolfe, 1963). Several authors (Bruce-Chwatt and Hayward, 1956; Hassett and Jenkins, 1951; Quraishi, 1968) have studied the uptake of phosphorus-32 by mosquitoes, and the accumulation and elimination of cesium-137 by A. aegypti has been reported by Guthrie (1969) and by Guthrie and Burzynski (1972).

Results and Discussion

Accumulation of Cesium-137 by Mosquito Larvae

The accumulation of radiocesium by A. aegypti larvae was performed for comparison with the other aedine species, as well as to compare the results of this radio-labeling technique with that of Guthrie (1969). Groups of one hundred newly hatched larvae were reared in medium containing 10,000 cpm/ml of carrier free cesium-137 at 26°C until pupation. Samples of ten larvae of each species were taken at least twice in each instar and beta counted. The mean radioactivity of larval instars 1 to 3 increased uniformly (Phase 1), then leveled off during the fourth instar (Phase 2). The results are given in Appendix A, Tables A-1 to A-5. Figure 1, a plot of the logarithm of cesium-137 activity versus time in hours, summarizes these two phases in the uptake by the five species. The best fit line for each phase was obtained by the least-squares method. The rate at which the larvae doubled their body-burden of cesium-137, T_d (doubling time), was different for the two phases. The slope of the least-squares fitted line is a measure of the rate of cesium-137 uptake. The doubling times of the two phases for each species are shown in Table 1.

Figure 1

Accumulation of ^{137}Cs by five aedine species of mosquito larvae reared at 26°C and sampled at successive periods. The regression lines of activity of the larvae for the two uptake phases are shown.

- - A. aegypti ▼ - A. atropalpus □ - A. dorsalis
○ - A. triseriatus △ - A. vexans

Figure 1

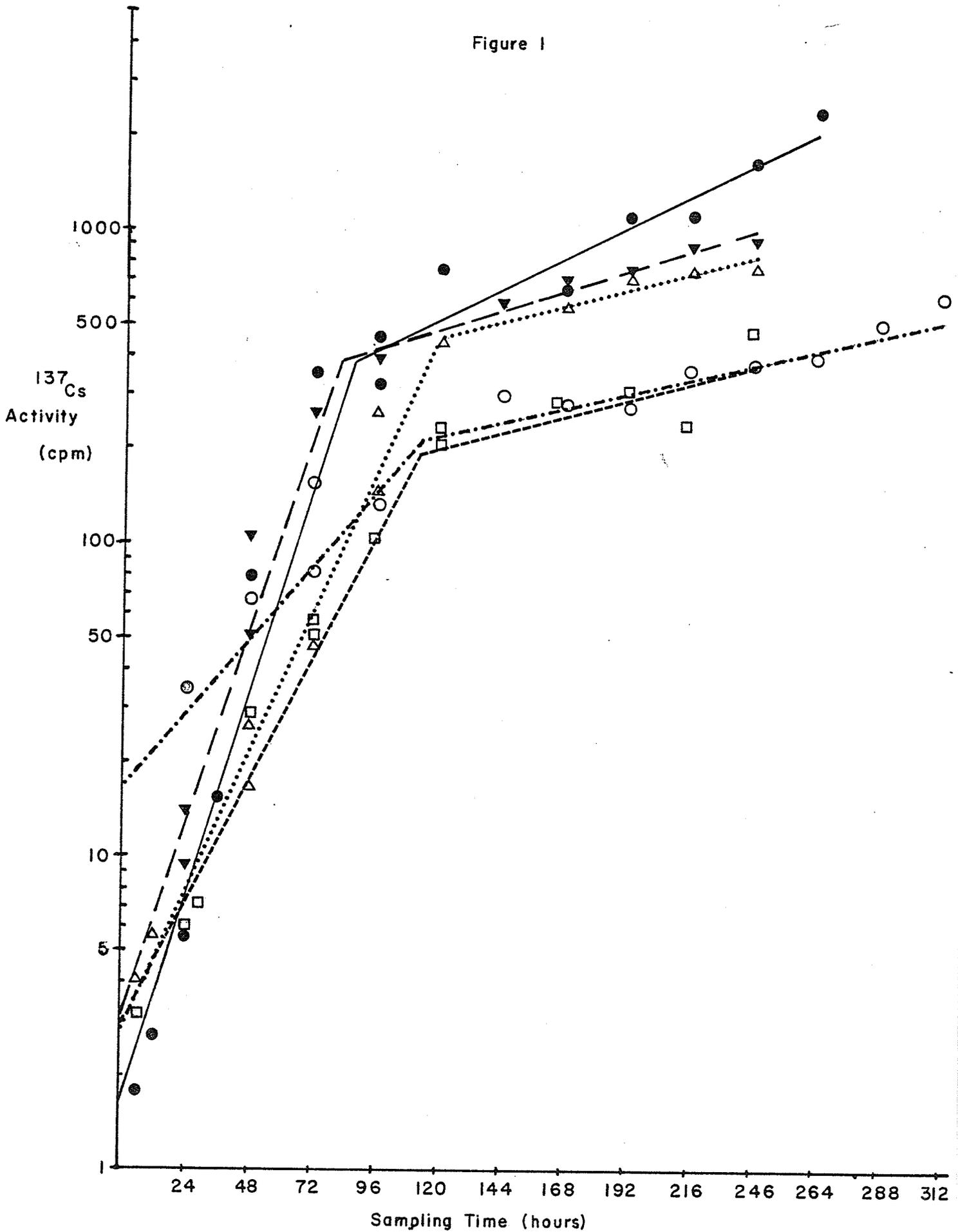


TABLE 1

Doubling times, T_d , of cesium-137 in hours for five aedine species of mosquito larvae. Development times in hours for the two phases is given.

Species	Phase 1 (Instars 1-3)		Phase 2 (Instar 4)	
	T_d	Develop. time	T_d	Develop. time
<u>Aedes aegypti</u>	11	96	74	48
<u>Aedes atropalpus</u>	12	120	116	72
<u>Aedes dorsalis</u>	19	120	131	72
<u>Aedes vexans</u>	17	96	142	72
<u>Aedes triseriatus</u>	31	144	154	96

The rate of uptake expressed as T_d is dependent on several factors: temperature, development time, and size and structure of the anal gills. Rearing temperature has a significant effect on radionuclide uptake (Davis, 1963). The T_d value obtained for A. aegypti in Phase 1 is less than that reported by Guthrie (1969) for a comparable phase of development. The rearing temperature used by Guthrie was 20°C, whereas that used in this experiment was 26°C.

In Table 1, there are apparent relationships between some of the species. A. aegypti and A. atropalpus have doubling times that are not statistically different, $P > 0.05$ in Phase 1. In Phase 2, however, T_d is significantly different for these species. This difference is attributed to the longer development time of A. atropalpus

in instar 4 (Table 1).

Another factor affecting the rate of nuclide uptake of these two species is the relative size and structure of their anal gills. The importance of the anal gills as a site of cation uptake has been shown by Wigglesworth (1938), Stobbart (1965) and others and will be further discussed in Chapter 4. Both A. aegypti and A. atropalpus have anal gills that are larger than the anal segment (up to four times the size) and broad in structure. The other three species have much narrower gills which are never larger than the anal segment. There is also a similar relationship between the A. dorsalis and A. vexans uptake phases (Table 1). A. dorsalis has anal gills only 1/3 as long as the anal segment while A. vexans has gills as long as the anal segment. Because of their importance in ionic control, shorter anal gills should result in a slower cesium-137 uptake rate and hence a longer T_d .

Elimination of Cesium-137 by Mosquito Larvae

Fourth instar larvae, that had been reared in cesium-137 medium, were transferred to non-labeled medium and samples of ten larvae were taken during the first 24 hours. The mean activity of the larvae decreased uniformly during the first 12 hours at which time it leveled off (Appendix B, Tables B-1 to B-5). Figures 2a to 2e show the logarithm of ^{137}Cs activity versus sampling time in hours. Considering Figure 2a in detail, it will be seen

Figure 2a

Elimination of ^{137}Cs by 4th instar A. aegypti larvae at 26°C . Points plotted are means \pm one standard deviation. Biological half life for assimilated and unassimilated ^{137}Cs is determined for the lines marked A and B respectively.

Figure 2a

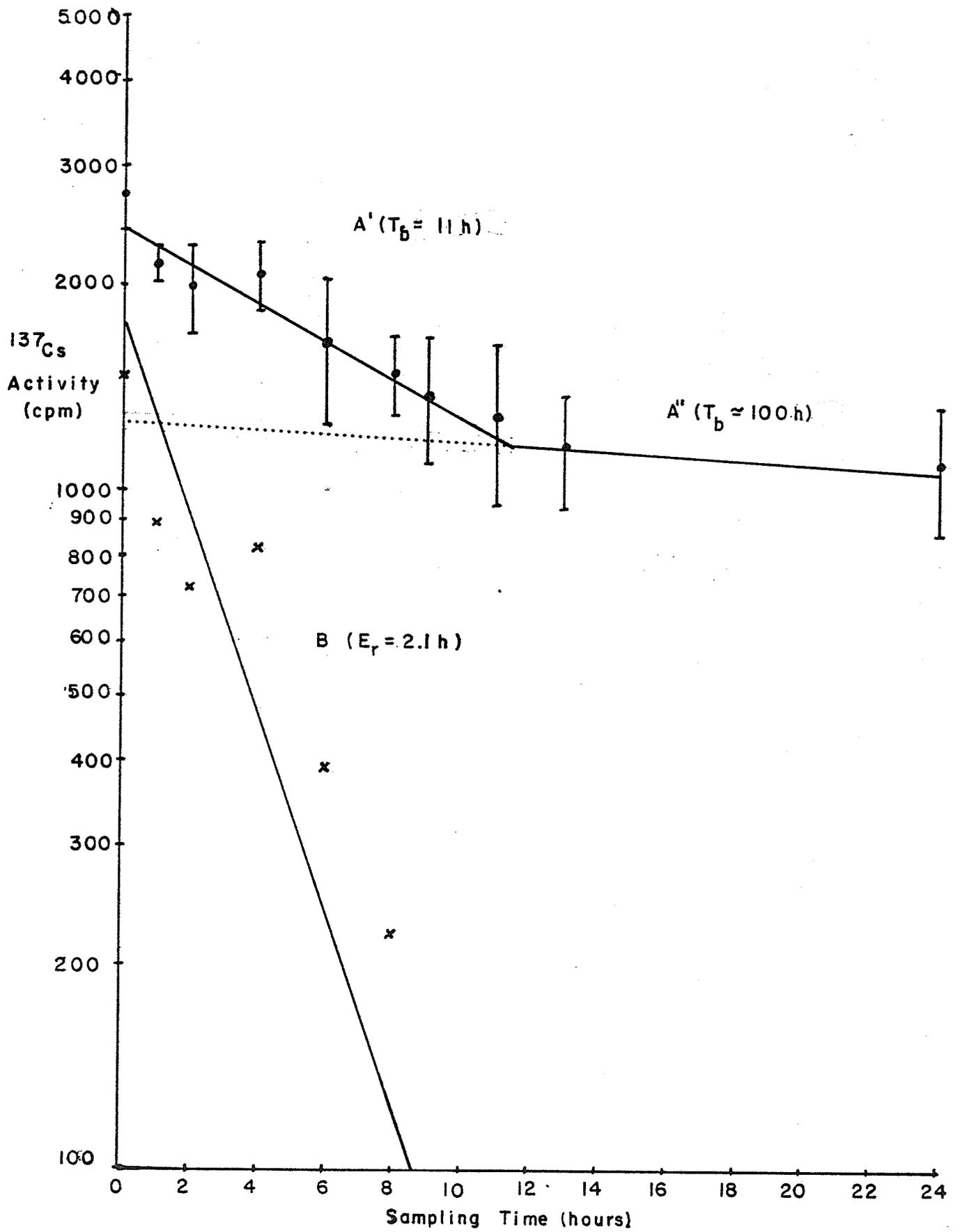


Figure 2b

Elimination of ^{137}Cs by 4th instar A. atropalpus larvae at 26°C . Points plotted are means \pm one standard deviation. Biological half life for assimilated and unassimilated ^{137}Cs is determined for the lines marked A and B respectively.

Figure 2b

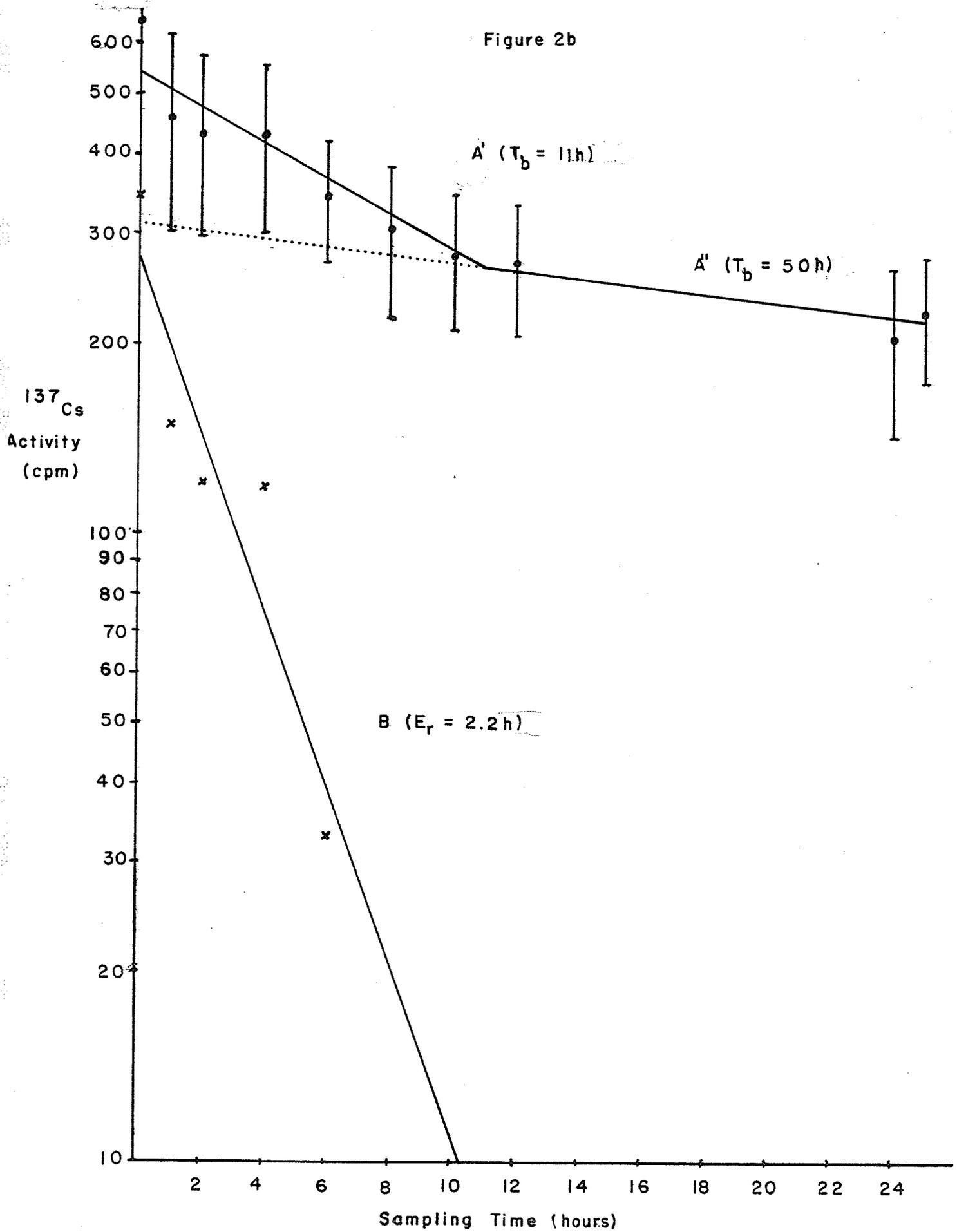


Figure 2c

Elimination of ^{137}Cs by 4th instar A. dorsalis larvae at 26°C . Points plotted are means \pm one standard deviation. Biological half life for assimilated and unassimilated ^{137}Cs is determined.

Figure 2c

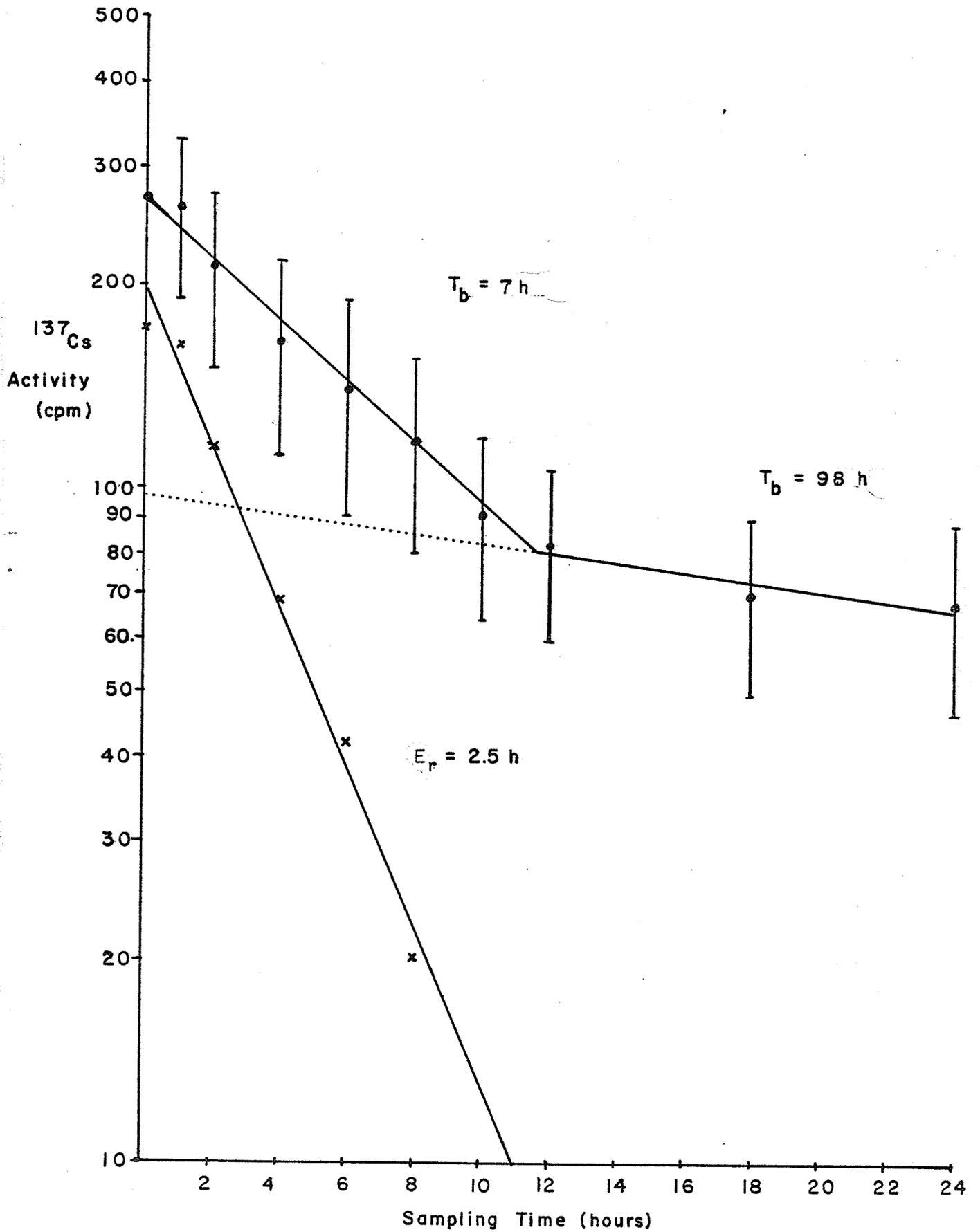


Figure 2d

Elimination of ^{137}Cs by 4th instar A. triseriatus larvae at 26°C . Points plotted are means \pm one standard deviation. Biological half life for assimilated and unassimilated ^{137}Cs is determined.

Figure 2d

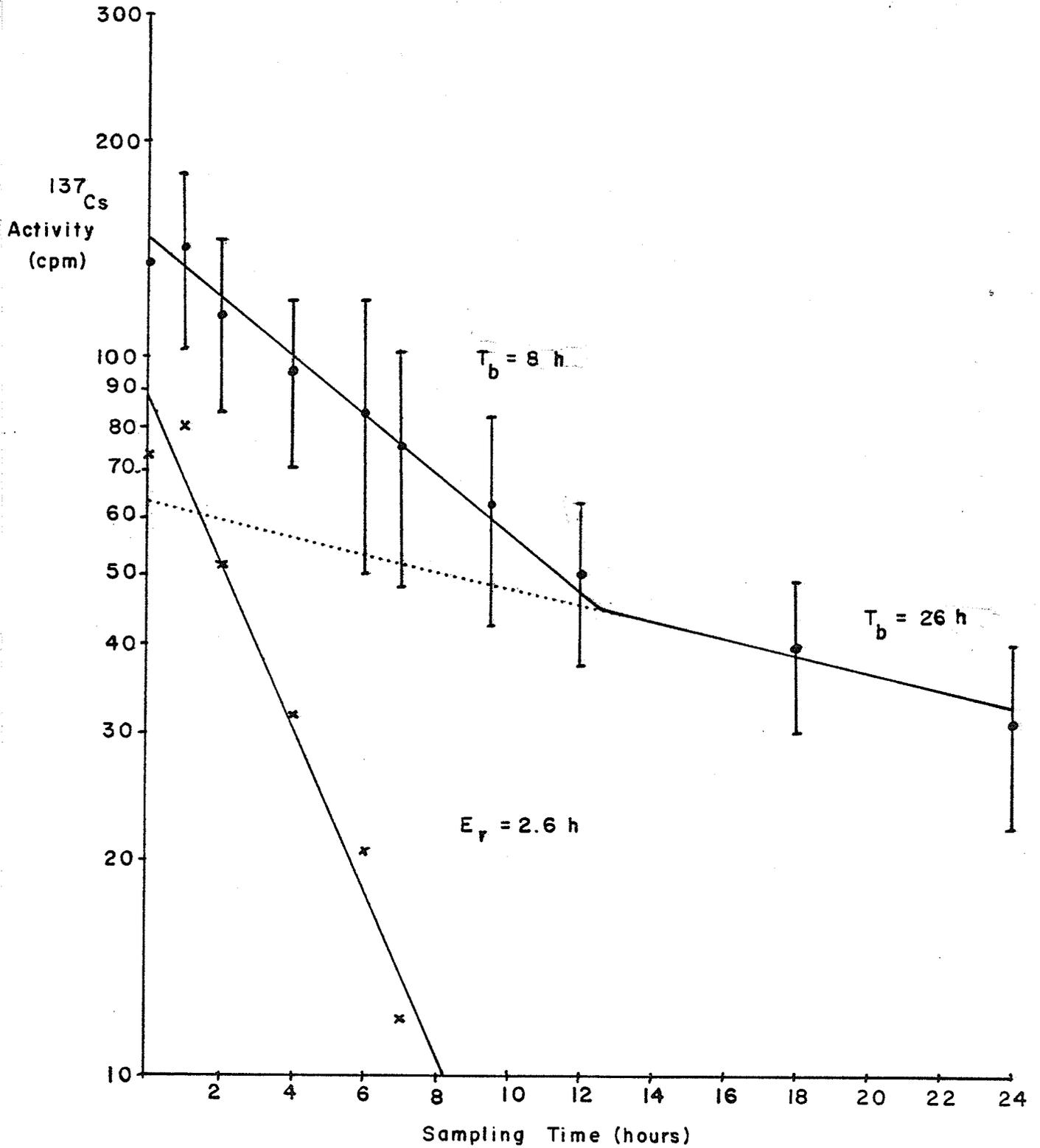
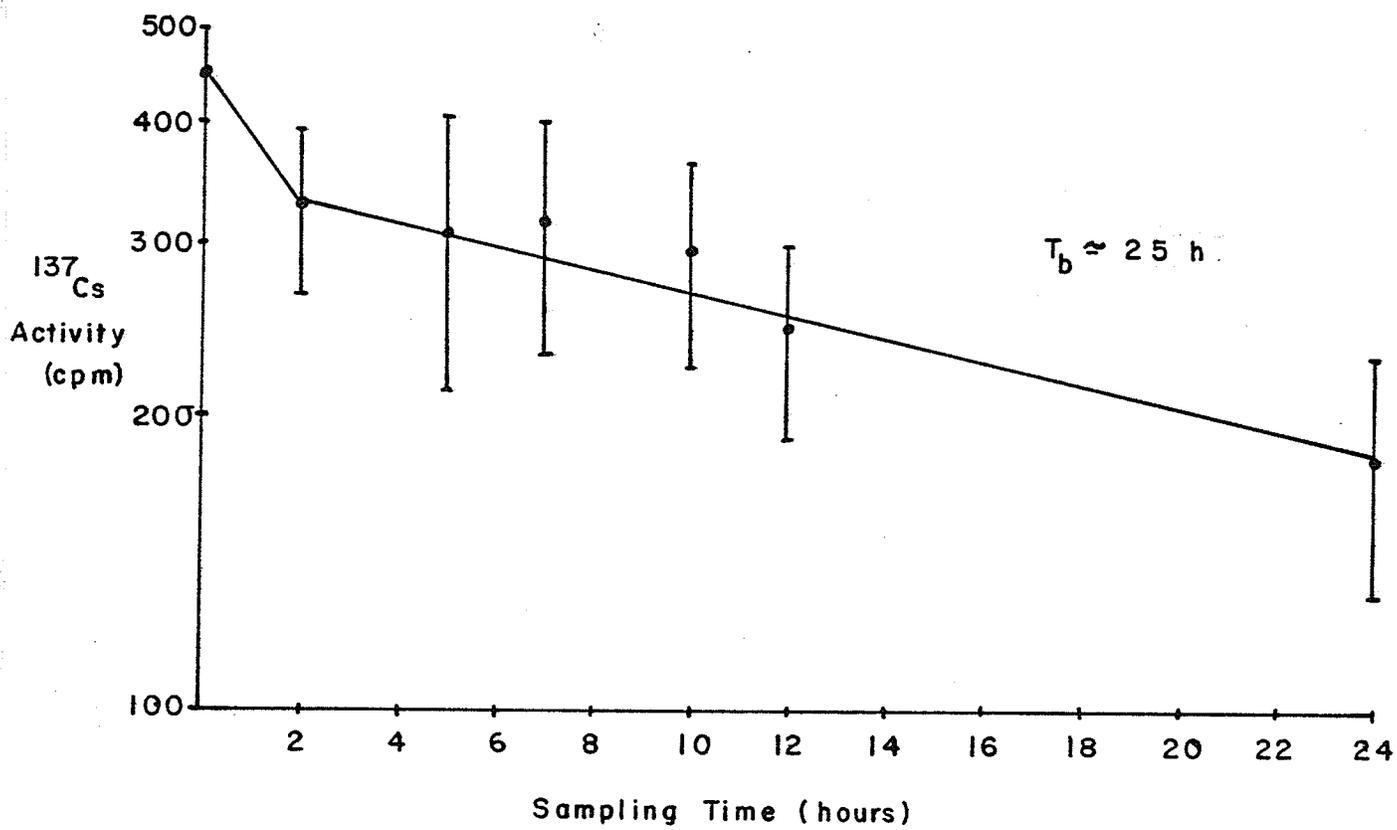


Figure 2e

Elimination of ^{137}Cs by 4th instar A. vexans larvae at 26°C . Points plotted are means \pm one standard deviation. Biological half life for assimilated ^{137}Cs is determined.

Figure 2 e



that the curve consists of two distinct components. One part (A') slopes steeply over the period 0-12 hours, and then tapers off (A"). According to Crossley (1960) the first component reflects the combined elimination rates (T_b) of assimilated and unassimilated radionuclide. Therefore, in order to obtain estimates of the elimination rates for these fractions, curve A" (Fig. 2a) was 'peeled' (Ore, 1963) by extrapolating to zero time, and the appropriate increment subtracted from the activities measured during the interval from 0 to 12 hours. The remainders are plotted as curve B in Figure 2a, from which the elimination of the unassimilated fraction of ingested radiocesium is calculated. The same procedure was used for the other aedine species. Curve A" represents the elimination of assimilated cesium-137. A summary of this data is given in Table 2.

TABLE 2

T_b and E_r (in hours) for the elimination of cesium-137 by mosquito larvae. A summary of Figures 2a to 2e and Appendix B. See text for explanation of T_b and E_r .

Species	T_b (assimilated)	E_r (unassimilated)
<u>A. aegypti</u>	100	2.1
<u>A. atropalpus</u>	50	2.2
<u>A. dorsalis</u>	98	2.5
<u>A. triseriatus</u>	26	2.6
<u>A. vexans</u>	25	--

The elimination rates of the unassimilated fraction of ingested radiocesium (E_r) for the five species are not significantly different, $P > 0.05$. E_r is indicative of the time required to empty the gut and is proportional to gut size. Since all the larvae were approximately the same during the fourth instar, these values should not be significantly different.

The biological half life of assimilated cesium-137 (T_b) shows species differences. T_b is an indication of the time required for the elimination of cesium-137 that has been incorporated into the tissues. This value varies for the different species because it is proportional to the permeability of the gut wall, metabolic activity, tissue structure, ionic balance control, etc. These factors vary in different species and are difficult to determine.

The T_b of 100 hours for A. aegypti at 26°C is in good agreement with Guthrie's (1969) value of 103 hours at 20°C. This is in contrast to the different T_d values for A. aegypti at the same temperatures. This finding suggests, therefore, that unlike uptake, elimination of ^{137}Cs may not be temperature dependent. Furthermore, it supports Wigglesworth's (1938) argument that the anal gills are not involved in elimination. Gut permeability and malpighian tubule function should be less temperature sensitive than the anal gills, the structures involved in the maintenance of ionic balance.

Uptake of Cesium-137 by Pupae

In the pupal stage an insect does not feed (Wigglesworth, 1964). The pupal stage is often dismissed as an inert resting stage between the larva and the adult. However, aquatic pupae are provided with a means of breathing and regulation to the surrounding water (Oldroyd, 1968).

Newly moulted pupae were placed in a medium containing $10,000 \pm 5\%$ cpm/ml carrier free cesium-137. After 24 hours the pupae were washed with distilled water and transferred to non-labeled water. The adults which emerged were killed immediately and attached to stainless steel planchets for beta counting. Pupal exuviae and the emergence water were also sampled and beta counted. A. aegypti pupae of both sexes served as controls, and were counted to obtain the background level. The data is summarized in Table 3.

The results of the pupal uptake study were generally inconclusive, due to the sample size. In all species except A. vexans, the activity of the females was significantly greater ($p = 0.05$) than that of the males. This finding reflects the larger mass of the females (Guthrie, 1969). The mean activities of males, females, and exuviae of each species were not significantly different ($p = 0.05$) from the controls. This result implies that little or no uptake of cesium-137 occurs during the pupal stage. The activity of the pupal exuviae is probably due to sorption of radiocesium.

TABLE 3

Cesium-137 uptake by mosquito pupae. Mean activity in counts per minute after 24 hours in cesium-137 medium.

Species	Sample	Mean Activity	Number
<u>Aedes aegypti</u>	male	0.53 ± 0.42	10
	female	1.06 ± 0.65	10
	exuviae	0.15 ± 0.06	10
<u>Aedes atropalpus</u>	male	0.95 ± 0.47	10
	female	1.32 ± 0.44	6
	exuviae	0.35 ± 0.26	10
<u>Aedes dorsalis</u>	male	0.83 ± 0.32	6
	female	1.57 ± 0.61	6
	exuviae	0.15 ± 0.08	6
<u>Aedes triseriatus</u>	male	1.56 ± 1.23	5
	female	1.70 ± 1.26	5
	exuviae	0.22 ± 0.19	5
<u>Aedes vexans</u>	male	4.96 ± 4.80	3
	female	1.36	1
	exuviae	1.06 ± 0.57	5
<u>Controls</u> (<u>A. aegypti</u>)	male	0.42 ± 0.58	6
	female	1.57 ± 1.95	6
	exuviae	0.14 ± 0.23	5

Conclusions

In the cesium-137 uptake studies, all five aedine species of mosquito larvae exhibited a two phase accumulation curve. The rate of uptake for each species depends on temperature, development time, and on size and structure of the anal gills. For those species which have the above factors in common, the accumulation rates are similar. Such is the case in Phase 1 (instars 1-3) for A. aegypti and A. atropalpus, and for A. dorsalis and A. vexans. The Phase 2 (instar 4) doubling time indicates a decrease in food consumption prior to moulting for all five species. The T_d values for a given temperature are not the same for all species within a genus.

Mosquito larvae eliminate cesium-137 in two distinct fractions: 1) assimilated radiocesium, and 2) unassimilated radiocesium. There was no significant difference in the elimination rates of unassimilated cesium-137 by the five species studied. The biological half life of assimilated cesium-137 showed species differences. These result from differences in the permeability of the gut, metabolic activity, and tissue structure, etc. The anal gills are not involved in elimination. The gut permeability and malpighian tubule function of A. aegypti are not temperature dependent, with respect to radiocesium.

The cesium-137 pupal uptake studies suggest that little or no uptake occurs during the pupal stage. The apparent uptake of radiocesium was probably due to surface contamination.

CHAPTER 4

THE EFFECT OF POTASSIUM ION AND THE REMOVAL OF THE ANAL
GILLS ON CESIUM-137 ACCUMULATION AND ELIMINATION BY
FOURTH INSTAR MOSQUITO LARVAEIntroduction

Cesium and potassium show similarities in metabolic processes because of the fact that both are in Group 1 of the periodic table. The ecological relationships of potassium and cesium have been reviewed by Davis (1963). There is a possibility of potassium competing with cesium in a manner similar to calcium and strontium competition. The amount of radiocesium accumulated by an organism may be affected by the concentration of potassium in its environment. Suppression of cesium-137 uptake by potassium has been reported for algae (Morgan and Meyer, 1953), higher plants (Menzell and Heald, 1955), and some vertebrates (Mraz, 1959). Its suppression of cesium-137 uptake by mosquito larvae has been reported by Guthrie and Burzynski (1972).

Aquatic invertebrates can control the volume and composition of their body fluids by ion regulation. Osmoregulation in larvae is achieved by the body surface and gut lining (both of which transport ions and water between the environment and the interior of the body) and the excretory organs. Excretory systems play an important part in maintaining the body fluid concentrations of

aquatic larvae by conserving or excreting substances already present in the blood.

Wigglesworth (1938) demonstrated the osmoregulatory function of the anal gills of mosquito larva, and Stobbart (1959, 1960) showed that about 90% of the ionic exchange takes place through these organs. When the anal gills were destroyed by treatment with a 5% NaCl solution, the Na^+ concentration in the larvae dropped drastically. If the gills were left intact but the gut was blocked, the Na^+ concentration was reduced by a "relatively small amount" (Stobbart, 1959). This suggests that most of the ion exchange takes place via the anal gills, and the remainder through the gut (Stobbart, 1959; Treherne, 1954). Thus, the influx via the anal gills is larger than efflux.

Methods

The following series of experiments were performed to determine the route of radiocesium accumulation by mosquito larvae. Newly emerged fourth instar larvae were divided into four groups which were treated as follows:

- (1) Normal larvae in ^{137}Cs labeled medium (N),
- (2) Normal larvae in ^{137}Cs labeled medium plus K^+ (NK^+),
- (3) Degilled larvae in ^{137}Cs labeled medium (D),
- (4) Degilled larvae in ^{137}Cs labeled medium plus K^+ (DK^+).

Fourth instar A. aegypti larvae were used because earlier instars did not survive degilling. The degilling methods

are described in Chapter 2. A 0.05M potassium chloride solution was used for the K^+ medium (Guthrie and Burzynski, 1972). Larvae were sampled every 2 hours during the first 12 hours and at 18 and 24 hours thereafter. The mean larval activities are given in Appendix C, Tables C-1, C-2, C-3.

The first two experiments were performed using chemically degilled larvae. The chemical method had adverse effects on larval survival; in some groups all the larvae died within 12 hours (Appendix C, Table C-1 and C-2). The surgical degilling method was used in the third experiment and resulted in high larval survival. Therefore, only the results of the third experiment are discussed. These data are plotted in Figure 3.

RESULTS AND DISCUSSION

Effect of K^+ and Degilling on Uptake of Cesium-137

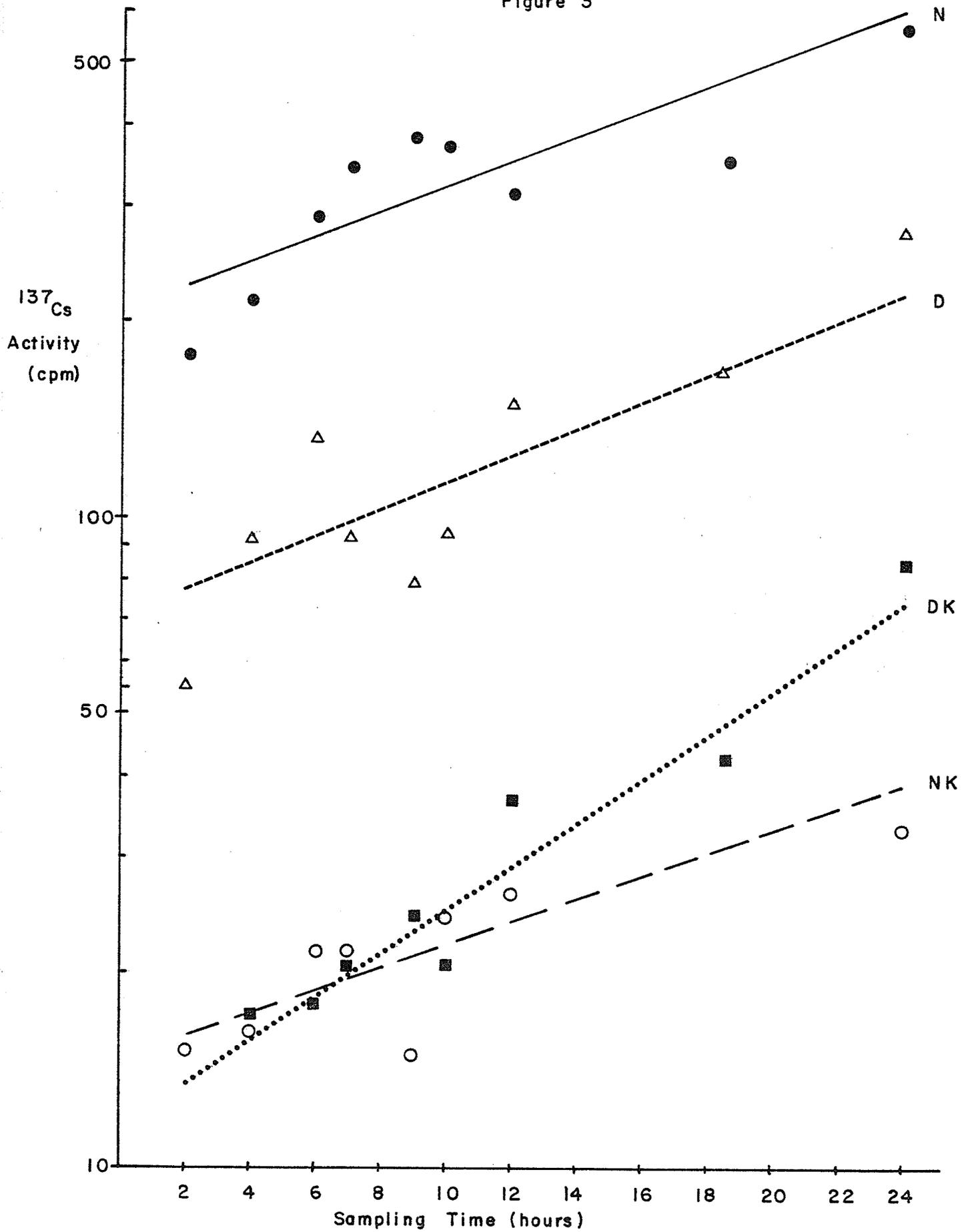
Comparing the normal larvae reared in K^+ free medium with those reared in K^+ medium, there is a drastic reduction in the amount of cesium-137 accumulated in the presence of potassium. If the major site of radiocesium uptake is the anal gill, this reduction in the presence of K^+ would be the result of competition for transport sites across the gill membrane, suggesting that the anal gills discriminate between K^+ and Cs^+ . This apparent discrimination may be the result of isotopic dilution because the accumulation rates are comparable ($p > 0.05$).

Figure 3

The effect of potassium ion and degilling on the uptake of ^{137}Cs by 4th instar A. aegypti reared at 26°C .

- - Normal larvae (N)
- - Normal larvae with K^+ (NK)
- △ - Degilled larvae (D)
- - Degilled larvae with K^+ (DK)

Figure 3



Comparison of degilled and normal larvae reared in K^+ free medium showed that their regression coefficients were not significantly different ($p > 0.05$). However, there was a difference in the magnitude of the cesium-137 body burden attained. For the degilled larvae, the site of uptake must be the gut. Since the reduction is less than has been previously reported (Stobbart, 1959), the assumption is made that the gut increased its uptake to compensate for the loss of transport via the anal gills. This assumption was substantiated in the next experiment.

When considering the accumulation curve for the degilled larvae in K^+ medium (Figure 3), the rate of accumulation is significantly greater ($p < 0.05$) than that for the other three treatments. The degilling process resulted in a greater radiocesium uptake via the gut in the presence of potassium. This observation suggests that the gut does not distinguish between potassium and cesium ions to the same extent as do the anal gills.

The Effect of Degilling on Uptake of Carbon Particles through the Gut

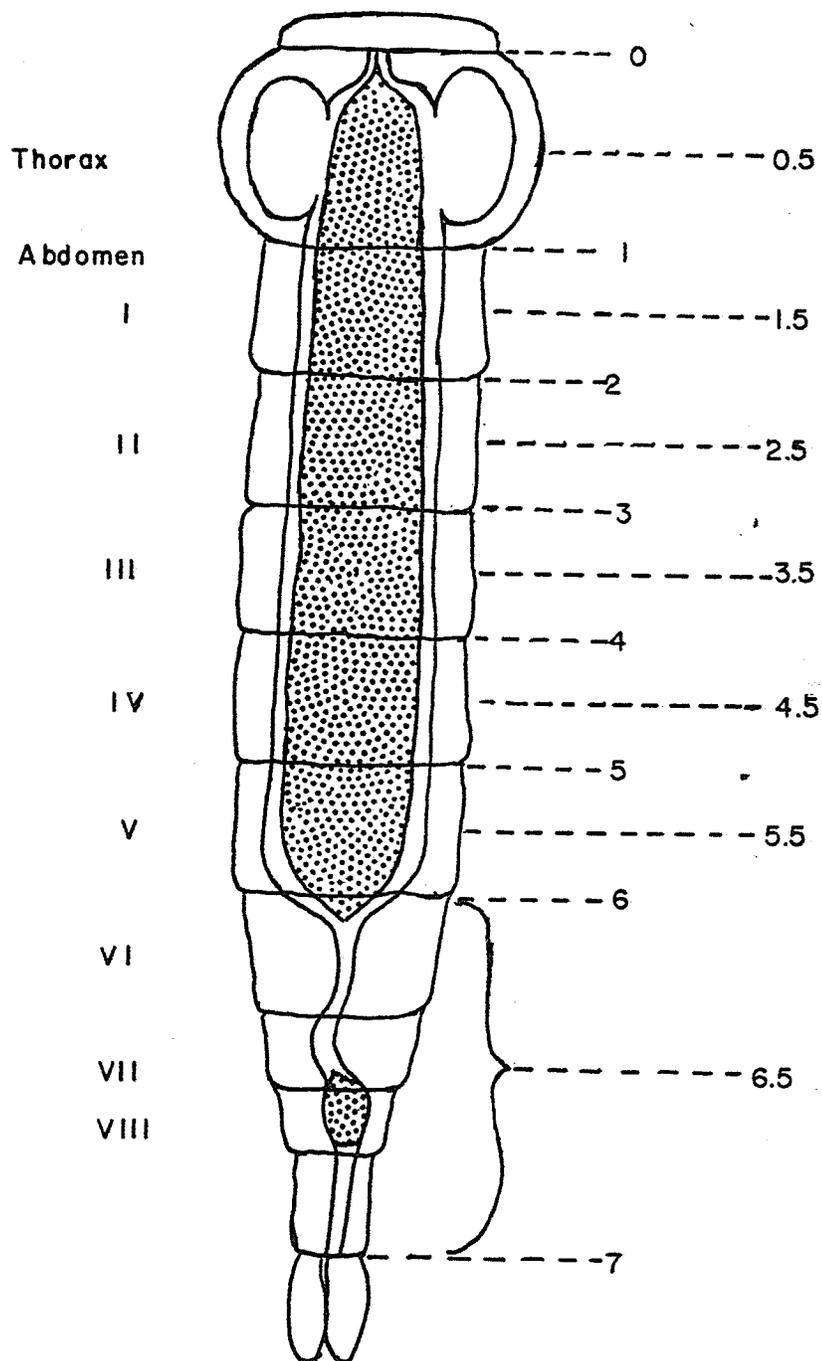
This experiment was performed to test the earlier finding that removal of the anal gills resulted in an increased uptake by the gut. Fourth instar larvae were degilled surgically and placed in a medium containing carbon particles. The larvae were periodically sampled and given a displacement value depending on the amount of carbon in

the gut. Figure 4 shows the displacement values of a larval gut filled with carbon. The mean displacement values of ten larvae sampled for 24 hours (Appendix C, Table C-4) is plotted as the logarithm versus time in hours (Fig. 5). Initially the degilled larvae accumulate carbon particles at a faster rate than the normal larvae, because of increased feeding activity. The higher rate for degilled larvae decreased with time and they were surpassed by the normal larvae whose guts become filled completely in less time. The initial larger rate of gut uptake by degilled larvae would result in a greater radiocesium accumulation which would explain the similarity between the two accumulation curves of the normal and degilled larvae in the K^+ free medium.

Effect of K^+ and degilling on elimination of cesium-137

The following experiment was performed to determine the effect of K^+ and removal of the anal gills on cesium-137 elimination by mosquito larvae. The larvae were reared in a cesium-137 medium until the fourth instar, degilled, and placed in distilled water and allowed to eliminate their radiocesium body burden. Figure 6 shows the least squares fitted lines of the regression of logarithm ^{137}Cs on time for the four treatments as used previously (Appendix C, Table C-5). Statistically there were no significant differences ($p > 0.05$) between the regression coefficients of the four treatments in the elimination rates. This result is to be expected since

Figure 4



Displacement values for a carbon gutted mosquito larva

Figure 5

The effect of degilling on carbon particle uptake by
4th instar A. aegypti larvae.

Figure 5

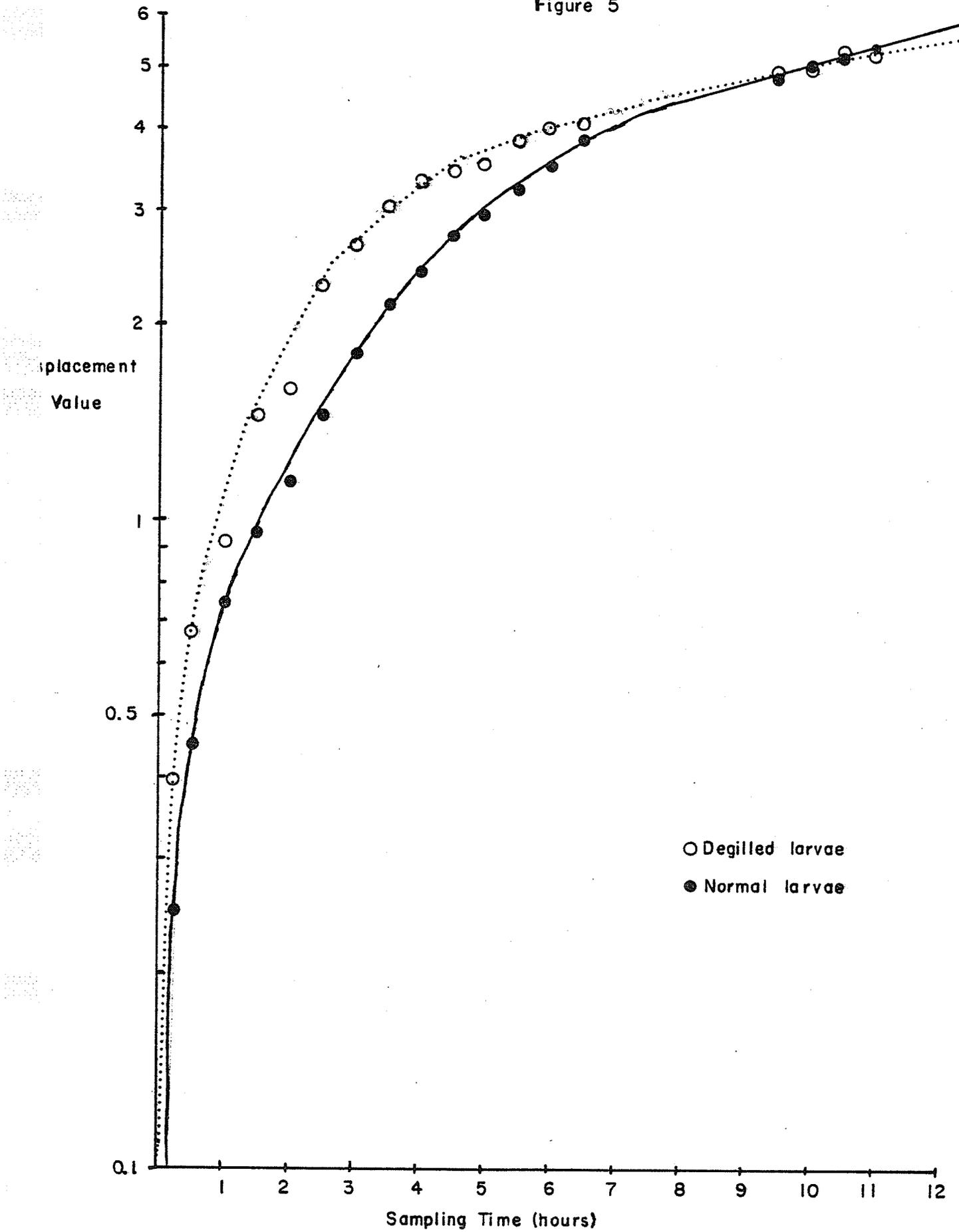
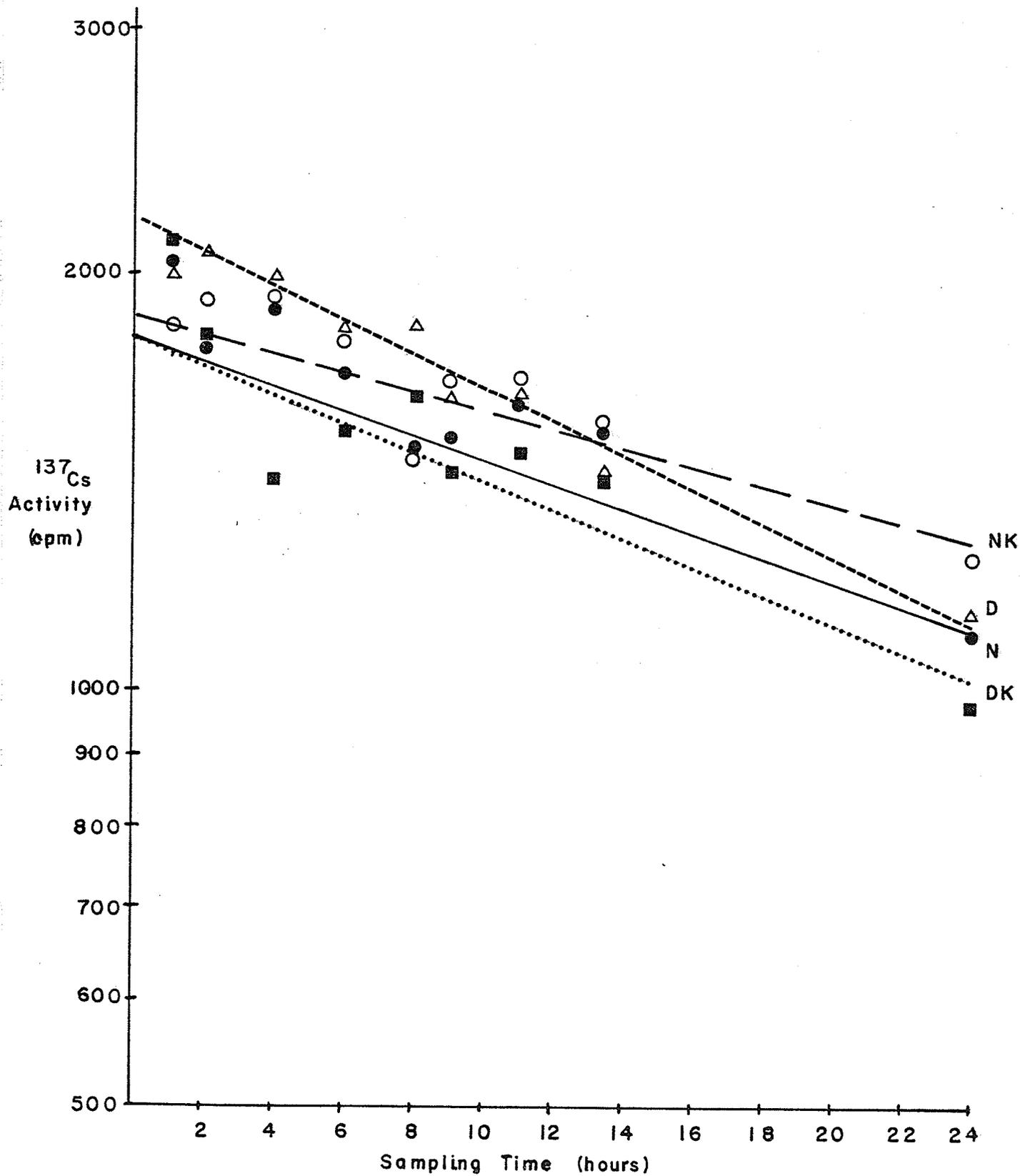


Figure 6

The effect of potassium ion and degilling on the elimination of ^{137}Cs by 4th instar A. aegypti larvae at 26°C . Regression lines for the larval activities are drawn.

- - Normal larvae (N) ○ - Normal larvae with K^+ (NK)
△ - Degilled larvae (D) ■ - Degilled larvae with K^+ (DK)

Figure 6



neither external K^+ concentration nor the anal gills should affect elimination via the malpighian tubules and hind gut.

Conclusions

From the studies with potassium ion and degilling, the following conclusions can be drawn. Potassium in the rearing medium reduces the body burden of cesium-137 achieved by mosquito larvae, but it does not affect the rate of accumulation of radiocesium. Degilling larvae also resulted in a reduction of the amount of ^{137}Cs accumulated but to a lesser extent than potassium ion. The gut uptake of degilled larvae increased with the result that there was no significant change in the rate of accumulation compared with normal larvae. The gut membrane of mosquito larvae may not be able to distinguish between potassium and cesium ions. Neither K^+ nor degilling has any affect on elimination of cesium-137 by mosquito larvae.

CHAPTER 5

UPTAKE AND ELIMINATION OF CESIUM-137 BY NOTONECTA
UNDULATA, A LARVAL MOSQUITO PREDATORIntroduction

Mosquito larvae are preyed upon by both arthropods and fish. Excluding fish, the most important predators of mosquito larvae are aquatic Hemiptera and Coleoptera, especially Notonectidae and Dytiscidae (Hinman, 1934). Christophers (1960) noted that Notonecta spp. actively destroy larvae.

Notonecta undulata Say is one of the most widely distributed backswimmers in North America. Hinman (1934) considered it an important mosquito predator in permanent and semi-permanent habitats. Adult backswimmers consistently selected mosquito larvae and pupae over six other prey types (Ellis and Borden, 1970).

The backswimmer Notonecta undulata Say was chosen to study the uptake of cesium-137 by a predator of mosquito larvae to determine the possibility of biological magnification along food chains, and to investigate the application of the radioactive tracer technique to measure food consumption by a predator insect with piercing-sucking mouthparts.

Methods

Adult backswimmers were collected in the spring soon after the ponds were free of ice. They were colonized

in the laboratory using the method described by Ellis and Borden (1969). Newly laid eggs hatched in about a week. The procedure for determining the accumulation and elimination rates of cesium-137 by both immature and adult backswimmers is described in Chapter 2.

Results and Discussion

Nymph Uptake

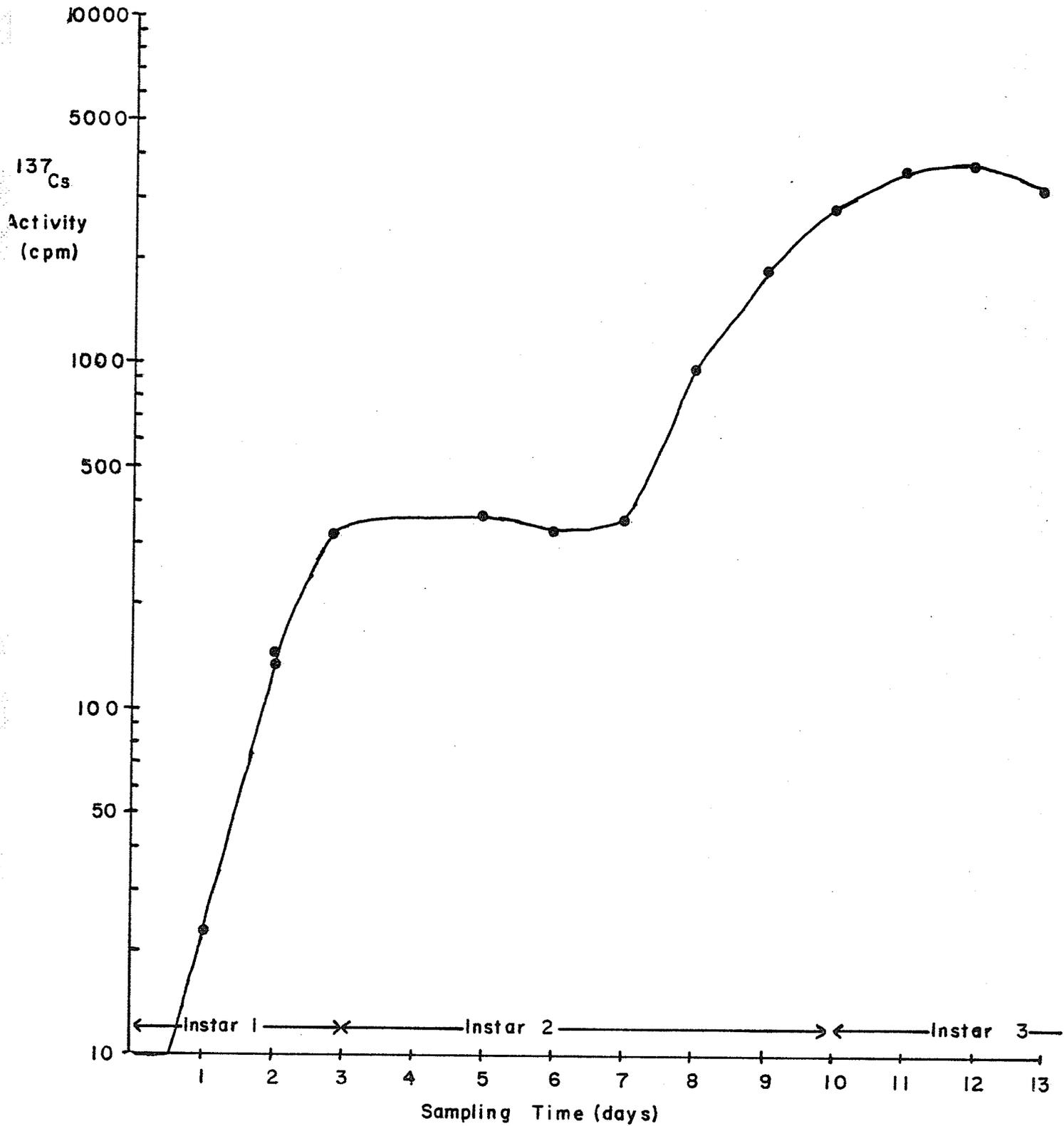
A group of 15 first instar nymphs were obtained from eggs hatched in the laboratory. The water temperature was maintained at 25°C during the experiment. The nymphs were reared individually in 100 ml beakers and fed ten radio-cesium labeled mosquito larvae, daily. Each nymph was counted on a gamma spectrometer and fed immediately afterwards. High mortality occurred during the molting and none of the backswimmers survived through the third instar. The daily handling of the nymphs for weighing and counting could also have attributed to the observed mortality. To reduce the amount of handling, weighing was discontinued and weights were only obtained after death. Prior to daily feeding a random sample of six mosquito larvae was taken from the 'feed-stock'. These specimens were weighed and then gamma, and beta counted. Samples of the larval exuviae and nymph rearing medium were also counted.

The logarithm of the mean nymph activities (Appendix D, Table D-1) was plotted against time in days (Figure 7). The plot adopts a step-shape, increasing

Figure 7

Uptake of ^{137}Cs by various instars of N. undulata fed ^{137}Cs labeled mosquito larvae.

Figure 7



rapidly during the early part of the first instar, then levelling off in the latter portion of the first instar. The same is also true for the 2nd instar. This type of graph indicates very rapid food consumption after molting and a reduction in food uptake prior to molting. This result agrees with the finding of Guthrie (1969), who studied the radiocesium uptake by immature Lethocerus americanus. The second experiment using nymphs was a disaster. All the nymphs died during the first instar.

Uptake and Elimination of Cesium-137 by Adult Notonecta undulata Say

Individual adult backswimmers from the laboratory colony, were gamma counted and fed cesium-137 labeled mosquito larvae daily. After reaching a steady-state, the adults were fed non-labeled mosquito larvae in order to determine their rate of cesium-137 elimination. The results (Appendix D, Table D-2) are plotted in Figures 8 and 9 which show the logarithm of cesium-137 activity versus time in days. The accumulation rate for cesium-137 expressed as doubling time (T_d) was 1.1 days. The backswimmers reached steady state after seven days of labeled feeding, that is, their cesium-137 body burden did not increase significantly. The value of T_d at steady state was calculated to be 27 days.

The elimination phase results are plotted in Figure 9. This figure suggests that radiocesium was

Figure 8

Uptake of ^{137}Cs by adult N. undulata fed ^{137}Cs labeled mosquito larvae. Means \pm one standard deviation are plotted. Biological doubling time (T_d) is shown.

Figure 8

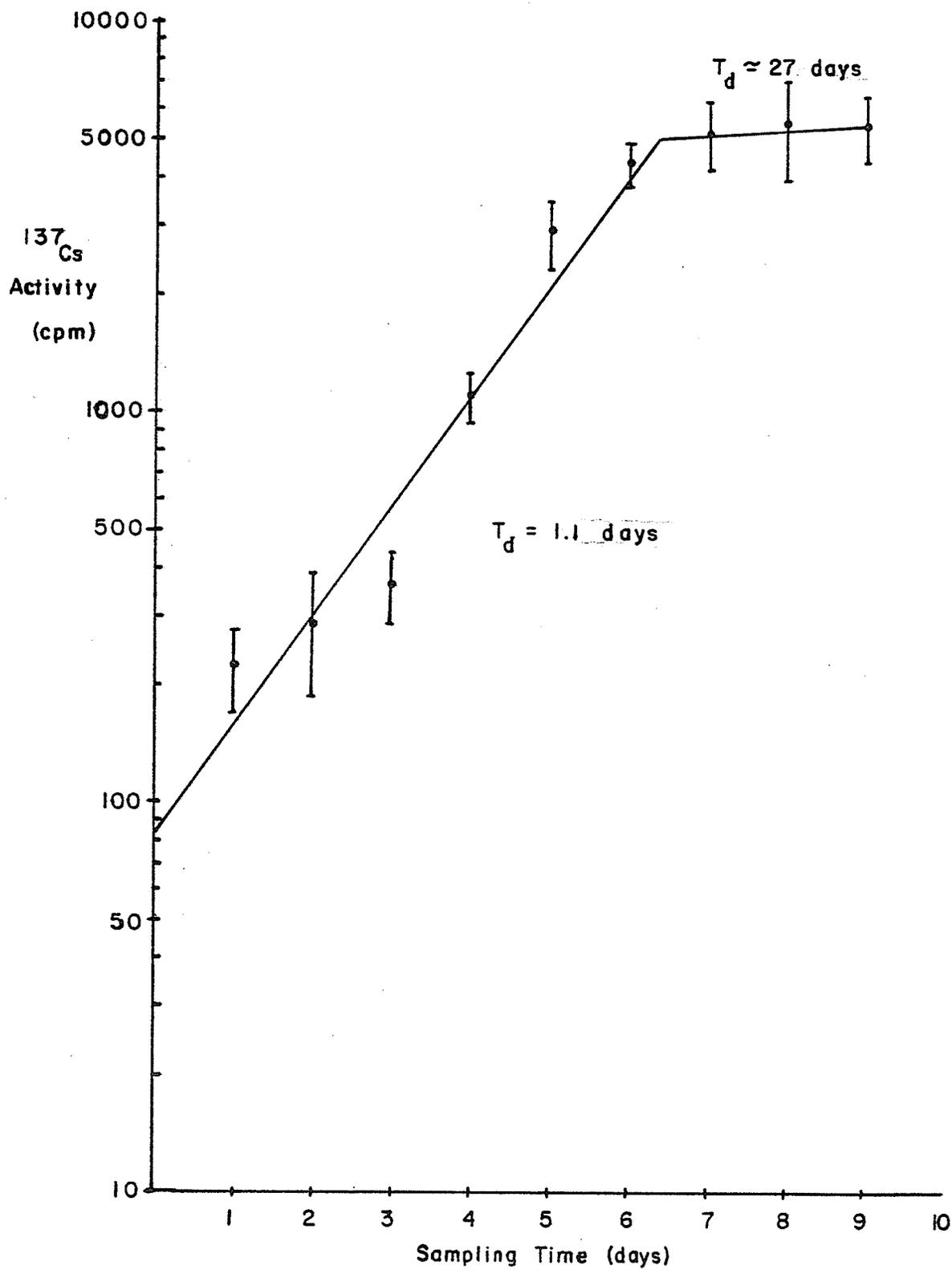
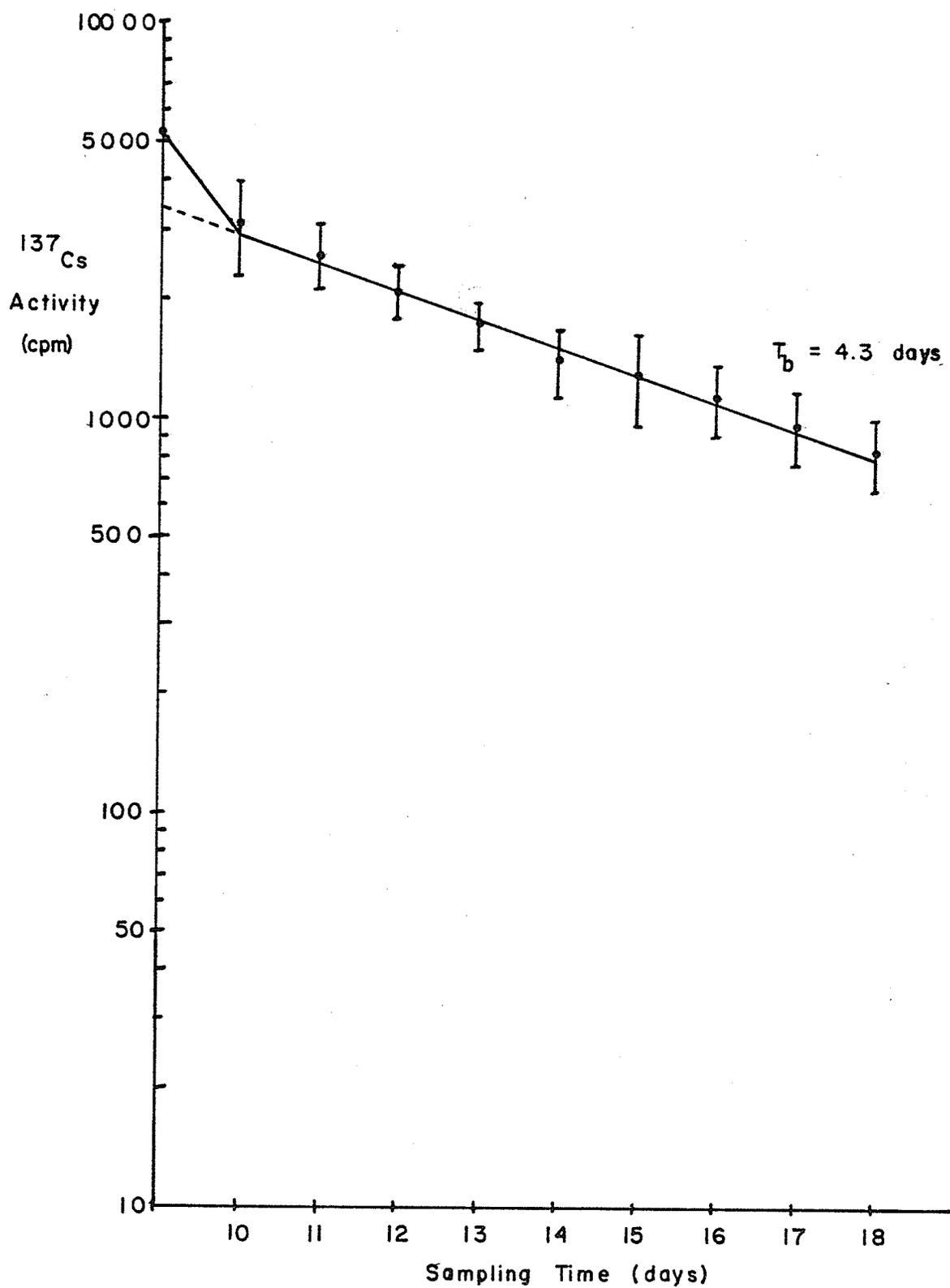


Figure 9

Elimination of ^{137}Cs by adult N. undulata. Means \pm one standard deviation are plotted. The adults were fed non-labeled mosquito larvae. Biological half life is determined.

Figure 9



eliminated at two rates by adult N. undulata, a finding similar to that obtained by Guthrie and Brust (1969) for adult L. americanus. The initial elimination phase is very rapid and has a biological half life, $T_b = 1.1$ days. This T_b value is assumed to represent the T_b of non-assimilated radiocesium and reflects the insect's 24 hour feeding schedule. A single elimination rate would be indicative of complete assimilation of cesium-137 (Crossley and Howden, 1961; Crossley, 1963). The latter part of the graph (Figure 9) has a longer half life, $T_b = 4.3$ days and represents elimination by the insect of assimilated radiocesium. It has been postulated that the biological half life for radiocesium is related to the insect's size (Crossley, 1963). That is, a small insect would have a shorter T_b than a larger one. This can be explained by the fact that the smaller insect has a higher metabolic rate than a large insect. Consequently, less time would be required to eliminate a given amount of label. The T_b of 4.3 days for N. undulata measured in this work and that of 10.8 days reported for a larger insect, L. americanus (Guthrie and Brust, 1969), supports Crossley's (1963) hypothesis.

Measurement of Food Consumption

Using the same methods as Guthrie and Brust (1969), the following calculations were performed with N. undulata adults.

$$I = Q_b \lambda_b + Q_g \lambda_g = (b \lambda_b + g \lambda_g) Q$$

where I is the ingestion or feeding rate, b is the fraction of Q (total body burden) taken into the body, and g is the fraction of Q remaining in the gut. From Figure 9, values of b and g at zero time are calculated to 0.54 and 0.46 respectively. Therefore $I = (0.54\lambda_b + 0.46\lambda_g) Q$. The elimination rates (λ 's) may be obtained from the slopes of the curves in Fig. 9:

$$\lambda_b = \frac{\ln 2}{T_b} = \frac{0.693}{4.3} = 0.163$$

$$\lambda_g = \frac{0.693}{1.1} = 0.63$$

Therefore $I_b = (0.54 \times 0.163)Q = 0.088Q$

$$I_g = (0.46 \times 0.63)Q = 0.290Q$$

The fraction of nuclide assimilated (A) is:

$$A = \frac{I_b}{I_b + I_g} = \frac{Q_b \lambda_b}{Q_b \lambda_b + Q_g \lambda_g}$$

Therefore $A = \frac{0.088Q}{(0.088 + 0.290)Q} = 0.23$

Thus, it is concluded that N. undulata adults assimilated less than 25% of the radiocesium and probably less than 25% of the food which they ingested.

Having estimated the fraction of ingested food assimilated into the body, the weight of mosquito larvae consumed daily to maintain the steady-state level of ^{137}Cs may be calculated using the following equation:

$$I = \frac{Q \lambda_b}{F}$$

where I = ingestion or feeding rate,

F = fraction of ingested prey assimilated into the body,

λ_b = elimination rate constant of the body compartment,
and

Q = total body-burden, i.e., specific activity of adult backswimmer.

For N. undulata adults, the pertinent data are:

$$Q = \frac{5200 \text{ cpm}}{72.3 \text{ g}} = 72 \text{ cpm/g adult}$$

$$T_b = 4.3 \text{ days (Fig. 9)}$$

$$F = 0.23$$

$$\lambda_b = 0.163$$

Substituting in the equation

$$I = \frac{72 \times 0.163}{0.23} = 51 \text{ cpm/g adult/day}$$

Larval mosquito consumption required to maintain this rate of cesium-137 intake was: $\frac{51}{3 \times 10^3} = 0.017 \text{ g larvae/g adult/day.}$

Conclusions

Notonecta undulata nymphs accumulated cesium-137 in a step-like fashion, with rapid radiocesium uptake soon after molting and a reduction in ^{137}Cs uptake prior to molting. This type of accumulation was not present with the adult backswimmers, which had a single accumulation

rate with a doubling time of 1.1 days. The elimination of cesium-137 by adult N. undulata had two distinct components: 1) non-assimilated radiocesium and 2) assimilated radiocesium, with biological half lives of 1.1 and 4.3 days respectively. The fraction of radionuclide assimilated was calculated to be 0.23, that is, less than 25% of the radiocesium and, presumably the ingested food, was assimilated. The larval mosquito consumption at steady-state was calculated to be 0.017 g larvae/g adult/day. The ^{137}Cs biological half life for N. undulata and that obtained for L. americanus by Guthrie and Brust (1969), 4.3 and 10.8 days respectively, supports Crossley's (1963) hypothesis that the smaller the insect the smaller the T_b . It is noteworthy that the fraction of ingested food assimilated by N. undulata, 0.23, is larger than that reported for L. americanus, 0.07 (Guthrie and Brust, 1969). It would appear, therefore, that Crossley's hypothesis may equally apply to the size of an insect and the fraction of its prey which is assimilated.

CHAPTER 6

SUMMARY

The accumulation and elimination of cesium-137 by several aedine species of mosquito larvae and a common predator has been studied.

All five aedine species of mosquito larvae showed two distinct ^{137}Cs accumulation rates (T_d). A decrease in food consumption in the 4th instar prior to molting was observed. The T_d values at the same temperature are not the same for all species within a genus. Mosquito larvae also eliminated cesium-137 in two phases. The elimination rate of unassimilated radiocesium was the same for all the larval mosquito species studied. The anal gills are not involved in elimination. Elimination is less temperature sensitive than accumulation. Little or no accumulation of radiocesium takes place during the pupal stage.

Potassium ion in the rearing medium reduced the amount of cesium-137 accumulated by mosquito larvae but it has little effect on the rate of accumulation. Removal of the anal gills also resulted in a reduction of the amount of ^{137}Cs accumulated but to a lesser extent than K^+ . When larvae are degilled, they increase their gut uptake to compensate for the loss of ion uptake via the anal gills. The gut membrane does not appear to distinguish between K^+ and Cs^+ as do the anal gills. Neither K^+ nor degilling have any effect on the elimination of cesium-137 by

mosquito larvae.

N. undulata nymphs show a reduction in food consumption prior to each molt. Adult backswimmers have a single uptake rate (T_d) for cesium-137, $T_d = 1.1$ days. As with the mosquito larvae, N. undulata adults have two elimination rates. $T_b = 1.1$ days for unassimilated cesium-137 and $T_b = 4.3$ days for assimilated radiocesium. The fraction of radiocesium assimilated was 0.23 and larval mosquito consumption at steady-state was 0.017 g larva/g adult/day. Crossley's (1963) hypothesis that the rate of elimination is inversely proportional to the size of the insect is supported. It is suggested that it equally applies to food assimilation. That is, the fraction of ingested food assimilated by an aquatic predator with piercing-sucking mouthparts is inversely proportional to the size of the insect predator.

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APPENDIX

APPENDIX A

TABLE A-1

Accumulation of cesium-137 by Aedes aegypti larvae reared in $^{137}\text{CsCl}$ medium. Specific activity of medium was 8432 cpm/ml. Rearing temperature = 26°C. ^{137}Cs activity is reported as the mean cpm \pm one standard deviation total-beta activity. Wet weights are reported as mean \pm one standard deviation.

Time (hours)	Instar	Mean Weight (mg.)	Mean activity (cpm)	Mean cpm/mg	Number
6	1st	-	1.79 \pm 0.70	-	10
12	1st	-	2.72 \pm 0.80	-	10
24	1st	0.08 \pm 0.018	5.69 \pm 2.08	71.1	10
30	2nd	0.14 \pm 0.02	15.5 \pm 3.5	110.7	10
48	3rd	0.57 \pm 0.06	79.1 \pm 16.5	138.8	10
72	3rd	0.71 \pm 0.14	347.6 \pm 98.0	489.6	10
96	3rd	0.77 \pm 0.17	323.3 \pm 49.8	419.9	10
96	4th	1.26 \pm 0.21	457.0 \pm 91.8	362.7	10
120	4th	1.74 \pm 0.45	757.0 \pm 152.3	435.1	10
168	4th	1.80 \pm 0.33	652.6 \pm 191.2	362.6	10
192	4th	1.89 \pm 0.30	1125.1 \pm 220.0	595.3	10
216	4th	1.96 \pm 0.53	1125.5 \pm 242.0	574.2	10
240	4th	2.61 \pm 0.37	1664.1 \pm 245.1	637.6	10
264	4th	2.84 \pm 0.63	2376.7 \pm 772.8	836.9	10

Regression line data:

Phase 1 (Instars 1-3)

$r = 0.9560$

$b = 0.5011$

$m = 0.0620$

Phase 2 (Instar 4)

$r = 0.8809$

$b = 5.1294$

$m = 0.0094$

APPENDIX A

TABLE A-2

Accumulation of cesium-137 by Aedes atropalpus larvae reared in $^{137}\text{CsCl}$ medium, specific activity was 8000 cpm/ml. Rearing temperature = 26°C . ^{137}Cs activity is reported as cpm total-beta activity. Activity and wet weights are reported as mean \pm one standard deviation.

Time (hours)	Instar	Mean Weight (mg.)	Mean Activity (cpm)	Mean cpm/mg	Number
24	1st	-	9.39 \pm 3.18	-	9
24	2nd	-	14.05 \pm 2.78	-	10
48	2nd	0.24 \pm 0.09	51.17 \pm 14.81	238.8 \pm 64.7	10
48	3rd	0.52 \pm 0.12	104.86 \pm 37.08	176.8 \pm 61.5	10
72	3rd	0.70 \pm 0.16	262.7 \pm 86.98	398.6 \pm 80.1	10
96	3rd	1.32 \pm 0.21	393.6 \pm 141.4	298.2 \pm 157.9	10
144	4th	1.56 \pm 0.43	587.7 \pm 242.6	376.0 \pm 131.6	10
168	4th	2.14 \pm 0.34	680.2 \pm 218.7	315.4 \pm 73.4	10
192	4th	1.92 \pm 0.23	741.1 \pm 176.1	391.6 \pm 102.4	10
216	4th	1.84 \pm 0.31	883.1 \pm 233.6	475.2 \pm 73.1	10
240	4th	1.66 \pm 0.30	927.1 \pm 304.4	550.1 \pm 131.8	10

Regression line data:

Phase 1

$$r = 0.9306$$

$$b = 1.2038$$

$$m = 0.0583$$

Phase 2

$$r = 0.9849$$

$$b = 5.4677$$

$$m = 0.0060$$

APPENDIX A

TABLE A-3

Accumulation of cesium-137 by Aedes dorsalis larvae reared at 26°C in $^{137}\text{CsCl}$ medium, specific activity was 8000 cpm/ml. ^{137}Cs activity is reported as counts per minute total-beta activity. Wet weights and activities are reported as mean \pm one standard deviation.

Time (hours)	Instar	Mean Weight (mg.)	Mean Activity (cpm)	Mean cpm/mg	Number
4.5	1st	-	3.17 \pm 0.81	-	10
22	1st	0.055 \pm 0.007	5.93 \pm 1.13	107.8	10
28.5	1st	0.06 \pm 0.01	7.07 \pm 1.45	117.8	10
48	2nd	0.17 \pm 0.02	28.4 \pm 4.4	167.1	10
72	2nd	0.27 \pm 0.04	50.4 \pm 10.3	186.7	9
72	3rd	0.40 \pm 0.04	57.4 \pm 8.0	143.5	10
96	3rd	0.61 \pm 0.18	104.0 \pm 32.8	170.5	10
120	3rd	0.92 \pm 0.08	205.9 \pm 85.9	223.8	6
120	4th	1.26 \pm 0.12	236.2 \pm 141.6	187.5	6
165	4th	1.52 \pm 0.17	281.6 \pm 68.6	185.3	10
192	4th	1.78 \pm 0.30	304.9 \pm 90.1	171.3	10
213	4th	1.65 \pm 0.30	239.9 \pm 85.3	145.4	10
238	4th	3.81 \pm 0.53	479.0 \pm 133.8	125.7	10

Regression line data:

Phase 1

$$r = 0.9704$$

$$b = 0.4768$$

$$m = 0.0161$$

Phase 2

$$r = 0.5016$$

$$b = 2.0252$$

$$m = 0.0023$$

APPENDIX A

TABLE A-4

Accumulation of cesium-137 by Aedes triseriatus larvae reared at 26°C in $^{137}\text{CsCl}$ medium, specific activity was 6582 cpm/ml. ^{137}Cs activity is shown as cpm total-beta activity. Activities and wet weights are reported as mean \pm one standard deviation.

Time (hours)	Instar	Mean Weight (mg.)	Mean Activity (cpm)	Mean cpm/mg	Number
24	1st	-	34.69 \pm 7.79	-	9
48	1st	-	23.0 \pm 9.34	-	8
48	2nd	-	66.6 \pm 31.2	-	10
72	2nd	0.22 \pm 0.03	82.7 \pm 18.4	375.9	10
72	3rd	0.39 \pm 0.05	156.0 \pm 38.7	400.0	10
96	3rd	0.43 \pm 0.06	123.0 \pm 36.5	286.0	10
144	4th	0.92 \pm 0.12	299.9 \pm 108.7	326.0	7
168	4th	1.07 \pm 0.23	278.9 \pm 79.5	260.6	10
192	4th	1.29 \pm 0.21	273.4 \pm 109.5	211.9	10
216	4th	1.57 \pm 0.24	362.1 \pm 94.6	230.6	10
240	4th	1.76 \pm 0.41	376.0 \pm 143.5	213.6	10
264	4th	2.37 \pm 0.35	393.7 \pm 64.8	166.1	10
288	4th	2.35 \pm 0.25	504.6 \pm 207.3	214.7	10
312	4th	2.42 \pm 0.33	612.5 \pm 27.9	253.1	10

Regression line data:

Phase 1

$$r = 0.6918$$

$$b = 2.8419$$

$$m = 0.0221$$

Phase 2

$$r = 0.5901$$

$$b = 4.8416$$

$$m = 0.0045$$

APPENDIX A

TABLE A-5

Accumulation of cesium-137 by Aedes vexans larvae reared in $^{137}\text{CsCl}$ solution, specific activity was 8124 cpm/ml at 26°C. ^{137}Cs activity is given as cpm total-beta activity. Activities and wet weights are reported as mean \pm one standard deviation.

Time (hours)	Instar	Mean activity (cpm)	Number
6	1st	4.13 \pm 2.07	10
12	1st	5.68 \pm 2.43	10
24	1st	7.25 \pm 3.15	10
48	2nd	16.7 \pm 6.3	10
48	3rd	26.7 \pm 10.8	9
72	3rd	48.0 \pm 15.1	10
96	3rd	148.6 \pm 34.2	10
96	4th	263.1 \pm 84.2	8
120	4th	441.3 \pm 102.3	9
168	4th	573.0 \pm 140.7	10
192	4th	710.2 \pm 159.6	9
216	4th	753.1 \pm 163.2	9
240	4th	767.5 \pm 160.1	7

Regression line data:

Phase 1

$$r = 0.9908$$

$$b = 1.0888$$

$$m = 0.0421$$

Phase 2

$$r = 0.9660$$

$$b = 5.5350$$

$$m = 0.0049$$

APPENDIX B

TABLE B-1

Elimination of ^{137}Cs by 4th instar A. aegypti larvae reared in non-labeled medium after moulting into the 4th instar. Radiocesium activity is reported in counts per minute (cpm) \pm one standard deviation. The rearing temperature was 26°C .

Time (hours)	Mean activity (cpm)	Number
0	2734.4 \pm 275.3	5
1	2151.3 \pm 124.5	7
2	1982.8 \pm 296.6	6
4	2084.6 \pm 230.2	5
6	1651.1 \pm 397.9	8
8	1484.2 \pm 203.6	9
9	1380.3 \pm 297.3	9
11	1282.6 \pm 345.0	5
13	1168.8 \pm 229.5	4
24	1093.1 \pm 232.9	10

Regression line data:

Assimilated & unassimilated
(0-12 h)

$$r = - 0.9278$$

$$b = 7.4843$$

$$m = - 0.3306$$

Assimilated (12-24 h)
curve A "

$$r = - 1.0000$$

$$b = 7.1399$$

$$m = - 0.0069$$

Unassimilated (0-12 h) curve B

$$r = - 0.8158$$

$$b = 7.7933$$

$$m = - 0.0641$$

APPENDIX B

TABLE B-2

Elimination of ^{137}Cs by 4th instar A. atropalpus larvae at 26°C . Larvae transferred to non-labeled medium in early 4th instar. Radiocesium activity is shown as mean \pm one standard deviation.

Time (hours)	Mean activity (cpm)	Number
0	565.2 \pm 182.6	10
1	459.9 \pm 159.9	10
2	433.4 \pm 143.3	9
4	430.3 \pm 127.7	8
6	344.3 \pm 74.7	10
8	305.2 \pm 81.2	9
10	278.4 \pm 68.3	10
12	270.1 \pm 64.5	10
24	204.3 \pm 62.2	10
25	224.9 \pm 51.2	10
28	218.0 \pm 70.9	10
48	160.4 \pm 44.4	10

Regression line data:

Assimilated & unassimilated
(0-12 h)

$$r = - 0.9434$$

$$b = 6.2932$$

$$m = - 0.0652$$

Assimilated (12-48 h)

$$r = - 0.9646$$

$$b = 5.7415$$

$$m = - 0.0140$$

Unassimilated (0-12 h) curve B

$$r = - 0.9259$$

$$b = 5.6228$$

$$m = - 0.3226$$

APPENDIX B

TABLE B-3

Elimination of ^{137}Cs by 4th instar A. dorsalis larvae at 26°C . Larvae were transferred to non-labeled medium in the 4th instar. Radiocesium activities are shown as mean \pm one standard deviation.

Time (hours)	Mean activity (cpm)	Number
0	271.3 \pm 70.2	10
1	261.0 \pm 68.1	10
2	213.0 \pm 62.2	10
4	165.7 \pm 53.4	9
6	139.9 \pm 51.2	10
8	117.8 \pm 38.6	10
10	91.3 \pm 27.5	10
12	82.1 \pm 22.3	10
18	69.5 \pm 20.4	10
24	67.5 \pm 21.3	10

Regression line data:

Assimilated & unassimilated
(0-12 h)

$$r = - 0.9941$$

$$b = 5.5923$$

$$m = - 0.1038$$

Assimilated (12-24 h)

$$r = - 0.9268$$

$$b = 4.5809$$

$$m = - 0.0071$$

Unassimilated (0-12 h) curve B

$$r = - 0.9939$$

$$b = 5.2815$$

$$m = - 0.2720$$

APPENDIX B

TABLE B-4

Elimination of ^{137}Cs by 4th instar A. triseriatus larvae at 26°C . Larvae transferred to non-labeled medium in the 4th instar. Radiocesium activities are shown as mean \pm one standard deviation.

Time (hours)	Mean activity (cpm)	Number
0	136.1 \pm 33.7	10
1	143.0 \pm 38.1	10
2	114.3 \pm 32.1	9
4	94.6 \pm 25.3	10
6	83.4 \pm 36.3	10
7	74.9 \pm 27.1	9
9.5	62.4 \pm 20.8	10
11	46.5 \pm 14.9	10
12	50.1 \pm 12.7	10
18	39.5 \pm 9.4	10
24	31.1 \pm 8.8	10
30	23.3 \pm 6.4	10
48	18.8 \pm 7.3	10

Regression line data:

(0-12 h) curve A'

$$r = - 0.9880$$

$$b = 2.1531$$

$$m = - 0.0399$$

(12-48 h) curve A''

$$r = - 0.9555$$

$$b = 1.7984$$

$$m = - 0.0118$$

Unassimilated (0-12 h) curve B

$$r = - 0.9819$$

$$b = 1.9417$$

$$m = - 0.1140$$

APPENDIX B

TABLE B-5

Elimination of ^{137}Cs by 4th instar A. vexans larvae at 26°C . The larvae were transferred to non-labeled medium in the 4th instar. Radiocesium activities are shown as mean \pm one standard deviation.

Time (hours)	Mean activity (cpm)	Number
0	449.9 \pm 101.2	7
2	327.5 \pm 66.8	6
5	309.7 \pm 98.2	6
7	317.9 \pm 82.4	6
10	294.9 \pm 70.1	6
12	246.4 \pm 55.2	5
24	182.5 \pm 49.3	6

Regression line data:

(0-12 h) curve A'

$$r = - 0.8571$$

$$b = 1.8970$$

$$m = - 0.0106$$

(0-24 h curve A''

$$r = - 0.6997$$

$$b = 5.9497$$

$$m = - 0.0335$$

APPENDIX C

TABLE C-1

Effect of degilling and K^+ on cesium-137 uptake by 4th instar A. aegypti larvae reared at $26^{\circ}C$. Chemically degilled larvae were used. Specific activity of the ^{137}Cs medium = 10^3 cpm/ml. Activity and wet weights are reported as mean \pm one standard deviation.

Time (hours)	Mean weight (mg)	Mean activity (cpm)	Mean cpm/mg	Number
<u>Normal larvae in K^+ free medium</u>				
1	0.99	7.02 ± 3.08	7.09	10
2	0.81	16.75 ± 3.74	19.11	9
4	0.75	17.73 ± 5.16	27.08	9
6	0.87	27.72 ± 11.7	31.86	10
8	0.77	28.82 ± 11.9	34.66	9
9	0.86	32.51 ± 15.5	37.80	10
11	0.75	30.22 ± 13.2	46.24	9
13	0.68	38.26 ± 18.5	56.26	10
24	0.91	59.24 ± 14.8	65.27	10
<u>Normal larvae in K^+ medium</u>				
1	0.89	2.78 ± 0.87	3.12	10
2	0.91	4.75 ± 2.16	5.22	10
4	0.81	5.07 ± 1.26	6.26	10
6	0.75	5.91 ± 1.36	7.88	10
8	0.83	7.08 ± 1.88	9.29	9
9	0.99	7.38 ± 1.81	7.45	10
11	0.85	7.11 ± 2.25	8.36	10
13	0.82	8.17 ± 2.61	9.96	10
24	0.71	17.2 ± 4.7	22.66	9

TABLE C-1 Continued

Time (hours)	Mean weight (mg)	Mean activity (cpm)	Mean cpm/mg	Number
<u>Degilled larvae in K⁺ medium</u>				
1	0.74	2.16 ± 0.8	2.92	10
2	0.71	3.8 ± 0.8	5.35	10
4	0.77	6.19 ± 1.2	7.48	9
6	0.79	9.36 ± 2.08	11.85	10
8	0.73	11.56 ± 2.60	15.84	10
11	0.67	11.67 ± 4.16	17.42	10
13	0.76	16.71 ± 4.96	21.99	10
<u>Degilled larvae in K⁺ free medium</u>				
1	0.60	8.68 ± 5.09	14.47	8
2	0.70	14.74 ± 4.78	21.06	8
4	0.76	15.61 ± 4.60	20.54	10
6	0.71	17.60 ± 4.42	24.79	10
8	0.81	18.97 ± 3.35	23.42	10
11	0.69	19.78 ± 6.80	28.67	7

APPENDIX C

TABLE C-2

Effect of degilling and K^+ on cesium-137 uptake by 4th instar A. aegypti larvae reared at 26°C. Chemically degilled larvae were used. Specific activity of the ^{137}Cs medium = 10^4 cpm/ml. Activity and wet weights are reported as mean \pm one standard deviation.

Time (hours)	Mean weight (mg)	Mean activity (cpm)	Mean cpm/mg	Number
<u>Normal larvae in K^+ free medium</u>				
1	0.89	92.4 \pm 41.0	103.8	10
2	1.04	196.3 \pm 110	188.8	9
4	1.05	256.4 \pm 168.3	244.2	9
6	0.92	263.7 \pm 80.9	286.6	6
8	0.89	270.4 \pm 158.1	201.2	5
11	0.87	380.2 \pm 256.4	436.7	8
12	0.94	533.7 \pm 225.3	567.7	7
24	0.76	1608.4 \pm 350.0	2116.5	5
<u>Normal larvae in K^+ medium</u>				
1	0.86	20.7 \pm 9.0	24.1	10
2	0.96	34.5 \pm 9.4	35.9	10
4	0.96	51.6 \pm 13.2	53.8	10
6	0.99	58.8 \pm 15.4	59.4	10
8	1.00	83.2 \pm 21.9	83.2	10
9	0.94	81.0 \pm 36.6	86.2	10
11	0.91	101.3 \pm 19.8	111.3	10
12	0.91	100.9 \pm 21.8	110.9	10
24	0.95	271.5 \pm 73.9	285.9	9

TABLE C-2 continued

Time (hours)	Mean weight (mg)	Mean activity (cpm)	Mean cpm/mg	Number
<u>Degilled larvae in K⁺ free medium</u>				
1	0.88	98.2 ± 64.4	102.5	10
2	1.04	156.5 ± 33.1	150.5	9
4	0.92	217.0 ± 60.1	235.9	10
6	0.86	307.3 ± 106.2	357.3	10
8	0.86	325.0 ± 160.2	377.9	9
9	0.98	387.4 ± 154.3	395.3	9
11	0.73	421.0 ± 124.8	576.7	10
12	0.76	496.3 ± 147.5	653.0	10
24	0.65	868.3 ± 304.0	1335.8	5
<u>Degilled larvae in K⁺ medium</u>				
1	0.93	18.6 ± 5.9	20.0	9
2	0.97	26.5 ± 4.6	27.3	10
4	1.01	48.3 ± 12.8	47.8	9
6	0.86	85.3 ± 24.1	99.3	10
8	0.74	98.0 ± 6.5	132.4	3
9	1.02	114.2 ± 38.2	112.0	5
11	0.96	170.4 ± 48.9	177.5	10
12	0.63	173.9 ± 38.7	276.0	6

APPENDIX C

TABLE C-3

Effect of degilling and K^+ on cesium-137 uptake by 4th instar A. aegypti larvae reared at $26^\circ C$. Surgically degilled larvae were used. Specific activity of the ^{137}Cs medium = 10^4 cpm/ml. Activity are reported as mean \pm one standard deviation.

Time (hours)	Mean activity (cpm)	Number
<u>Normal larvae in K^+ free medium</u>		
1	7.0 \pm 5.3	10
2	178.8 \pm 65.8	9
4	214.3 \pm 118.7	10
6	287.8 \pm 109.6	10
7	344.4 \pm 148.6	10
9	379.3 \pm 126.3	10
10	375.2 \pm 235.1	10
12	313.5 \pm 142.3	10
18.5	351.0 \pm 134.6	8
24	557.5 \pm 235.1	10
30	753.9 \pm 296.4	10
<u>Normal larvae in K^+ medium</u>		
1	18.4 \pm 9.2	9
2	15.2 \pm 7.1	10
4	16.3 \pm 4.7	10
6	21.6 \pm 7.1	10
7	21.8 \pm 6.0	10
9	14.7 \pm 4.3	10
10	24.4 \pm 6.1	10
12	26.4 \pm 6.7	10
24	33.2 \pm 12.8	10
30	51.7 \pm 25.5	10

TABLE C-3 continued

Time (hours)	Mean activity (cpm)	Number
<u>Degilled larvae in K⁺ medium</u>		
1	8.1 ± 3.8	10
4	17.2 ± 8.9	9
6	17.8 ± 9.8	7
7	20.5 ± 12.2	10
9	24.6 ± 14.1	10
10	20.5 ± 6.4	10
12	37.2 ± 25.6	10
18.5	42.6 ± 21.2	3
24	85.0 ± 33.2	10
30	63.9 ± 28.8	6
<u>Degilled larvae in K⁺ free medium</u>		
1	5.02 ± 2.73	9
2	55.7 ± 35.7	10
4	93.0 ± 76.3	10
6	134.1 ± 104.9	10
7	93.7 ± 54.3	9
9	78.9 ± 60.5	8
10	93.9 ± 57.7	9
12	151.5 ± 92.8	9
18.5	168.7 ± 70.2	7
24	274.9 ± 255.3	9
30	197.3 ± 164.6	8

APPENDIX C

TABLE C-4

Uptake of carbon particles by normal and degilled A. aegypti larvae. Displacement values as per Fig. 4.

Mean \pm one standard deviation shown. Sample size (n) = 10.

Time (hours)	Normal larvae	Degilled larvae
0.25	0.25 \pm 0.20	0.40 \pm 0.21
0.5	0.45 \pm 0.28	0.68 \pm 0.33
1	0.75 \pm 0.37	0.93 \pm 0.44
1.5	0.95 \pm 0.54	1.45 \pm 0.60
2	1.15 \pm 0.78	1.58 \pm 1.19
2.5	1.45 \pm 0.64	2.30 \pm 1.03
3	1.80 \pm 0.86	2.65 \pm 1.06
3.5	2.15 \pm 1.11	3.05 \pm 1.36
4	2.40 \pm 1.22	3.35 \pm 1.42
4.5	2.75 \pm 1.06	3.46 \pm 1.62
5	2.95 \pm 1.43	3.55 \pm 1.17
5.5	3.25 \pm 1.23	3.82 \pm 1.54
6	3.50 \pm 1.20	4.00 \pm 1.43
6.5	3.85 \pm 1.40	4.09 \pm 1.30
9	4.80 \pm 1.21	4.90 \pm 1.07
10	5.05 \pm 0.96	5.05 \pm 1.21
10.5	5.15 \pm 0.91	5.30 \pm 1.17
11	5.25 \pm 0.95	5.20 \pm 1.16
23.5	7.00 \pm 0.00	6.19 \pm 1.25

Regression line data:

Normal

r = 0.9920

b = 0.3695

m = 0.4755

Degilled

r = 0.9601

b = 0.9993

m = 0.4352

APPENDIX C

TABLE C-5

Effect of degilling and K^+ on cesium-137 elimination by 4th instar A. aegypti larvae reared at $26^\circ C$. Activity and wet weights are reported as mean \pm one standard deviation.

Time (hours)	Mean weight (mg)	Mean activity (cpm)	Mean cpm/mg	Number
<u>Normal larvae in K^+ free medium</u>				
1	1.07 \pm 0.28	2036 \pm 125	1906.4	10
2	1.00 \pm 0.21	1743 \pm 177	1745.4	9
4	1.07 \pm 0.27	1920 \pm 123	1798.1	10
6	0.90 \pm 0.21	1697 \pm 111	1890.1	10
8	1.02 \pm 0.30	1484 \pm 145	1453.9	9
9	0.90 \pm 0.22	1510 \pm 173	1677.7	9
11	1.06 \pm 0.22	1618 \pm 150	1532.0	10
13	0.95 \pm 0.26	1548 \pm 164	1608.0	8
24	0.85 \pm 0.19	1093 \pm 66	1284.8	10
<u>Normal larvae in K^+ medium</u>				
1	1.02 \pm 0.30	1873 \pm 121	1829.5	10
2	1.12 \pm 0.12	1917 \pm 178	1705.3	10
4	1.12 \pm 0.26	1923 \pm 229	1717.1	10
6	1.13 \pm 0.19	1794 \pm 237	1592.1	10
8	0.98 \pm 0.23	1458 \pm 274	1492.4	10
9	1.07 \pm 0.13	1572 \pm 74	1474.7	10
11	1.12 \pm 0.15	1682 \pm 87	1501.6	10
13	1.10 \pm 0.12	1555 \pm 87	1413.2	10
24	0.83 \pm 0.17	1242 \pm 27	1492.9	9

Regression line data:

<u>N</u>	<u>NK⁺</u>
r = -0.77839	r = -0.81379
b = 3.28646	b = 3.29288
m = -0.00989	m = -0.00758

APPENDIX C

TABLE C-5
(continued)

Time (hours)	Mean weight (mg)	Mean activity (cpm)	Mean cpm/mg	Number
<u>Degilled larvae in K⁺ free medium</u>				
1	1.03 ± 0.30	1973 ± 515	1941.8	9
2	0.97 ± 0.21	2053 ± 234	2127.0	10
4	0.96 ± 0.17	1984 ± 183	2059.8	10
6	0.97 ± 0.22	1813 ± 274	1861.3	10
8	1.06 ± 0.24	1845 ± 181	1743.8	10
11	1.01 ± 0.25	1637 ± 203	1622.5	7
13	0.86 ± 0.21	1434 ± 151	1666.1	8
24	0.86 ± 0.18	1145 ± 322	1562.5	3
<u>Degilled larvae in K⁺ medium</u>				
1	1.04 ± 0.23	2107 ± 239	2027.4	10
2	0.94 ± 0.15	1771 ± 175	1886.0	10
4	0.86 ± 0.11	1408 ± 165	1636.4	9
6	0.86 ± 0.21	1529 ± 98	1771.3	9
8	1.08 ± 0.12	1736 ± 158	1604.8	10
9	0.96 ± 0.20	1442 ± 146	1501.7	10
11	0.84 ± 0.19	1472 ± 175	1763.2	10
13	0.91 ± 0.14	1446 ± 291	1585.1	10
24	0.75 ± 0.20	969 ± 367	1287.0	8

Regression line data:

$$\begin{aligned} \overline{D} \\ r &= -0.97866 \\ b &= 3.33008 \\ m &= -0.01133 \end{aligned}$$

$$\begin{aligned} \overline{DK} \\ r &= -0.83915 \\ b &= 3.29140 \\ m &= -0.01152 \end{aligned}$$

APPENDIX D

TABLE D-1

Uptake of ^{137}Cs by N. undulata nymphs that were fed ten radiocesium labeled mosquito larvae daily. The cesium-137 activity is reported in mean cpm \pm one standard deviation.

Time (days)	Instar	Mean activity (cpm)	Number
1	1st	22.9 \pm 7.66	15
2	1st	134 \pm 13.6	9
2	2nd	146.6 \pm 50.3	9
3	2nd	318.7 \pm 76.9	9
5	2nd	360 \pm 130.8	9
6	2nd	320.5 \pm 29.6	6
7	2nd	348	1
8	2nd	959	1
9	2nd	1861	1
10	3rd	2778	1
11	3rd	3531	1
12	3rd	3679	1
13	3rd	3149	1

APPENDIX D

TABLE D-2

Uptake and elimination of ^{137}Cs by adult N. undulata. Ten radiocesium labeled mosquito larvae were fed daily to each adult. Cesium-137 activity is reported in mean cpm \pm one standard deviation.

Time (days)	Mean activity (cpm)	Number
1	224.7 \pm 53.0	10
2	284.7 \pm 100.5	10
3	363.7 \pm 74.7	7
4	1108.1 \pm 175.5	7
5	2886.4 \pm 564.8	5
6	4290.6 \pm 554.4	5
7	5155.4 \pm 1228.5	5
8	5511.9 \pm 1606.7	4
9	5332.3 \pm 1068.7	3
Start of non-labeled feeding		
10	3119.5 \pm 853.6	3
11	2566.8 \pm 506.3	3
12	2073.4 \pm 359.5	3
13	1731.3 \pm 227.2	3
14	1408.8 \pm 283.8	3
15	1282.5 \pm 332.2	3
16	1125.3 \pm 227.8	3
17	963.5 \pm 196.4	3
18	825.1 \pm 167.9	3

Regression line data:

^{137}Cs uptake (1-6 h)

$$r = 0.9452$$

$$b = 4.4456$$

$$m = 0.6391$$

Elimination (10-18 h)

$$r = -0.9281$$

$$b = 9.5993$$

$$m = -0.1628$$