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The Influence of Potassium, Temperature and E. Coli on the Accumulation and Retention of Cesium-137 by Limnodrilus Hoffmeisteri

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THE INFLUENCE OF POTASSIUM, TEMPERATURE AND *E. COLI* ON THE ACCUMULATION AND RETENTION OF CESIUM-137 BY *LIMNODRILUS HOFFMEISTERI*

by

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William Erl Steger

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INTRODUCTION

Cesium-137 is one of the radionuclides produced in fair abundance by the fission process. Since it has a half-life of 30.5 years, emits beta and gamma radiation upon decay, and is a biologically active element, it is one of the more important fission by-products. Nuclear generating facilities are using the fission process to generate electricity. These facilities are located near water which is used for cooling purposes. Also, they are located in populated areas where organic enrichment of the aquatic ecosystem and, consequently, tubificids are most common. Effluents from these plants may contain ^{137}Cs, which may find their way to the tubificid worms. The accumulation and retention of ^{137}Cs by Limnodrilus hoffmeisteri has never been studied.

Because cesium and potassium are both chemically and physiologically similar (Bryan, 1963; Davis and Foster, 1958; Davis, 1961; and Kornberg, 1960 and 1961), it is accumulated in living organisms and may be passed along in food chains. One aquatic food chain has been studied with other isotopes (Whitten and Goodnight, 1967) and went from bacteria to tubificids to fish. In this food chain, the worms played a key role. Stromberg and Goodnight (1971) found that tubificid worms accumulated more Phosphorous-32 when Escherichia coli were present because the bacteria accumulated the isotope and the worms fed upon them. They also studied the effect of temperature on the accumulation process. They found that

an increase in the rate of accumulation occurred with higher temperatures, but equilibrium concentration of ^{32}P was not affected with the higher temperature.

This study is an investigation of some experimental conditions and their influence on the accumulation and retention of ^{137}Cs by Limnodrilus hoffmeisteri. The conditions studied include solutions with and without potassium, solutions at 10°C and 20°C, and solutions with and without E. coli.

LITERATURE REVIEW

Biology of Tubificid Worms

These small annelid worms range in size from 2 millimeters in length when hatched to 4-15 millimeters when fully grown. Their life cycle is usually two years. Kennedy (1966a) used three groupings to characterize the age of Limnodrilus worms: 1. immature worms; no penis sheath, 2. mature worms; penis sheath present, 3. breeding worms; spermatophores present. His studies suggest that these worms matured from six months to two years and would breed in the first and second year of life. Most worms died after breeding while some returned to an immature stage, matured and bred again. The most reliable characteristic of the worm used for taxonomic purposes is the distal end of the penis sheath. In Limnodrilus hoffmeisteri the penis sheath is trumpet shaped (Kennedy, 1969a), and can be observed simply by slide mounting.

These worms occur in lakes, rivers and streams throughout the world. Brinkhurst (1960) described 18 species from Britain. Some 45 species have been identified in North America by Brinkhurst (1965). Finogenova (1970) has presented information concerning the distribution of these worms in the Soviet region of of the Danube and other water bodies. A new genus and species has been described by Cook (1970) from the Antartica.

Ecology of the Worms

High densities of tubificids occur in rivers and lakes receiving sewage or other organic enrichment (Brinkhurst, 1965, 1966a, 1966b, 1970; Goodnight and Whitley, 1963). Population densities of over a million per square meter have been reported in heavily polluted waters (Brinkhurst and Kennedy, 1965; Brinkhurst, 1970). Whitten (1966) stated that these worms can make up to 80% of the biomass in organically polluted areas. The occurrence of large numbers of tubificids in polluted waters is usually attributed to the respiratory anatomy (Palmer, 1966) and physiology of the worms enabling them to operate at very low oxygen tensions and even anaerobic conditions (Kennedy, 1966b; and Dales, 1963). Because of these extreme environmental conditions, natural enemies such as leeches, fish and some triclad species cannot exist; consequently, the tubificid populations increase uncontrolled.

In addition to predation and respiration adaption, these annelids have been found to have some species-specific characteristics. Brinkhurst (1964) conducted an experiment in which he fed bacteria to the worm. The bacteria had been isolated from worm-inhabited sediments and from the worms themselves. After a one week digestion period, only one bacteria could be found in the media and gut. The surviving species was different for each worm.

This suggested to the experimentor a difference in digestive enzymes in the species studied.

A study on the defecation rate of three tubificid species by Appleby and Brinkhurst (1970) showed that the metabolic rate was effected by temperature and the effect differed from species to species. Data obtained from studies by Berg, Janasson and Ockelman (1962), Aston (1968), and Palmer (1968) on four different tubificids indicated different rates of oxygen consumption under similar conditions.

These studies indicate species specific physiological differences which seem to be related to the distribution and niche the worms occupy (Brinkhurst, personal communication).

Limnodrilus hoffmeisteri and Tubifix tubifix have been shown to be resistant to such pollutants as lead, zinc and sodium pentachlorophenate at levels where other organisms were killed (Goodnight and Whitley, 1963). A study conducted by Fowler and Goodnight (1962) on the respiration of T. tubifix showed that one part per million (ppm) of sodium pentachlorophenate and copper sulphate produce significant changes in the worms' respiration without disturbing the behavior of the worms. Aston (1973) has written the most recent article concerning tubificid worms and pollution stressing the ability of the worms to resist deleterious chemicals present in some aquatic environments.

Cesium-137

The importance of Radiocesium (Cesium-137 and ^{137}Cs) in the

environment has only recently been realized (Davis, 1961). There is a high yield of ^{137}Cesium during the fission reaction. Cesium-137 half-life is about 30 years and upon decay produces a gamma ray of 0.662 M.E.V. and beta particles of 0.514 and 1.8 M.E.V.. In addition, ^{137}Cs is both chemically and physiologically similar to potassium (Davis, 1961).

The single most important source of ^{137}Cs is from the fission bomb. Increasingly more important is the use of fission reactors to generate electricity. Effluents from these plants may contain ^{137}Cs. These plants are being located near populated areas where organic pollution and tubificids are most common. The International Joint Commission on the Pollution of Lake Erie, Lake Ontario and the International Section of the St. Lawerence River (Campbell, 1969a, 1969b, 1969c; and Heeney and Herton, 1970) have cited nuclear reactors, waste processing plants, industry, medicine and research as five main sources of radioactive substances being dumped into these waterways. Tubificid distribution has been shown to be correlated with municipalities, reactors and industrial sites (Brinkhurst, Hamilton, and Herrington, 1968; Brinkhurst, 1970; Hiltunen, 1969). In a study on Lake Erie, Lerman and Taniguchi (1971) have shown that 90% of the ^{137}Cs was "sorbed" on the first 9-11 centimeters of sediment. Tubificid worms occupy this layer of sediments (Brinkhurst, 1960).

Because of the physical and chemical similarities between ^{137}Cs and potassium, their physiological actions are similar. Kornberg (1960, 1961) pointed out that these similarities should

not be overdrawn because biological processes do not discriminate between these two elements in a constant and predictable manner.

The similitude between these two elements was recognized as far back as 1882 when Ringer compared the effects of potassium, rubidium and cesium on frog heart action. In an experiment, Loeb (1921) found that sea urchin eggs required potassium to develop into the free living blastula stage and that the potassium requirement could be replaced by cesium. Davis (1961) discussed the relationships between cesium and potassium in organisms. Potassium and cesium were absorbed from the rat gastrointestinal tract and a deficiency of potassium increased the absorption and decreased the elimination of cesium. The reverse was also true in that an increase in potassium ions decreased the absorption and increased the excretion of cesium. Bryan (1963) studied the accumulation of ^{137}Cs in brackish water invertebrates in relation to the regulation of potassium. In 0.01% seawater, he found that potassium concentrations increased, but under similar conditions, cesium concentrations increased to higher concentrations than those of potassium. In 1963 Bryan's study on the accumulation of ^{137}Cs in several decapod crustaceans showed a selective excretion of ^{137}Cs in relation to that of potassium. In these species, body surface area was more important in the accumulation and loss of potassium, while the amount of muscle tissue seemed to be the limiting factor in the accumulation and loss of cesium.

Tubificid Accumulation Studies

The accumulation and retention of Calcium-45 by the tubificid worm, Rhizodrilus limasus, were studied by Tomiyama, Kobayashi and Ishio (1965a, 1965b). Calcium-45 was taken up from solution and an equilibrium was reached in a 10-15 hour period. When stable calcium ion concentration of the media was equal to the radioisotope, Calcium-45 was eliminated at the same rate at which it was absorbed. Higher concentrations of stable calcium increased the rate of excretion while lower concentrations slowed the rate of excretion. They also showed that EDTA, a chelating agent, reduced the accumulation of Calcium-45, but in some cases the calcium salt of EDTA was absorbed by the worms.

Whitten (1966, doctoral dissertation) studied the accumulation of eight radionuclides by Limnodrilus worms. Concentration factors were determined for both the stable element and its radioisotope. These methods were in good agreement in most cases. Where differences occurred, the stable element analysis was higher and was believed to be the result of incomplete equilibrium between the stable isotope and the radioisotope during the test period. Whitten and Goodnight (1967) found that the concentration factors for Strontium-89 and Calcium-45 were dependent upon the stable calcium concentration of the mediums. A near linear reduction in the accumulation of the radioisotope was observed when the stable calcium ion concentration increased over the range of concentrations tested. The comparative behavior of Strontium-89

and Calcium-45 was also studied (Whitten and Goodnight, 1967). A discrimination against Strontium was found and it decreased when the Calcium ion concentration of the medium was increased.

The effect of temperature on the accumulation of Phosphorous-32 by Limnodrilus worms was studied by Stromberg and Goodnight (1971). An increase in the rate of accumulation occurred with higher temperatures but there was no clear indication of a change in the equilibrium concentrations of Phosphorous-32 with the higher temperature.

The effect of temperature may be a more involved relationship on the concentration of radioisotopes under natural conditions. The effect is probably on the rate of biological processes (Davis and Foster, 1958). During cold weather fish in Columbia consumed less plant food and, consequently, less radioactivity. A similar situation applies to insects, crustaceans and mollusks (Polikarpov, 1966). Dormant stages of insects contained less radioactivity than actively feeding stages (Davis and Foster, 1958). Glaser (1961) conducted an investigation to study the effects of temperature on the concentration factors of Iodine-131 for Drussensia polymorpha. The difference between 3°C and 20°C was a ratio of 1:4. There was no statistical significance between 3°C and 13°C. Plaice fry studied by Boroughs, Chipman and Rice (1957) showed a decrease in the amount of Strontium-89 concentration at lower temperatures. Only a third of the amount concentrated at 20°C was concentrated at 10°C. Little has been published on the effects of temperature on the accumulation and retention of the radioisotopes.

Transfer of Radioisotopes in Simple Food Chains

Whitten and Goodnight (1967) conducted studies on the accumulation of radiophosphorous by Limnodrilus worms from a .01% Knops solution, bacteria and sediments. These worms accumulated more phosphorous from labeled Escherichia coli than from Knops solution or sediments. It was thought that the bacteria changed the inorganic phosphate to a more available organic form for the worms.

Stromberg and Goodnight (1971) studied the transfer of Phosphorous-32 from E. coli and Chlamydomas pyrenoidosa to Limnodrilus worms. Their data indicated that the worms accumulated more Phosphorous-32 in the presence of E. coli than from the algae. The bacteria and algae competed with the worms for Phosphorous-32 as the worms ingested them. This paradox was explained in the finding that these worms did not have an endogenous cellulase and could not digest the cellulitic cell wall to absorb the bound radiophosphorous.

Once the isotope is accumulated by the worm, it is then available to predators of the worms. Whitten's doctoral dissertation showed that Phosphorous-32 would be transferred to fish through labeled sludge worms. Although tubificid worms are seldom identified in the stomach of fish, Kennedy (1969b) has shown that dace, Leuciscus leuciscus, feed upon tubificid worms. Other predators of these worms include fish like Procladius, Cryptochoronomus and Acanthorcyclops, as well as many leeches and some triclad species

(Brinkhurst, 1964). Since there are many predators of tubificid worms, isotopes concentrated by them could be passed along in food chains and contribute significantly to the contamination of other living organisms.

METHODS AND MATERIALS

For clarity, this portion of the paper has been divided into seven sections. These sections are: 1. Methods of Culture, 2. Accumulation Procedures, 3. Retention Procedures, 4. Competition Experiments, 5. Temperature Experiments, 6. *E. coli* Experiments, 7. Statistical Methods.

Methods of Culture

The worms used in all experiments were obtained from the Kalamazoo River about 300 meters downstream from the Kalamazoo sewage treatment plant. During the course of experimentation, three collections were made. At the collecting site, dip nets were used to collect substrate containing the worms. The substrate was rinsed with river water until most of the small sediments were washed away. This concentrated mass of sediments and worms was placed in a gallon container, filled with river water and transported to the laboratory.

Three or four gallon containers were filled during a collection. The mass of sediments and worms was transported to the laboratory where each gallon container was emptied into rectangular shaped aquaria; substrata was covered with water. The aquaria were covered with aluminum foil and were allowed to sit undisturbed overnight. In the morning, large balls of worms could be removed easily from the corners of the aquaria. This migration and clumping in the corners of the aquaria were thought to be due to peutrifi-

cation, overcrowding and oxygen depletion of the environment. These clumps of worms were placed in large finger bowls containing 0.01% Knops solution and stored at 10°C. This was considered the stock supply of worms. Solutions were changed daily and worms were not kept for more than three weeks in this manner.

These collections of worms were found to contain approximately 40% L. hoffmeisteri. The remainder of the sample consisted mostly of Tubifex tubifex and immature worms. The immature worms could be separated from the T. tubifex and L. hoffmeisteri because of the difference in size. T. tubifex and L. hoffmeisteri were initially separated by observing setae under a dissecting scope. The T. tubifex has long hair setae while the L. hoffmeisteri has short setae (Brinkhurst, 1960). In addition to the setae characteristics, mature L. hoffmeisteri were about twice the size of T. tubifex and were darker in color. The color difference was observed to be intestinal content. Once separation was complete, samples of these worms were cleared with Bouins fixative and observed under a microscope for the trumpet shape penis sheath (Brinkhurst, 1960; Kennedy, 1969a). Later in time, L. hoffmeisteri and T. tubifex could be separated by observation of setae and only random samples of worms were slide mounted and observed.

The Escherichia coli culture was obtained from the Biology Department at Western Michigan University. It was cultured according to Gunnison and Goodnight (1971), and was harvested in 0.01% Knops solution. Once the cells were resuspended, one milliliter of the culture was serially diluted and plated on Trypti-

case Soy Agar (BBL). A viable count was obtained and was 2×10^{10} cells/ml.

Accumulation Procedures

From the stock culture of worms, groups of approximately a hundred worms were placed in sterile finger bowls which had been previously coated with Desicoate (Beckman). This solution helps prevent adsorption of ions to the glass surface. One hundred ml of sterile 0.01% Knops solution was added to each finger bowl and each finger bowl was covered with Saran Wrap to prevent evaporation of the solution (Whitten, 1966). A rubberband was stretched around the finger bowl to hold the wrap securely in place. The media of each finger bowl was changed daily to prevent fouling. After one week of acclimation to experimental conditions, 1.0 microcurie of Cesium-137 was added to each bowl in the 100 ml of Knops solution. For the remainder of the accumulation, the solutions were not changed. At the appropriate sampling time, the worms were removed from the radioactive solution, rinsed for one to two minutes in a carrier Knops solution containing 50 mg of Cesium Chloride per liter of Knops, and blotted on a filter paper. Groups of ten worms were placed into pre-weighed scintillation vials and weighed again. Wet weight of the worms was obtained by the difference. One ml of 1N Sodium Hydroxide was added to each of these ten vials and the vials were placed in a 50°C water bath for four hours. After complete digestion, sufficient BBS-2 acid

solubilizer (Beckman) was added to each vial to neutralize the digestate. After neutralization and clearing (20 minutes), 15 ml of the scintillation cocktail was added to each vial and shaken vigorously. The cocktail was composed of four grams of PPO, 150 mg POPOP brought to 1,000 milliliters with scintillation grade toluene. These samples were then counted on a Packard 3310 Tri-Carb Liquid Scintillation Spectrophotometer for a sufficient period of time so that counting error was less than 5%. Sample counts were corrected for background, and half-life and counting efficiency. Counting efficiency was determined by both internal and external standardization methods (Chase and Rabinowitz, 1970). The counts were converted to disintegrations per minute (DPM) per gram wet weight of worm by the formula:

$$\text{DPM per g wet weight} = \frac{\text{Corrected counts per minute}}{\text{E\% x wet weight of sample.}}$$

The studies which were conducted at 20°C had sampling times at 1, 3, 6, 12, 24, 48, 72, and 96 hours for both accumulation and retention. Experiments at 10°C were sampled less frequently. The sampling times for 10°C studies were 6, 12, 24, 48, 72, and 96 hours for accumulation and retention.

Retention Procedures

The worms were irradiated at the same time and in the same manner as in the uptake experiments. After 96 hours of exposure to the radioactive solution, the worms were carefully transferred to fresh sterile Knops solution. Again the bowls were covered

with Saran Wrap and a rubberband was stretched around it. Samples of worms were taken at prescribed times and treated as in the same manner described in the Accumulation Procedures.

Competition Experiments

The media used in the potassium free experiment (Experiment 1) was made from water which had been double-distilled. The second distillation was in glass. The ionic strength of the media was maintained by adjusting the remaining ions in the 0.01% Knops solution. The media also contained 20 mg/liter of chloramphenicol to control microbiological contamination. The media that contained two parts per million (ppm) of potassium (Experiments 2-5) was made with double-distilled water as was all media used in these experiments. Again, ionic strength was maintained by adjusting the remaining ions in the solution. All other methods were identical to the methods presented in the Accumulation and Retention Procedures Sections.

Temperature Experiments

The methods for the experiments at 10°C and 20°C (Experiments 4 and 5) were the same as in the Accumulation and Retention Procedures Sections. The media was ionically adjusted, contained 2 ppm of potassium and contained 20 mg/liter of chloramphenicol.

E. coli Experiments

One ml of the bacterial culture was pipetted into each of

twenty-eight sterile finger bowls. Ninety-nine ml of the sterile irradiated Knops solution containing 2 ppm of potassium without chloramphenicol was added to each of these finger bowls. The worms were then carefully introduced as in the other studies. Both the 10°C and 20°C accumulation studies were conducted in this fashion. E. coli were not added in the retention studies. The remainder of these studies was performed as previously described in the Accumulation and Retention Procedures Sections.

Statistical Methods

The experimental data were converted to DPM/gm wet weight of the worms (Appendices 1-10) and were encoded on IBM cards. Before curve fitting and analyses, eleven pieces of data were eliminated by the Outlier's Test in the Pharmacopeia of the United States, 1970. The statistical analyses were performed using computer programs provided by the Mathematical Services Unit at The Upjohn Company, Portage, Michigan.

Accumulation and retention curves were fit for each experiment using a non-linear least squares estimation program. The following equation was assumed to describe the accumulation curves (Whitten and Goodnight, 1967). The equation was:

$$\gamma = \alpha(1-e^{\beta t})$$

where: γ = DPM per hour per gm wet weight of worms (dependent variable); α = asymptotic value of the curve; e = the base of the natural logarithm; β = the rate constant in reciprocal hours as t is in hours, and t = time in hours (independent variable). The

equation assumed to describe the retention curves (Whitten and Goodnight, 1967) was the expression:

$$\gamma = \alpha e^{\beta t}.$$

The non-linear program obtained least square estimates for the α and β parameters for each accumulation and retention curve. Ninety-five percent confidence intervals were also calculated by the program for each of the parameters. The α parameter is the asymptotic value for the curve. When an accumulation curve at the end of the 96-hour sampling period was approximately equal to the α parameter, the accumulation of the isotope was then at equilibrium. The β parameter is the rate at which the isotope was accumulated or retained by the worms. The 95% confidence intervals for the α and β parameters are compared to determine significant differences between the accumulation experiments and between the retention experiments.

Figure 4 graphically displays the 95% confidence intervals for the α and β parameters for the accumulation experiments, while Figure 8 shows the same parameters and their intervals for the retention experiments. These rectangles which define the intervals are the largest possible areas that the intervals could occupy. Actually, the true area of the combined intervals for each experiment is smaller and probably contained in the rectangle. Thus, comparisons of these areas is a conservative test for significant differences between experiments. If the areas do not overlap, the experiments are different. Overlapping of these areas indicates non-significant differences.

Retention curves were developed using the 96-hour accumulation data as the zero hour retention data. Initially, there were no differences between these experiments because they were started at the same time and in the same incubator. Samples were randomly removed for the accumulation study and remaining samples were used for retention. Consequently, 96-hour accumulation samples were zero hour retention samples.

The experimental data were converted to natural logarithms before analyses. The data were analyzed by three tests. These tests were Bartlett's test for homogeneity of variances (a Chi-square test), analysis of variance (ANOVA) and Newman-Keuls test. The Bartlett's test was used to determine if sample variances were equal. In most instances, the Bartlett's test showed that experimental variances were not equal. Because sample sizes for each sampling time were small ($\leq$ 10, one sample = 20), and have long-tailed distributions, positive kurtosis, this test probably gave many results of heterogeneity (Snedecor and Cochran, 1967). The results of this test should be viewed cautiously. The analysis of variance determined if there were any significant differences between the experiments. Once differences were shown, the Newman-Keuls test compared each experimental mean for significant differences. This test was chosen because it is capable of comparing more than two means and reduces the probability of making a Type 1 error to five percent for all comparisons made at any one sampling time. These three tests were performed on the data for each experiment at every sampling time.

The standard error of the means was never greater than 20%, and the relative standard deviation was always less than 5%. The level of statistical significance throughout this investigation was 95%.

The curve fitting provided information about the amount and rate of ^{137}Cs accumulation by these worms. It also determined equilibrium values for each condition. The tests compared the amount of isotope in the worms for each experiment at the various sampling times.

RESULTS

This portion of the paper has been divided into two sections. The first section reports the data of the accumulation experimentations and the second section reports the data of the retention experiments. Each of these sections is further divided into competition, temperature and *E. coli* experiments.

Accumulation: Competition Experiments

The accumulation by the worms maintained in the potassium free Knops solution is shown in Figure 1 (also see Table 1 for experimental conditions). Accumulation proceeded exponentially and reached equilibrium within 96 hours. In the Knops solution with 2 ppm potassium, accumulation by the worms was also exponential, but did not reach equilibrium during the study. Equilibrium was not achieved because the accumulation curve at 96 hours (Figure 2) was not equal to the asymptotic value of the curve in Table 2. The estimates for the α and β parameters and their 95% confidence intervals of these two experiments are also shown in Table 2. When the intervals for the α parameters are compared, a significant difference in the amount of isotope accumulated results. Comparing the intervals for the β parameters indicates no significant differences in the rates of accumulation. Figure 4 shows that these experiments are significantly different. The fact that the amount of accumulation is significantly different was also verified

by the Newman-Keuls test. At every sampling time the amount of ^{137}Cs accumulated by the worms in the potassium free solution was greater than the amount accumulated by the worms in the 2 ppm solution (Tables 3-11). The presence of the 2 ppm potassium reduced accumulation by some 3.5 fold, affected equilibrium, but did not significantly affect the rate of accumulation.

Accumulation: Temperature Experiments

Figure 2 shows the accumulation of ^{137}Cs by the worms at both 20°C and 10°C. The accumulation at 20°C was characterized by an exponential curve while the 10°C accumulation proceeded almost linearly. Equilibrium was not attained by the worms at either temperature because accumulation curves at 96 hours did not equal asymptotic values of the curves (Figure 2, Table 2). Because the accumulation at 10°C was nearly linear, the data did not fit the equation for accumulation, $y = \alpha(1-e^{\beta t})$, because this equation assumes an exponential and asymptotic curve. Consequently, there is no 95% confidence interval for the α parameter for this experiment. The comparisons by the Newman-Keuls test show a significant difference between the amount of isotope accumulated at 20°C and 10°C at every sampling time (Tables 3-11). The rate of accumulation was significantly reduced by the lower temperature. This can be seen when the 95% confidence interval for each of the β parameters is compared in Table 2.

Accumulation: E. coli Experiments

The type of accumulation curves obtained when E. coli were present in Knops solution is shown in Figure 3. The two curves shown are the accumulations for the worms at 20°C and 10°C in the presence of E. coli. The accumulation at 20°C is exponential and did not reach equilibrium during the study. This can be seen when Figure 3 and its asymptotic value in Table 2 are compared. This comparison shows that they are not equal so equilibrium was not attained. The accumulation at 10°C was also exponential and approached equilibrium at 96 hours. Accumulation is reduced when the E. coli are present in the Knops solution, as seen when Figures 2 and 3 and the asymptotic values for each curve are compared (Table 2). This suggests that the E. coli competed with the worms for available ^{137}Cs. Even though the 95% confidence intervals for the parameters at 20°C overlap (Figure 4), the general trend in the curves appears to be that the worms accumulated less ^{137}Cs when E. coli were present. No comparison can be made of the α parameters at 10°C because a 95% confidence interval was not determined for the 10°C experiment without E. coli. However, the rate of accumulation is more rapid at 10°C when E. coli are present and equilibrium was established sooner. The amount of ^{137}Cs accumulation in 96 hours at 10°C was greater in the experiment without E. coli than in the E. coli experiment. Again, this suggests E. coli competed

with the worms for available ^{137}Cs.

The Newman-Keuls test shows that there are some differences between the 20°C solutions with and without E. coli. Two differences occur at the one and three hour samples (Tables 3, 4 and 11). The amount accumulated at one and three hours was different, but as accumulation proceeded, the curves approached each other, resulting in no significant differences at the 6, 24, 48 and 96 hour samples (Tables 5, 7, 9, 10 and 11). Figure 4 shows that the 95% confidence intervals for these experiments overlap and consequently are not significantly different.

At 10°C, there are no statistically significant results at the various sampling times except at the 12 and 96 hour samples. At 12 hours the accumulation in the E. coli solution is greater than the solution without E. coli (Figures 2 and 3). At 96 hours equilibrium was approached in the E. coli solution but accumulation in the solution without E. coli was still linear and not at equilibrium.

FIGURE 1

Accumulation of Cesium-137 by L. hoffmeisteri at 20°C. The 0.01% modified Knops solution contained chloramphenicol (20 mg/L) and was potassium free (Experiment 1). Points are determined by the computer. Open circles are experimental mean values.

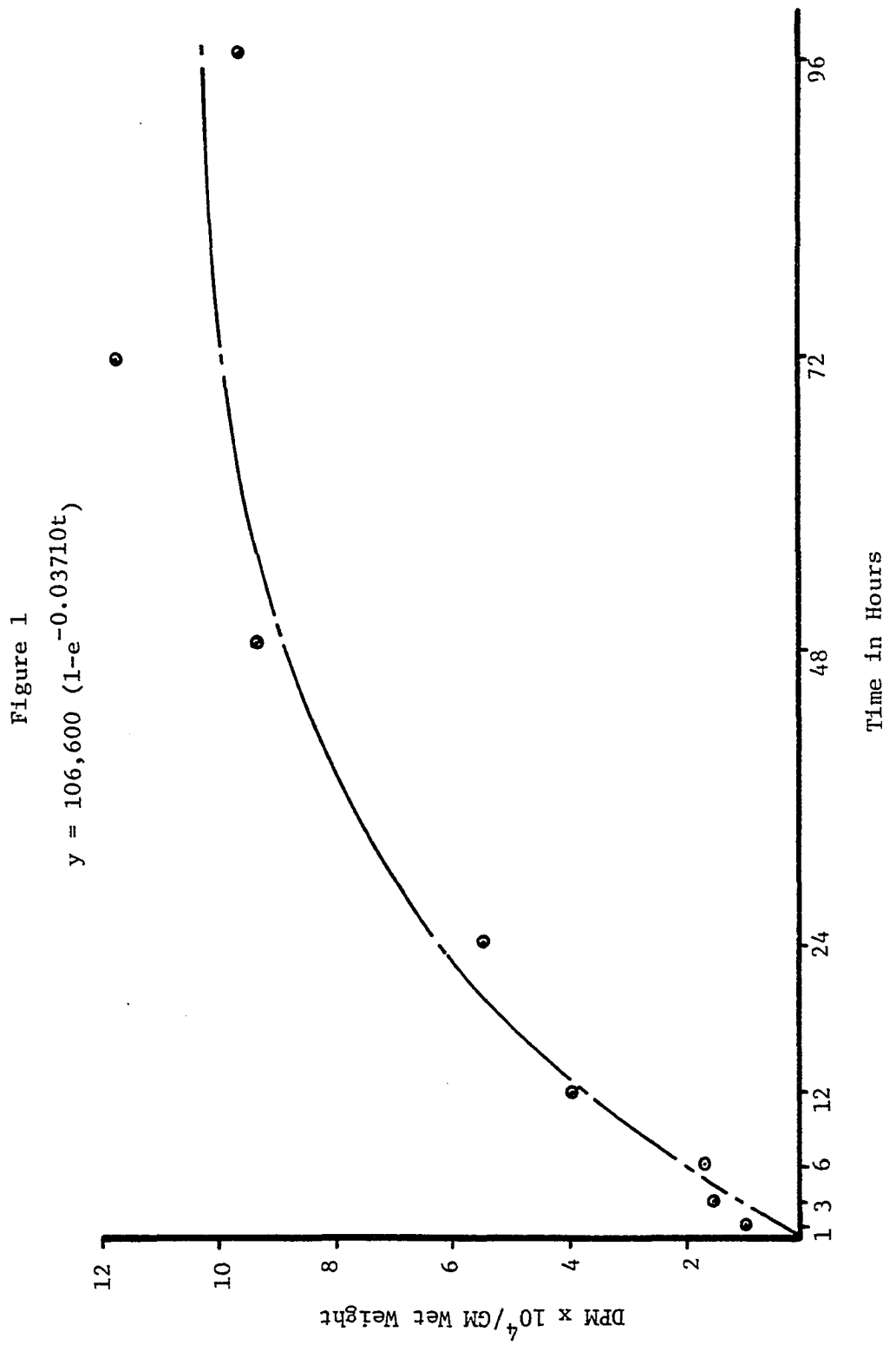

Figure 1

$y = 106{,}600\ (1-e^{-0.03710t})$

FIGURE 2

Accumulation of Cesium-137 by L. hoffmeisteri at 20°C and 10°C (Experiment 5 and 4, respectively). The 0.01% modified Knops solution contained chloramphenicol (20 mg/L) and 2 ppm potassium. Points and solid triangles are values determined by the computer. Open circles and open triangles are experimental mean values.

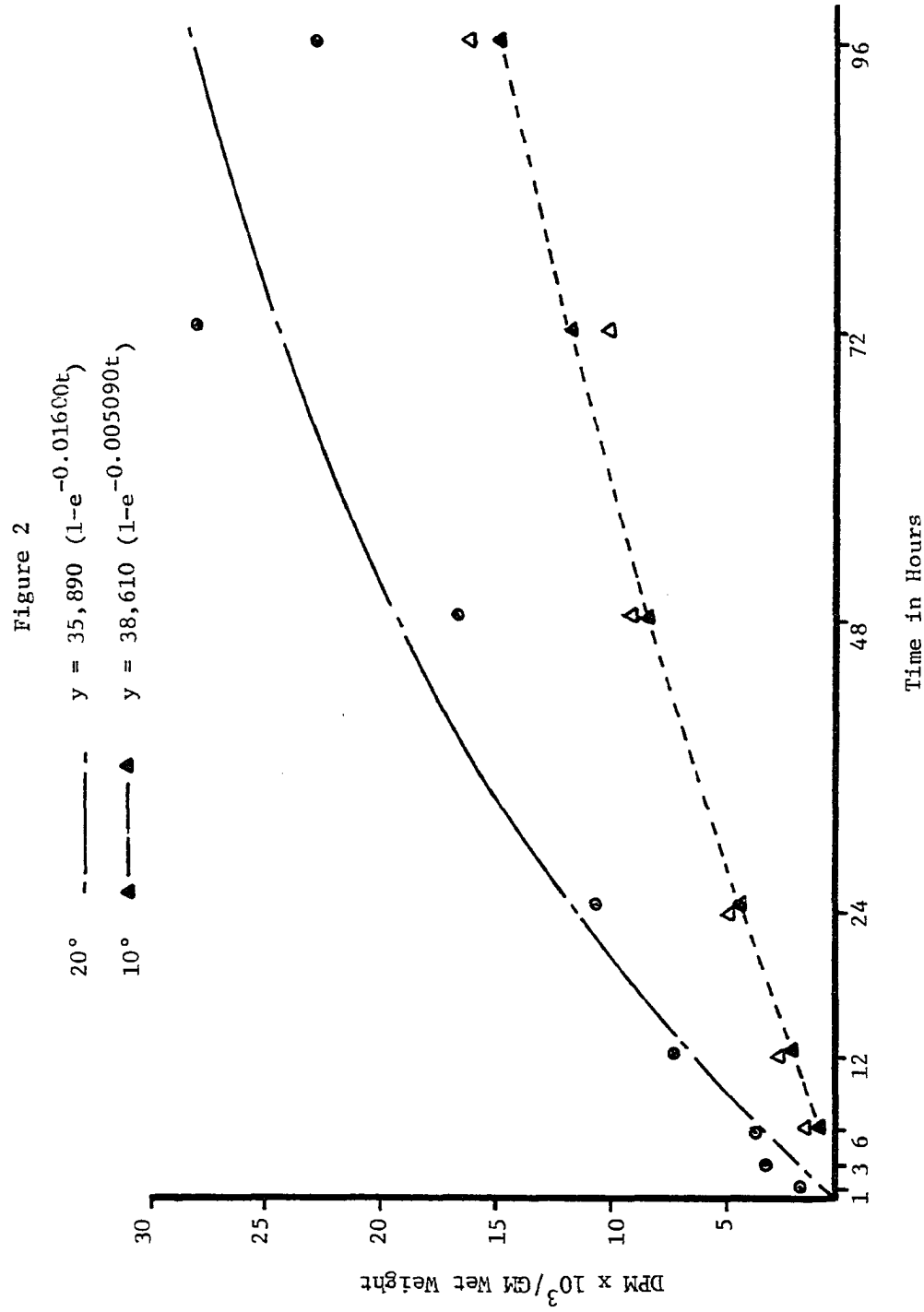

FIGURE 3

Accumulation of Cesium-137 by L. hoffmeisteri at 20°C and 10°C (Experiments 3 and 2, respectively). The 0.01% modified Knops solution contained E. coli and 2 ppm potassium. Points and solid triangles are values determined by the computer. Open circles and open triangles are experimental mean values.

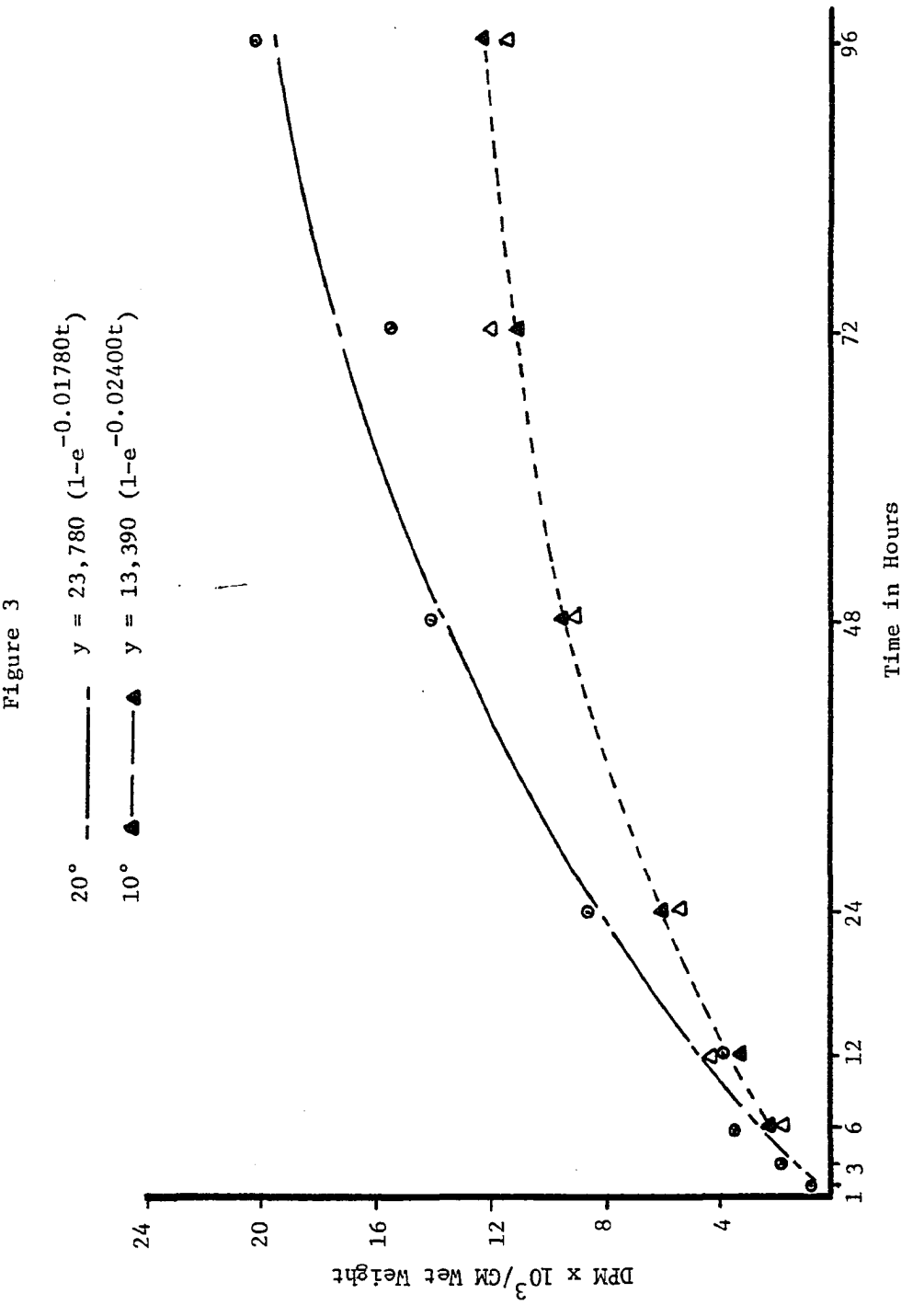

Figure 3

FIGURE 4

Graphic display of the 95% confidence intervals for the α and β parameters for each of the accumulation experiments. Experiment 4 is not shown because 95% confidence intervals for the α parameter were not obtained. Within each rectangle is the number which describes the conditions of the experiment (see Table 1, page 33).

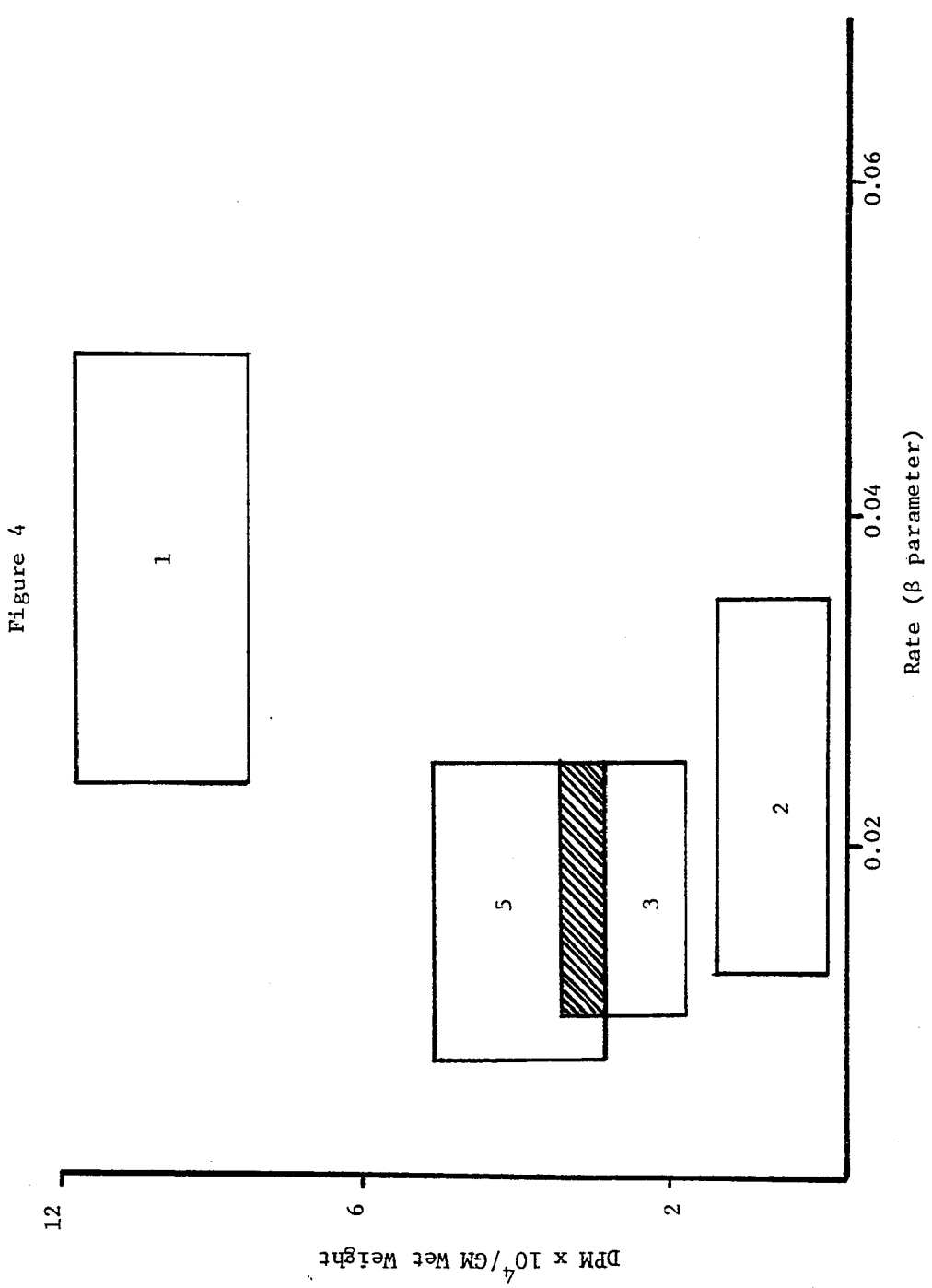

Figure 4

Table 1. Experimental conditions for the accumulation of Cesium-137 by L. hoffmeisteri. The number presented with each condition corresponds to that condition throughout the statistical analyses.

Experimental Condition 1:	Potassium free 0.01% modified Knops solution maintained at 20°C and contained chloramphenicol.
Experimental Condition 2:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 10°C and contained E. coli.
Experimental Condition 3:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 20°C and contained E. coli.
Experimental Condition 4:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 10°C and contained chloramphenicol.
Experimental Condition 5:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 20°C and contained chloramphenicol.

Table 2. The α and β parameters for each of the accumulation experiments and their 95% confidence intervals, α = asymptotic value of the curve expressed in dmp/gm, and β = rate constant. Parameters are in ranked order.

Experiment	α parameter	95% confidence interval
2	13,390	10,630 - 16,150
3	23,780	18,440 - 29,120
5	35,890	24,870 - 46,920
4	38,610	None
1	106,600	94,870 - 118,230

Experiment	β parameter	95% confidence interval
4	5.090×10^{-3}	$4.660 - 5.526 \times 10^{-3}$
5	1.600×10^{-2}	$0.7202 - 2.481 \times 10^{-2}$
3	1.780×10^{-2}	$1.026 - 2.539 \times 10^{-2}$
2	2.400×10^{-2}	$1.252 - 3.539 \times 10^{-2}$
1	3.710×10^{-2}	$2.440 - 4.971 \times 10^{-2}$

Table 3. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for one hour of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation, and sample size for each experiment at one hour.

Bartlett's test for homogenity of variances = 13.14*

ANOVA Table

Source	DF	SS	MS	F
Experiments	2	38.94	19.47	117.3*
Error	25	4.148	0.1659	
Total	27	43.088		

Newman-Keuls Test

Standard deviation of experimental means = 0.1341 Degrees of freedom = 25

Experiment		3	5	1
	Mean	6.359	7.766	9.150
3	6.359	--	1.407*	2.791*
5	7.766	--	--	1.384*

Experiment	Mean	Standard Deviation	Sample Size
1	9.150	0.2438	10
3	6.359	0.3675	10
5	7.766	0.5852	8

*Significant differences at the 95% level of confidence.

Table 4. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for three hours of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at three hours.

Bartlett's test for homogenity of variances - 18.38*

ANOVA Table

Source	DF	SS	MS	F
Experiments	2	24.01	12.01	107.6*
Error	27	3.013	0.1116	
Total	29	27.023		

Newman-Keuls Test

Standard deviation of experimental means = 0.1056 Degrees of freedom = 27

Experiment		3	5	1
	Mean	7.525	7.948	9.599
3	7.525	--	0.423*	2.074*
5	7.948	--	--	1.651*

Experiment	Mean	Standard Deviation	Sample Size
1	9.599	0.1573	10
3	7.525	0.3339	10
5	7.948	0.4456	10

*Significant differences at the 95% level of confidence.

Table 5. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for six hours of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at six hours.

Bartlett's test for homogenity of variances = 23.94*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	32.03	8.008	158.2*
Error	44	2.227	0.05065	
Total	48	34.257		

Newman-Keuls Test

Standard deviation of experimental means = 0.0719 Degrees of freedom = 44

Experiment		4	2	3	5	1
	Mean	7.439	7.561	8.114	8.132	9.685
4	7.439	--	0.122	0.675*	0.693*	2.246*
2	7.561	--	--	0.553*	0.571*	2.124*
3	8.114	--	--	--	0.018	1.571*
5	8.132	--	--	--	--	1.553*

Experiment	Mean	Standard Deviation	Sample Size
1	9.685	0.1190	10
2	7.561	0.1339	10
3	8.114	0.2902	10
4	7.439	0.2696	10
5	8.132	0.2563	9

*Significant differences at the 95% level of confidence.

Table 6. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for twelve hours of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at twelve hours.

Bartlett's test for homogenity of variances = 15.59*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	49.27	12.32	220.6*
Error	45	2.513	0.05584	
Total	49	51.783		

Newman-Keuls Test

Standard deviation of experimental means = 0.0747 Degrees of freedom = 45

Experiment		4	2	3	5	1
	Mean	7.807	8.066	8.218	8.892	10.56
4	7.807	--	0.259*	0.411*	1.085*	2.75*
2	8.066	--	--	0.152	0.826*	2.49*
3	8.218	--	--	--	0.674*	2.34*
5	8.892	--	--	--	--	1.67*

Experiment	Mean	Standard Deviation	Sample Size
1	10.56	0.1399	10
2	8.066	0.2775	10
3	8.218	0.2650	10
4	7.807	0.1660	10
5	8.892	0.2913	10

*Significant differences at the 95% level of confidence.

Table 7. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for 24 hours of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 24 hours.

Bartlett's test for homogenity of variances = 22.02*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	36.87	9.217	108.3*
Error	45	3.830	0.8511	
Total	49	40.700		

Newman-Keuls Test

Standard deviation of experimental means = 0.0923 Degrees of freedom = 45

Experiment		4	2	3	5	1
	Mean	8.490	8.500	9.008	9.236	10.85
4	8.490	--	0.060	0.518*	0.746*	2.36*
2	8.550	--	--	0.458*	0.686*	2.30*
3	9.008	--	--	--	0.228	1.84*
5	9.236	--	--	--	--	1.62*

Experiment	Mean	Standard Deviation	Sample Size
1	10.85	0.3299	10
2	8.550	0.2451	10
3	9.008	0.3633	10
4	8.490	0.1251	10
5	9.236	0.3301	10

*Significant differences at the 95% level of confidence.

Table 8. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for 48 hours of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 48 hours.

Bartlett's test for homogenity of variances = 18.64*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	40.22	10.05	95.70*
Error	45	4.727	0.1051	
Total	49	44.947		

Newman-Keuls Test

Standard deviation of experimental means = 0.1025 Degrees of freedom = 45

Experiment		4	2	3	5	1
	Mean	8.985	9.047	9.455	9.694	11.44
4	8.985	--	0.062	0.470*	0.709*	2.46*
2	9.047	--	--	0.408*	0.647*	2.39*
3	9.455	--	--	--	0.239*	1.99*
5	9.694	--	--	--	--	1.75*

Experiment	Mean	Standard Deviation	Sample Size
1	11.44	0.1876	10
2	9.047	0.4248	10
3	9.455	0.4093	10
4	8.985	0.2994	10
5	9.694	0.2290	10

*Significant differences at the 95% level of confidence.

Table 9. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for 72 hours of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 72 hours.

Bartlett's test for homogenity of variances = 18.06*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	35.01	8.752	155.1*
Error	43	2.427	.05643	
Total	47	37.437		

Newman-Keuls Test

Standard deviation of experimental means = 0.0768 Degrees of freedom = 43

Experiment		4	2	3	5	1
	Mean	9.225	9.365	9.671	10.21	11.62
4	9.225	--	0.140	0.446*	0.987*	2.39*
2	9.365	--	--	0.356*	0.847*	2.25*
3	9.671	--	--	--	0.539*	1.95*
5	10.21	--	--	--	--	1.40*

Experiment	Mean	Standard Deviation	Sample Size
1	11.62	0.3556	9
2	9.365	0.2210	10
3	9.671	0.1630	9
4	9.225	0.1507	10
5	10.21	0.2491	10

*Significant differences at the 95% level of confidence.

Table 10. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for 96 hours of ^{137}Cs accumulation by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 96 hours.

Bartlett's test for homogenity of variances = 20.30*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	40.22	10.06	117.0*
Error	54	4.640	0.08592	
Total	58	44.860		

Newman-Keuls Test

Standard deviation of experimental means = 0.0890 Degrees of freedom = 54

Experiment		2	4	3	5	1
	Mean	9.327	9.659	9.889	10.12	11.42
2	9.327	--	0.332*	0.562*	0.80*	2.09*
4	9.659	--	--	0.230*	0.46*	1.76*
3	9.889	--	--	--	0.24*	1.53*
5	10.12	--	--	--	--	1.30*

Experiment	Mean	Standard Deviation	Sample Size
1	11.42	0.3218	20
2	9.327	0.1508	10
3	9.889	0.2274	10
4	9.659	0.2598	9
5	10.12	0.4030	10

*Significant differences at the 95% level of confidence.

Table 11. Summary of Bartlett's test (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for the accumulation experiments. S indicates significant differences at the 95% level of confidence and NS indicates no significant differences at the same level.

	ACCUMULATION TIME IN HOURS							
Test	1	3	6	12	24	48	72	96
Chi-Square	S	S	S	S	S	S	S	S
ANOVA	S	S	S	S	S	S	S	S
Newman-Keuls								
1 vs. 5	S	S	S	S	S	S	S	S
4 vs. 5	-	-	S	S	S	S	S	S
2 vs. 3	-	-	S	NS	S	S	S	S
2 vs. 4	-	-	NS	S	NS	NS	NS	S
3 vs. 5	S	S	NS	S	NS	NS	S	NS

Retention: Competition Experiments

Retention of ^{137}Cs by the worms in potassium free Knops solution is shown in Figure 5 (also see Table 12 for experimental conditions). During the experiment, retention was linear and the amount of ^{137}Cs retained was 84%. This and the slope indicate that there was very little loss of the isotope under these experimental conditions. Figure 6 shows the retention at 20°C when 2 ppm potassium was present in the Knops solution. This retention showed a more rapid loss of the isotope with about 67% of the isotope being retained during the study. A comparison of the α parameters for these retention curves in Table 13 indicates significant differences at the 95% level of confidence. The β parameters for these experiments were not significantly different (Table 13). The Newman-Keuls test shows significant differences between these two experiments at every sampling time (Tables 14-22). Figure 8 further substantiates that these two experiments are significantly different because the 95% intervals for the parameters do not overlap.

Retention: Temperature Experiments

The retention curves for the 20°C and 10°C experiments are presented in Figure 6. Both curves are linear and the rate of loss at 20°C is greater than at 10°C. About 67% of the isotope was

retained by the worms at 20°C, while 85% of the amount present at zero time was present at 96 hours in the 10°C study. This indicates that the 10°C retention of ^{137}Cs was greater than at 20°C. The α parameters are significantly different in these two studies but the rate parameters are not (Table 13). The Newman-Keuls test showed significant differences in the amount retained (α parameter) at 12 and 48 hours, but at other sampling times there were no significant differences (Tables 14-22). The graph of the 95% confidence intervals of the parameters shows that there is a significant difference between these two experiments when both parameters are compared simultaneously (Figure 8).

Retention: E. coli Experiments

The retention of ^{137}Cs by the worms in the 20°C and 10°C with E. coli Knops solution is shown in Figure 7. The 20°C curve indicates a rapid loss of the isotope with about 50% being retained at the end of the study. At 10°C the worms retained more of the isotope with about 87% remaining at 96 hours. There is no rapid loss of the isotope at 10°C temperature. The α and β parameters for these studies show a significant difference when the intervals are compared in Table 13. The Newman-Keuls test shows significant differences at three sampling times, 12, 24 and 48 hours, while the remaining sampling times do not (Tables 17, 18, 19 and 22). The curves approach each other at the 72 and 96 hour sampling times and are not different (Tables 20, 21 and 22 and

Figure 7). Figure 8 compares the 95% confidence intervals for the α and β parameter of the 20°C experiment with the same parameters of the 10°C experiment. This comparison shows that these experiments are significantly different at the 95% level of confidence.

Comparisons of the studies with and without E. coli at 20°C made by the Newman-Keuls test show that their only two sampling times occur when there are significant differences (Tables 14-22). The α parameters of these studies in Table 13 show that the amount retained is not significantly different. Also, the rate of loss is not different as indicated by the overlap of the β parameters. Figure 8 further shows that these two experiments are not significantly different from each other because of the overlap of 95% confidence interval (shaded area) of these two experiments.

At 10°C there is a significant difference between the amount retained in the studies with and without E. coli but no difference between the rates of loss (Table 13). The Newman-Keuls test shows that at three sampling times differences occur. Conversely, there are three times, two at the end of the studies, when there are no significant differences (Tables 14-22). These results indicate that the greatest differences in these retention curves occurred early in retention. As retention continued, these curves approach each other resulting in no significant differences. The graph of the 95% confidence intervals for the parameters of these experiments in Figure 8 shows that there are significant differences between these two experiments because the

areas which define these experiments do not overlap.

FIGURE 5

Retention of Cesium-137 by L. hoffmeisteri at 20°C (Experiment 1). The modified solution contained chloramphenicol (20 mg/L) and was potassium free. Points are values determined by the computer. Open circles are experimental mean values.

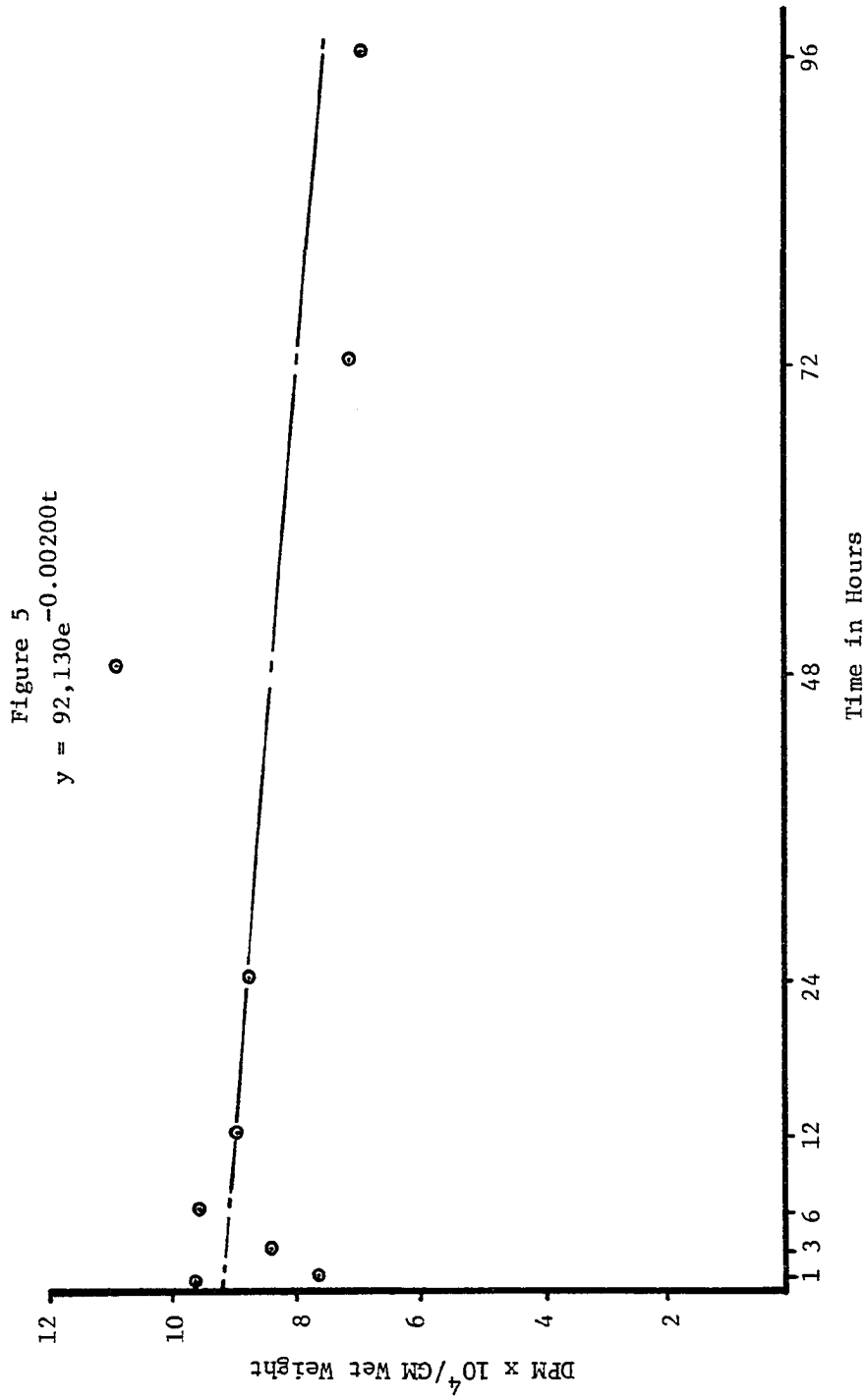

FIGURE 6

Retention of Cesium-137 by L. hoffmeisteri at 20° and 10° (Experiments 5 and 4, respectively). The 0.01% modified Knops solution contained chloramphenicol and 2 ppm potassium. Points and solid triangles are values determined by the computer. Open circles and open triangles are experimental mean values.

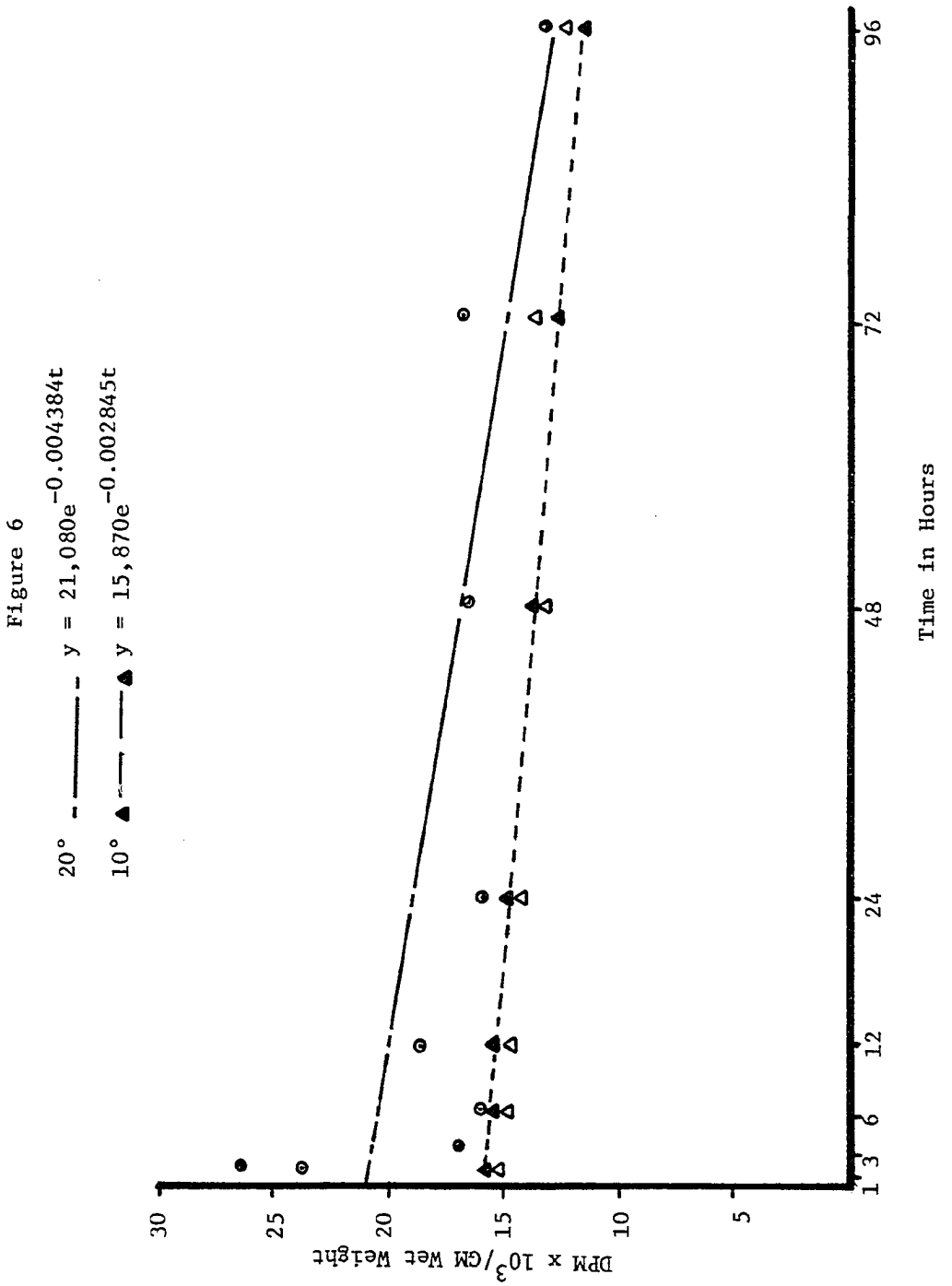

Figure 6

FIGURE 7

Retention of Cesium-137 by L. hoffmeisteri at 20°C and 10°C (Experiments 3 and 2, respectively). The 0.01% modified Knops solution contained 2 ppm potassium. Points and solid triangles are values determined by the computer. Open circles and open triangles are experimental mean values.

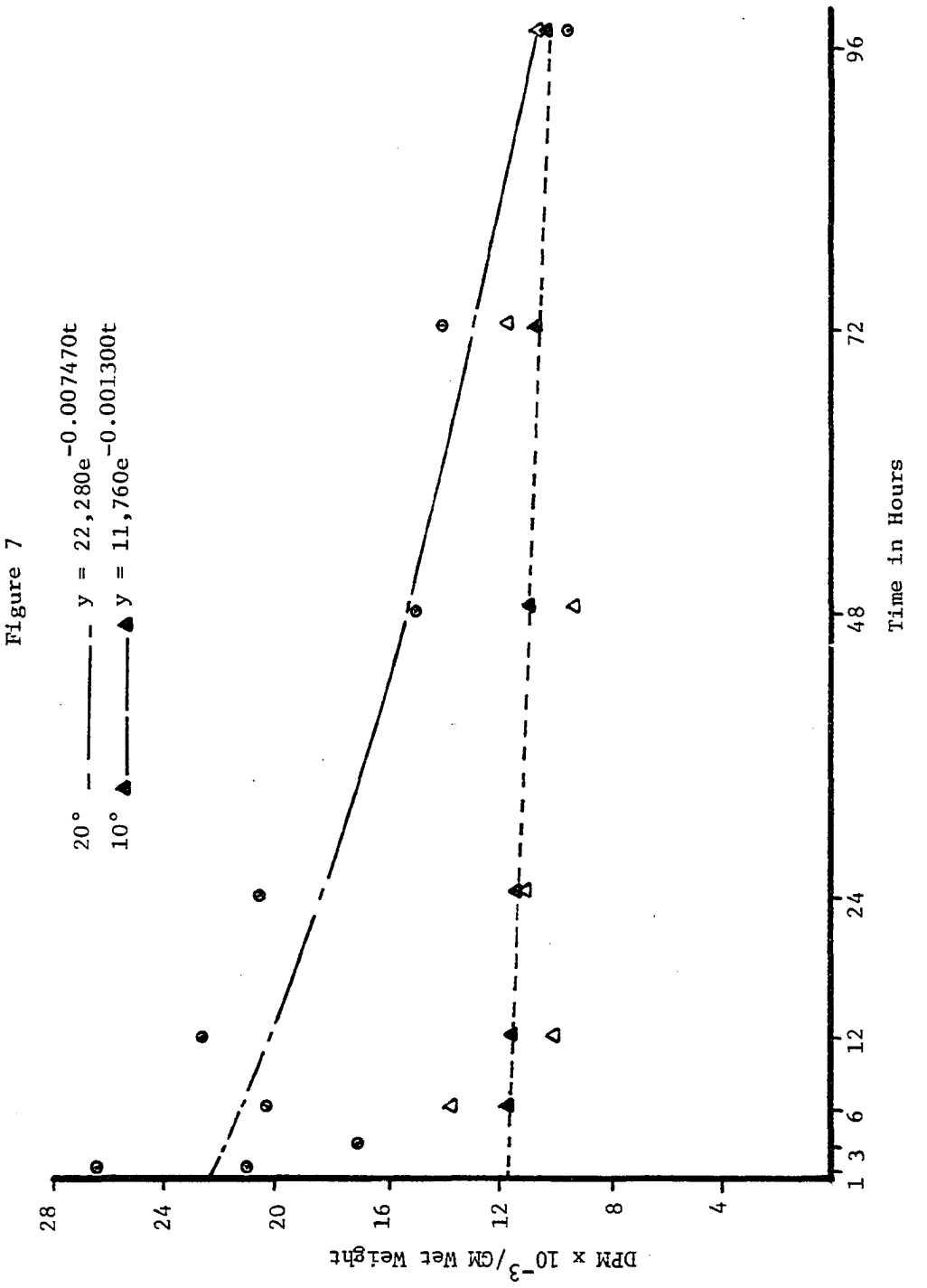

Figure 7

FIGURE 8

Graphic display of the 95% confidence intervals for the α and β parameters for each of the retention experiments. Within each rectangle is the number which describes the conditions of the experiment (see Table 12, page 56).

Figure 8

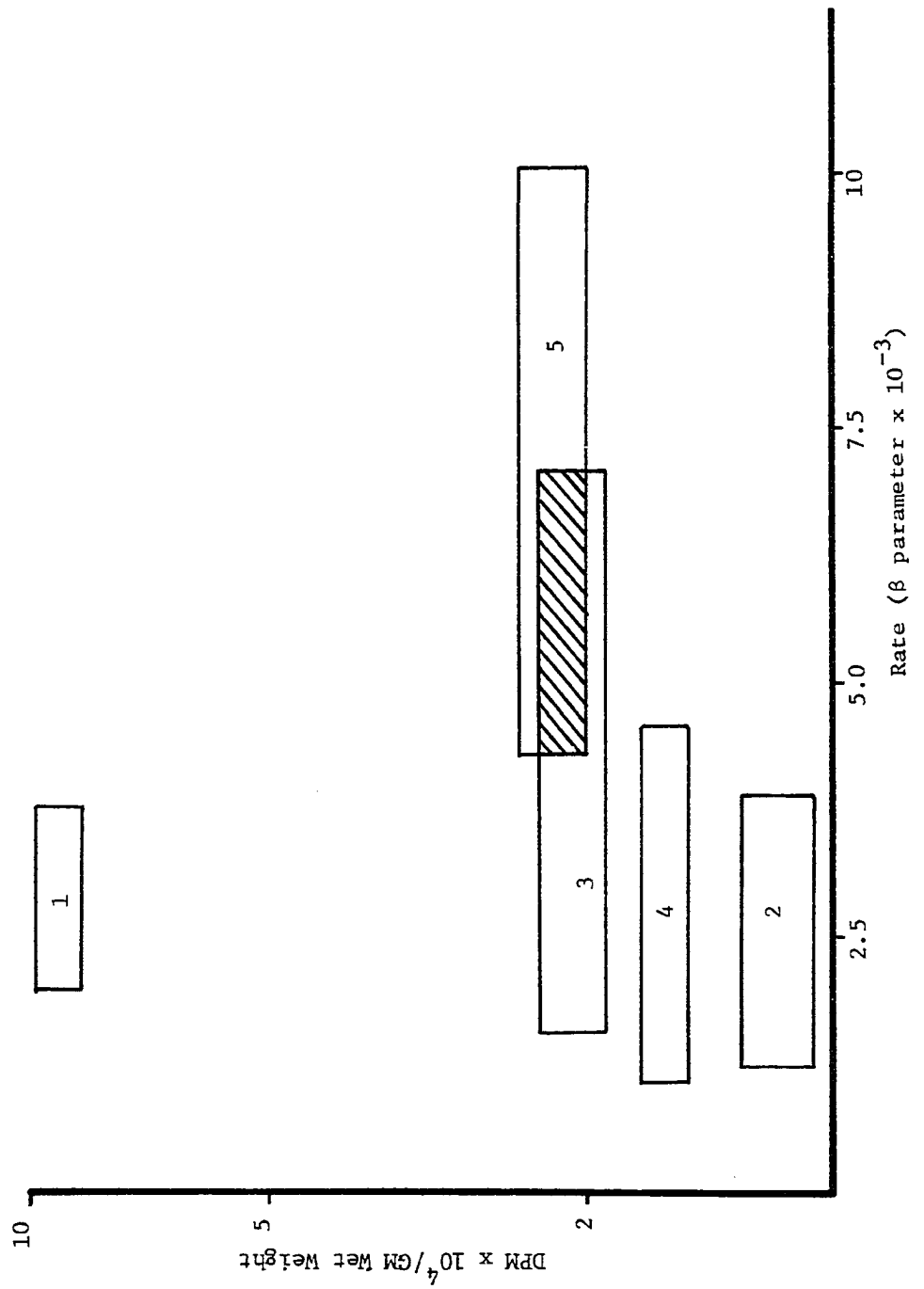

Table 12. Experimental conditions for the retention of Cesium-137 by L. hoffmeisteri. The number presented with each condition corresponds to that condition throughout the statistical analyses.

Experimental Condition 1:	Potassium free 0.01% modified Knops solution maintained at 20°C and contained chloramphenicol.
Experimental Condition 2:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 10°C (E. coli present during accumulation).
Experimental Condition 3:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 20°C (E. coli present during accumulation.
Experimental Condition 4:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 10°C and contained chloramphenicol.
Experimental Condition 5:	2 ppm of potassium in a 0.01% modified Knops solution maintained at 20°C and contained chloramphenicol.

Table 13. The α and β parameters for each of the retention experiments and their 95% confidence intervals. α = asymptotic value of the curve expressed in dpm/gm and β = rate constant. Parameters are in ranked order.

Experiment	α parameter	95% confidence interval
2	11,760	10,470 - 13,060
4	15,870	14,450 - 17,190
5	21,080	18,950 - 23,210
3	22,280	20,170 - 24,400
1	92,130	85,850 - 98,400

Experiment	β parameter	95% confidence interval
2	1.300×10^{-3}	$0.9870 - 3.860 \times 10^{-3}$
1	2.000×10^{-3}	$0.1916 - 3.817 \times 10^{-3}$
4	2.845×10^{-3}	$1.062 - 4.628 \times 10^{-3}$
5	4.384×10^{-3}	$1.597 - 7.172 \times 10^{-3}$
3	7.470×10^{-3}	$0.4338 - 1.060 \times 10^{-2}$

Table 14. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for one hour of ^{137}Cs retention by L. hoffmeisteri. Also presented are mean, standard deviation and sample size for each experiment at one hour.

Bartlett's test for homogenity of variances = 25.04*

ANOVA Table

Source	DF	SS	MS	F
Experiments	2	8.823	4.411	30.78*
Error	27	3.870	0.1433	
Total	29	12.693		

Newman-Keuls Test

Standard deviation of experiment means = 0.1197 Degrees of freedom = 27

Experiment		3	5	1
	Mean	10.07	10.10	11.24
3	10.07	--	0.03	1.17*
5	10.10	--	--	1.14*

Experiment	Mean	Standard Deviation	Sample Size
1	11.24	0.1415	10
3	10.07	0.4801	10
5	10.10	0.4236	10

*Significant differences at the 95% level of confidence.

Table 15. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for three hours of ^{137}Cs retention by L. hoffmeisteri. Also presented are mean, standard deviation and sample size for each experiment at three hours.

Bartlett's test for homogenity of variances = 3.054

ANOVA Table

Source	DF	SS	MS	F
Experiments	2	16.09	8.046	223.4*
Error	26	0.9364	0.03602	
Total	28	17.0264		

Newman-Keuls Test

Standard deviation of experiment means = 0.0611 Degrees of freedom = 26

Experiment		3	5	1
	Mean	9.729	9.763	11.31
3	9.729	--	0.034	1.59*
5	9.763	--	--	1.55*

Experiment	Mean	Standard Deviation	Sample Size
1	11.31	0.2124	10
3	9.729	0.2055	9
5	9.763	0.1461	10

*Significant differences at the 95% level of confidence.

Table 16. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for six hours of ^{137}Cs retention by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at six hours.

Bartlett's test for homogenity of variances = 16.90*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	26.92	6.730	74.38*
Error	45	4.072	0.09049	
Total	49	30.992		

Newman-Keuls Test

Standard deviation of experimental means = 0.0951 Degrees of freedom = 45

Experiment		2	5	4	3	1
	Mean	9.493	9.579	9.672	9.839	11.46
2	9.493	--	0.086	0.179	0.345	1.96*
5	9.579	--	--	0.094	0.260	1.88*
4	9.673	--	--	--	0.166	1.79*
3	9.839	--	--	--	--	1.62*

Experiment	Mean	Standard Deviation	Sample Size
1	11.46	0.1931	10
2	9.493	0.2764	10
3	9.839	0.4515	10
4	9.673	0.2405	10
5	9.579	0.2777	10

*Significant differences at the 95% level of confidence.

Table 17. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for 12 hours of ^{137}Cs retention by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 12 hours.

Bartlett's test for homogenity of variances = 27.77*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	27.65	6.914	134.4*
Error	43	2.212	0.05143	
Total	47	29.862		

Newman-Keuls Test

Standard deviation of experimental means = 0.0735 Degrees of freedom = 43

Experiment		2	4	5	3	1
	Mean	9.214	9.582	9.816	9.959	11.39
2	9.214	--	0.368*	0.603*	0.745*	2.18*
4	9.582	--	--	0.234*	0.377*	1.81*
5	9.816	--	--	--	0.142	1.58*
3	9.959	--	--	--	--	1.43*

Experiment	Mean	Standard Deviation	Sample Size
1	11.39	0.1499	10
2	9.214	0.2035	10
3	9.959	0.3716	10
4	9.582	0.1421	10
5	9.816	0.1741	8

*Significant differences at the 95% level of confidence.

Table 18. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for 24 hours of ^{137}Cs retention by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 24 hours.

Bartlett's test for homogenity of variances = 11.92*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	26.41	6.602	117.6*
Error	44	2.470	0.05615	
Total	48	28.880		

Newman-Keuls Test

Standard deviation of experimental means = 0.0758 Degrees of freedom = 44

Experiment		2	4	5	3	1
	Mean	9.311	9.547	9.658	9.893	11.36
2	9.311	--	0.236*	0.348*	0.582*	2.05*
4	9.547	--	--	0.111	0.346*	1.81*
5	9.658	--	--	--	0.235*	1.71*
3	9.893	--	--	--	--	1.47*

Experiment	Mean	Standard Deviation	Sample Size
1	11.36	0.2256	10
2	9.311	0.2927	10
3	9.893	0.2871	10
4	9.547	0.1966	9
5	9.658	0.1453	10

*Significant differences at the 95% level of confidence.

Table 19. Bartlett's test for homogenity of variances (Chi-square), analysis of variances (ANOVA) and Newman-Keuls test for 48 hours of ^{137}Cs retention by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 48 hours.

Bartlett's test for homogenity of variances = 5.611

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	37.36	9.340	186.5*
Error	45	2.254	0.05008	
Total	49	39.614		

Newman-Keuls Test

Standard deviation of experimental means = 0.0708 Degrees of freedom = 45

Experiment		2	4	3	5	1
	Mean	9.119	9.463	9.586	9.714	11.57
2	9.119	--	0.344*	0.467*	0.595*	2.45*
4	9.463	--	--	0.122	0.251*	2.11*
3	9.586	--	--	--	0.128	1.99*
5	9.714	--	--	--	--	1.86*

Experiment	Mean	Standard Deviation	Sample Size
1	11.57	0.2394	10
2	9.119	0.1699	10
3	9.586	0.2249	10
4	9.463	0.2768	10
5	9.714	0.1925	10

*Significant differences at the 95% level of confidence.

Table 20. Bartlett's test for homogenity of variances (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for 72 hours of ^{137}Cs retention by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 72 hours.

Bartlett's test for homogenity of variances = 16.72*

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	22.03	5.508	86.48*
Error	44	2.802	0.06369	
Total	48	24.832		

Newman-Keuls Test

Standard deviation of experimental means = 0.0807 Degrees of freedom = 44

Experiment		2	4	3	5	1
	Mean	9.356	9.494	9.502	9.707	11.16
2	9.356	--	0.137	0.145	0.350*	1.80*
4	9.494	--	--	0.008	0.213	1.67*
3	9.502	--	--	--	0.205	1.66*
5	9.707	--	--	--	--	1.45*

Experiment	Mean	Standard Deviation	Sample Size
1	11.16	0.1990	10
2	9.356	0.1260	9
3	9.502	0.3141	10
4	9.494	0.2982	10
5	9.707	0.2648	10

*Significant differences at the 95% level of confidence.

Table 21. Bartlett's test for homogenity of variances (Chi-square), analysis of variances (ANOVA) and Newman-Keuls test for 96 hours of ^{137}Cs retention by L. hoffmeisteri. Also presented are the mean, standard deviation and sample size for each experiment at 96 hours.

Bartlett's test for homogenity of variances = 8.735

ANOVA Table

Source	DF	SS	MS	F
Experiments	4	26.28	6.569	117.0*
Error	45	2.527	0.05616	
Total	49	28.807		

Newman-Keuls Test

Standard deviation of experimental means = 0.0749 Degrees of freedom = 45

Experiment		3	2	4	5	1
	Mean	9.137	9.218	9.365	9.562	11.10
3	9.137	--	0.081	0.227	0.425*	1.96*
2	9.218	--	--	0.146	0.344*	1.88*
4	9.365	--	--	--	0.197	1.73*
5	9.562	--	--	--	--	1.53*

Experiment	Mean	Standard Deviation	Sample Size
1	11.10	0.2786	10
2	9.218	0.3003	10
3	9.137	0.2132	10
4	9.365	0.1745	10
5	9.562	0.1927	10

*Significant differences at the 95% level of confidence.

Table 22. Summary of Bartlett's test (Chi-square), analysis of variance (ANOVA) and Newman-Keuls test for the retention experiments. S indicates significant results at the 95% level of confidence and NS indicates no significant differences at the same level.

	Retention Time in Hours							
Test	1	3	6	12	24	48	72	96
Chi-square	S	NS	S	S	S	NS	S	NS
ANOVA	S	S	S	S	S	S	S	S
Newman-Keuls								
1 vs. 5	S	S	S	S	S	S	S	S
4 vs. 5	-	-	NS	S	NS	S	NS	NS
2 vs. 3	-	-	NS	S	S	S	NS	NS
2 vs. 4	-	-	NS	S	S	S	NS	NS
3 vs. 5	NS	NS	NS	NS	S	NS	NS	S

DISCUSSION

Influence of Potassium

The accumulation of ^{137}Cs by the worms in potassium free modified Knops solution was characterized by a rapid accumulation of the isotope from solution. The asymptotic value of the curve (α parameter) is very close to the computer predicted curve after 96 hours of accumulation. This comparison indicates equilibrium was approximated in this study. The study shows that the worms are able to accumulate the isotope from solution. Davis and Foster (1958) noted that radionuclides may be accumulated by aquatic organism by three processes. These processes are: adsorption to exposed surfaces, assimilation of ingested materials and absorption through membranes. It seems unlikely that these worms accumulated the isotope by ingestion of solid materials because of observations of the Knops solution and the clusters of worms at the various sampling times showed that both were free of foreign materials. The chloramphenicol was present at the concentration used because it is bacteriostatic to microorganisms most likely to be introduced by handling. All worms were washed in a Knops solution that contained 50 mg/l of stable cesium to remove isotope adhering to the cuticle of the worms. Whitten and Goodnight (1967) showed through radioautographs that small amounts of Calcium-45 and Strontium-89 remained adsorbed to the cuticular surface, even after the worms were washed in Knops

solution. They explained that mucoproteins which lubricate the cuticle form $CaCO_3$ complexes which may have bound some of the isotopes. If small amounts of the isotope were adsorbed to the cuticle and not removed by washing, it seems unlikely that it would contribute significantly to the total amount accumulated by the worms because there is no known function for cesium in mucoprotein complex formation. The most likely explanation for the accumulation of the isotope is absorption through membranes.

When 2 ppm potassium was present in solution, the accumulation of the isotope was reduced by some 3.5 fold when compared to the potassium free experiment (Figures 1 and 2). The asymptotic value and the computer predicted curve indicate that equilibrium was also affected by the presence of the potassium. These comparisons as well as the analyses and graphic representation of the confidence intervals for the α and β parameters of each experiment all indicate significant differences between these two experiments. These differences are due to the presence of the 2 ppm potassium in the Knops solution.

More ^{137}Cs was retained by the worms in the potassium free solutions versus the worms in the 2 ppm potassium solutions. Eighty-four percent of the radionuclide was retained by the worms in the potassium solutions. Most of the isotope was retained by the worms when potassium was not present in solution.

This is in agreement with the study of Davis (1961). He found that potassium and cesium were absorbed and a deficiency

of potassium increased the absorption and decreased the elimination of the cesium. He also noted that when potassium ions increased in concentration, it decreased the absorption and increased the excretion of cesium.

Many investigators have studied the effects of the presence of a stable isotope on the accumulation of its radioactive counterpart (Tomiyama, Kobayashi and Ishio, 1956a and 1956b; Kornberg, 1960 and 1961; Bryan, 1963; Whitten, 1966; and Whitten and Goodnight, 1967). In all of these investigations, the radioisotope accumulation was reduced when the stable isotope was present. Because of the physical and chemical similarities between ^{137}Cs and potassium, their physiological actions are similar (Davis and Foster, 1958; Davis, 1961; Kornberg, 1960 and 1961; Bryan, 1963). As Kornberg (1960 and 1961) pointed out, these similarities should not be overdrawn because biological processes do not discriminate between these two elements in a constant and predictable manner. Bryan study on brackish water invertebrates in 1963 found that cesium concentrations increased to higher levels than did potassium. Body surface area was more important in the accumulation and loss of potassium while the amount of muscle tissue seemed to be a limiting factor in the accumulation and loss of cesium.

The experiments on the accumulation and retention of ^{137}Cs by the worms in solutions with and without potassium demonstrate the similarities between cesium and potassium. The worms accumu-

lated and retained more cesium when there was no potassium present. The presence of 2 ppm of potassium reduced the amount of cesium accumulation by 3.5 fold and decreased the amount of the isotope retained by these worms. If potassium were not similar to cesium, its presence in solution should not alter the accumulation and retention of the isotope by the worms. However, potassium in solution greatly modified both accumulation and retention curves. This indicates that potassium was competing with cesium for absorption by the worms. These findings again demonstrate the biological importance of ^{137}Cs because of the physiological importance of potassium in living organisms.

Influence of Temperature

These experiments, 4 and 5, and their analyses show that there is a significant temperature effect on the accumulation and retention of ^{137}Cs by L. hoffmeisteri. At 20°C the worms accumulated the isotope exponentially. Accumulation at 10°C was nearly linear. The Newman-Keuls test compared these two experiments and showed significant difference in the amount of isotope accumulated at every sampling time (see summary, Table 12). Approximately twice as much cesium was accumulated at 20°C than at 10°C (Figure 2). Retention of the isotope at both temperatures was linear (Figure 6). Although there were some differences at the various sampling times, Figure 8 shows that these experiments were different when both parameters for each experiment are compared. The retention curve

in Figure 6 indicates a more rapid loss of the isotope at 20°C with retention approaching the 10°C curve at 96 hours. The rates of loss are clearly not different, but the equilibrium values are. These findings are in good agreement with the work of Stromberg and Goodnight (1971). They studied the effect of temperature on the accumulation of Phosphorous-32 by Limnodrilus worms. They found that an increase in the rate of accumulation occurred at the higher temperature but there was no clear indication of a change in the equilibrium of ^{32}P with the higher temperature.

Davis and Foster (1958) noted that the effect of temperature may be a more involved relationship on the concentration of radioisotopes under natural conditions. The effect is probably on the rate of biological processes. During cold weather fish in the Columbia River consumed less food and, consequently, less radioactivity. A similar condition was noted in insects, crustaceans and mollusks by Polikarpov, 1966. Glaser in 1961 found that temperature affected the concentration factor of Iodine-131 in Drussensia polymorpha. The concentration ratio between 3°C and 20°C was 1:4, respectively. There were no differences between the 3°C and 13°C concentration factors. It was shown by Boroughs, Chipman and Rice (1957) that Plaice fry concentrated less Strontium-89 at a lower temperature. Only a third of the amount concentrated at 20°C was concentrated at 10°C.

In Limnodrilus hoffmeisteri, the amount of ^{137}Cs accumulated was decreased at a lower temperature. The rates of accumulation were significantly different between these two experiments, but equilibrium values were not. If equilibrium were significantly altered by different temperatures, some physical process could be hypothesized in controlling cesium accumulation by these worms. But it was not, suggesting that enzyme kinetics were reduced by the lower temperature and consequently, so were biological processes responsible for the accumulation and retention of cesium in these worms.

Influence of E. coli

The purpose of these studies was to determine the influence of E. coli on the accumulation and retention of ^{137}Cs by the tubificid worm, L. hoffmeisteri. The analyses of the accumulation of ^{137}Cs by the worms with and without E. coli at 20°C show that these experiments were not significantly different. Figure 8 also demonstrates that these studies were not different because of the overlap (shaded area) of the rectangles, which describe the intervals of each experiment. Comparing curves and α parameters of these two experiments indicate slightly more ^{137}Cs was accumulated by the worms when E. coli were not present in the Knops solution. At 10°C both of the parameters for each experiment (with and without E. coli) are different. Clearly the worms at 10°C without E. coli accumulated more isotope and at a different

rate. Because slightly more isotope was accumulated by the worms at 20°C without E. coli being present in solution and significantly more isotope was accumulated by these worms at 10°C without E. coli, indicates that the amount of isotope available to the worms in the studies without E. coli was greater than the amount available to the worms in the studies with E. coli (Figures 2, 3 and 4). These findings indicate that the bacterium competed with the worms for the available cesium. Bacteria are known to accumulate radionuclides (Davis and Foster, 1958; Gunnison and Goodnight, 1971). The retention studies indicate the same findings. Retention rates were not different for these studies at either temperature. What was significantly different was the amount of isotope in these worms at the beginning of the retention experiments (Figures 6, 7 and 8).

These findings appear to be in contradiction with the findings of other investigators who have studied E. coli and tubificid worms with other isotopes. Whitten and Goodnight (1967) studied the accumulation of ^{32}P by Limnodrilus worms from solution, bacteria and sediments. These worms accumulated more phosphorous from labeled E. coli than from solution or sediments. It was thought that the bacteria changed the inorganic phosphate to an organic form more useful to the worms. In studies performed by Stromberg and Goodnight (1971) the transfer of ^{32}P from E. coli and Chlamydomas pyrenoidosa to Limnodrilus worms showed that the worms accumulated more ^{32}P from the bacteria than from the algae. The bacteria and algae competed with the worms for the isotope as

the worms ingested them. It was found that these worms did not have an endogenous cellulase to digest the cellulitic cell wall of the algae to absorb the bound radiophosphorous.

In the present studies, the worms were speciated and only worms belonging to the genus species Limnodrilus hoffmeisteri were used. In the aforementioned studies, worms of the genus Limnodrilus were studied. Brinkhurst and Chara (1969) studied some of the potential nutritional resources by three tubificid worms. Seven species of bacterium were fed to the worms. After one week of exposure, only one species could be found in the media and worm gut. The surviving specie was different for each worm. This suggested to the experimentor that a difference in digestive enzymes occurred in each species studied. This study indicated specific physiological differences that may be related to the distribution and niche the species of worms occupy.

Because Limnodrilus hoffmeisteri did not accumulate ^{137}Cs better when E. coli were present in solution, indicates that E. coli did not play a significant role in these accumulation studies and that these worms collected from the Kalamazoo River may not have been able to digest the E. coli. The inability to digest the bacterium may be related to the niche that these worms occupy in the Kalamazoo River or to different digestive enzymes present in L. hoffmeisteri compared with other Limnodrilus worms.

SUMMARY

1. Limnodrilus hoffmeisteri worms were able to accumulate Cesium-137 from a 0.01% modified Knops solution that was potassium free. The most likely process of accumulation was absorption.

2. When 2 ppm potassium was present in a 0.01% modified Knops solution, the amount of Cesium-137 accumulated by the worms was reduced by 3.5 fold when compared to the potassium free experiment. The rate of accumulation was not appreciably affected and equilibrium was not attained during the study period.

3. A greater amount of the radioisotope was retained in the potassium free experiment versus the 2 ppm experiment. The rates of loss were not significantly different.

4. Accumulation curves were determined for the worms at 20°C and 10°C. The lower temperature reduced the accumulation of radioisotope by approximately two-fold. Equilibrium did not appear to be affected, but the shapes of the curves were. Accumulation at 20°C was exponential, while nearly linear at 10°C.

5. A greater percentage of the radioisotope was retained at the lower temperature. At 20°C less ^{137}Cs was retained and by the end of the study approached the 10°C retention curve.

6. There were no significant differences between the accumulation experiments at 20°C with and without E. coli. However, the worms appeared to accumulate slightly more radioisotope without E. coli. At 10°C more isotope was accumulated when E. coli were not present in the Knops solution.

It appeared that the bacteria competed with the worms for the available radioisotope. Possibly the worms were not able to digest these bacteria.

7. There was no clear indication that the retention experiments at 20°C with and without E. coli were different. At 10°C both experiments were different in the amount of radioisotope retained, but not in the rate of loss. This probably reflects differences in the amount of the radioisotope available to the worms during accumulation due to competition with E. coli.

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Appendix 1

Experimental Data for the Accumulation of ^{137}Cs by L. hoffmeisteri in Potassium Free Modified Knops Solution with Chloramphenicol at 20°C (Experiment 1)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
1	7,703	24	101,539
1	7,995	24	58,867
1	9,989	24	37,315
1	10,368	24	58,321
1	5,693	24	41,762
1	10,279	48	76,055
1	9,652	48	82,829
1	13,285	48	89,413
1	12,380	48	81,359
1	9,239	48	83,621
3	15,134	48	80,705
3	15,216	48	116,459
3	17,758	48	120,476
3	18,119	48	88,134
3	12,081	48	125,832
3	13,585	72	64,063
3	18,204	72	117,367
3	13,868	72	168,617
3	12,698	72	118,589
3	12,505	72	159,210
6	13,371	72	119,381
6	13,870	72	69,490
6	17,099	72	84,865
6	17,681	72	152,387
6	17,938	96	73,323
6	14,705	96	78,125
6	18,905	96	94,670
6	16,589	96	102,248
6	16,933	96	75,520
6	14,633	96	73,373
12	40,732	96	75,061
12	47,550	96	68,895
12	37,663	96	112,360
12	37,104	96	64,167
12	40,886	96	183,738
12	31,030	96	112,366
12	32,788	96	113,533
12	36,757	96	59,792
12	36,754	96	144,564
12	47,742	96	67,482
24	39,827	96	60,670
24	44,949	96	102,100
24	70,646	96	150,201
24	55,475	96	106,586
24	35,062		

Appendix 2

Experimental Data for the Accumulation of ^{137}Cs by L. hoffmeisteri in 2 ppm Potassium Modified Knops Solution with E. coli at 10°C (Experiment 2)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
6	1,805	48	6,824
6	2,724	48	6,839
6	1,985	48	5,820
6	1,840	48	6,809
6	2,000	48	7,061
6	1,747	48	6,175
6	1,907	48	17,717
6	1,734	48	6,484
6	1,726	48	15,872
6	1,912	48	13,243
12	2,484	72	17,683
12	4,549	72	9,894
12	2,889	72	11,277
12	2,361	72	11,720
12	5,219	72	12,063
12	3,302	72	9,645
12	2,665	72	9,340
12	3,075	72	16,360
12	2,439	72	9,629
12	4,032	72	11,885
24	6,621	96	11,571
24	7,257	96	12,490
24	4,725	96	9,747
24	7,928	96	11,462
24	4,368	96	10,377
24	4,962	96	10,028
24	4,203	96	16,157
24	4,013	96	10,472
24	4,756	96	11,396
24	4,355	96	9,914

Appendix 3

Experimental Data for the Accumulation of ^{137}Cs by *L. hoffmeisteri* in 2 ppm Potassium Modified Knops Solution with *E. coli* at 20°C (Experiment 3)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
1	298	24	8,894
1	802	24	16,968
1	499	24	7,680
1	608	24	6,930
1	464	24	9,559
1	488	24	5,412
1	770	24	7,837
1	423	24	5,979
1	1,000	24	5,514
1	778	24	12,305
3	1,430	48	14,437
3	2,928	48	8,522
3	1,396	48	13,790
3	1,836	48	7,412
3	1,075	48	8,188
3	2,301	48	15,393
3	1,622	48	9,432
3	2,237	48	22,046
3	1,655	48	14,701
3	3,016	48	23,753
6	3,754	72	18,250
6	2,601	72	15,107
6	4,629	72	15,520
6	2,986	72	12,908
6	2,466	72	14,422
6	3,102	72	12,464
6	5,451	72	19,054
6	2,243	72	17,262
6	3,190	72	19,351
6	4,306	96	20,890
12	3,137	96	21,101
12	3,224	96	22,115
12	3,564	96	20,859
12	7,100	96	23,326
12	2,739	96	14,414
12	3,206	96	28,504
12	3,427	96	16,018
12	3,527	96	13,689
12	4,074	96	20,771
12	4,417		

Appendix 4

Experimental Data for the Accumulation of ^{137}Cs by L. hoffmeisteri in 2 ppm Potassium Modified Knops Solution with Chloramphenicol at 10°C (Experiment 4)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
6	1,020	48	9,742
6	1,423	48	12,838
6	2,093	48	7,568
6	1,381	48	7,322
6	2,617	48	4,404
6	1,919	48	7,043
6	2,135	48	6,611
6	1,536	48	9,625
6	1,852	48	7,253
6	1,584	48	10,630
12	2,816	72	12,295
12	2,372	72	9,843
12	2,363	72	10,436
12	3,384	72	10,926
12	2,772	72	8,227
12	2,092	72	8,871
12	2,500	72	13,286
12	2,482	72	8,691
12	2,246	72	9,748
12	1,867	72	10,242
24	4,090	96	15,133
24	5,008	96	14,496
24	4,687	96	22,517
24	5,396	96	17,628
24	4,988	96	12,137
24	4,073	96	11,611
24	4,616	96	24,399
24	5,392	96	13,278
24	4,664	96	14,275
24	6,090		

Appendix 5

Experimental Data for the Accumulation of ^{137}Cs by L. hoffmeisteri in 2 ppm Potassium Modified Knops Solution with Chloramphenicol at 20°C (Experiment 5)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
1	5,851	24	8,623
1	1,091	24	15,347
1	2,812	24	6,586
1	2,378	24	6,560
1	2,910	24	11,069
1	1,877	24	7,456
1	3,815	24	15,800
1	1,074	24	12,877
3	3,628	48	16,101
3	2,470	48	15,200
3	7,047	48	18,873
3	3,353	48	12,938
3	2,230	48	15,373
3	1,784	48	18,988
3	2,644	48	14,098
3	1,619	48	10,806
3	4,313	48	20,493
3	2,125	48	23,154
6	4,415	72	27,139
6	4,387	72	22,244
6	2,266	72	36,975
6	4,627	72	28,809
6	3,167	72	30,674
6	3,126	72	35,039
6	2,536	72	23,037
6	3,030	72	36,800
6	3,937	72	20,875
12	7,150	72	18,398
12	8,043	96	19,746
12	6,678	96	19,843
12	7,146	96	35,701
12	4,087	96	14,631
12	6,529	96	19,041
12	6,162	96	40,082
12	10,180	96	41,906
12	7,426	96	33,816
12	12,118	96	14,881
24	10,595	96	28,192
24	12,688		

Appendix 6

Experimental Data for the Retention of ^{137}Cs by L. hoffmeisteri in Potassium Free Modified Knops Solution with Chloramphenicol at 20°C (Experiment 1)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
0	73,323	6	74,198
0	78,125	6	105,074
0	94,670	6	124,640
0	102,248	6	98,217
0	75,520	6	79,761
0	73,373	6	108,624
0	75,061	6	81,539
0	68,895	6	97,590
0	112,360	6	119,226
0	64,167	6	73,574
0	183,738	12	97,991
0	112,366	12	92,519
0	113,533	12	99,799
0	59,792	12	105,364
0	144,564	12	79,677
0	67,482	12	100,076
0	60,670	12	77,628
0	102,100	12	65,729
0	150,201	12	81,770
0	106,586	12	96,290
1	69,658	24	99,658
1	82,562	24	122,662
1	57,800	24	70,243
1	84,639	24	62,631
1	79,284	24	87,983
1	81,909	24	70,304
1	65,340	24	104,749
1	75,783	24	80,328
1	94,825	24	109,664
1	75,335	24	73,459
3	111,119	48	81,040
3	77,209	48	98,390
3	59,420	48	70,118
3	91,912	48	125,648
3	94,441	48	108,051
3	60,804	48	152,557
3	102,380	48	91,238
3	72,464	48	103,389
3	75,113	48	128,767
3	91,421	48	130,882

Appendix 6 (Cont'd)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
72	77,231	96	71,455
72	60,928	96	76,356
72	100,273	96	49,518
72	55,707	96	39,376
72	79,673	96	59,447
72	81,814	96	68,463
72	69,316	96	91,862
72	76,080	96	64,730
72	61,500	96	59,345
72	52,623	96	101,849

Appendix 7

Experimental Data for the Retention of ^{137}Cs by *L. hoffmeisteri* in 2 ppm Potassium Modified Knops Solution at 10°C (Experiment 2)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
0	11,571	24	10,030
0	12,490	24	9,007
0	9,747	24	8,405
0	11,462	24	11,783
0	10,377	24	11,427
0	10,028	48	9,282
0	16,157	48	7,703
0	10,472	48	13,726
0	11,396	48	8,829
0	9,914	48	9,596
6	10,403	48	9,090
6	20,549	48	7,953
6	11,724	48	9,942
6	21,787	48	8,782
6	12,591	48	7,617
6	11,406	72	12,681
6	12,037	72	14,958
6	10,082	72	10,258
6	11,143	72	11,874
6	15,981	72	11,294
12	9,247	72	9,919
12	11,944	72	10,399
12	12,032	72	11,493
12	9,349	72	12,052
12	11,594	96	7,216
12	10,131	96	8,544
12	10,038	96	9,678
12	6,024	96	13,142
12	10,319	96	7,474
12	11,365	96	11,066
24	7,890	96	9,876
24	13,439	96	16,282
24	19,145	96	7,051
24	15,537	96	14,733
24	8,484		

Appendix 8

Experimental Data for the Retention of ^{137}Cs by L. hoffmeisteri in 2 ppm Potassium Modified Knops Solution at 20°C (Experiment 3)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
0	20,890	12	13,511
0	21,101	12	22,841
0	22,115	12	13,700
0	20,859	12	27,417
0	23,326	24	35,445
0	14,414	24	23,557
0	28,504	24	19,713
0	16,018	24	15,301
0	13,689	24	24,549
0	20,771	24	14,488
1	13,313	24	17,358
1	32,748	24	14,162
1	20,805	24	23,129
1	18,728	24	18,031
1	40,478	48	13,696
1	54,976	48	16,898
1	14,580	48	11,785
1	30,993	48	9,792
1	23,163	48	16,009
1	14,205	48	16,802
3	14,116	48	21,899
3	11,394	48	15,611
3	18,830	48	13,951
3	14,277	48	12,453
3	17,265	72	22,762
3	18,520	72	12,052
3	20,023	72	9,541
3	22,091	72	8,845
3	17,440	72	14,558
6	7,846	72	16,119
6	28,238	72	11,320
6	11,283	72	10,489
6	26,684	72	13,997
6	11,876	72	20,397
6	26,396	96	9,514
6	18,272	96	8,047
6	22,284	96	11,176
6	24,887	96	9,168
6	25,282	96	9,292
12	30,414	96	8,971
12	16,989	96	9,932
12	23,269	96	5,917
12	22,374	96	9,231
12	14,087	96	13,582
12	40,516		

Appendix 9

Experimental Data for the Retention of ^{137}Cs by L. hoffmeisteri in 2 ppm Potassium Modified Knops Solution with Chloramphenicol at 10°C (Experiment 4)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
0	15,133	24	11,919
0	14,496	24	11,816
0	22,517	24	12,704
0	17,628	24	12,670
0	12,137	48	11,935
0	11,611	48	13,727
0	24,399	48	10,346
0	13,278	48	11,100
0	14,275	48	9,383
6	17,118	48	22,427
6	16,610	48	15,537
6	12,216	48	16,518
6	14,177	48	9,334
6	14,335	48	13,246
6	12,002	72	12,432
6	28,132	72	9,393
6	15,832	72	11,465
6	17,823	72	8,878
6	15,163	72	21,955
12	15,649	72	10,345
12	12,407	72	18,713
12	13,305	72	13,814
12	14,496	72	14,847
12	14,363	72	16,433
12	14,274	96	13,521
12	13,578	96	8,645
12	18,569	96	10,906
12	17,786	96	10,127
12	11,972	96	12,390
24	14,587	96	10,679
24	13,138	96	16,240
24	16,073	96	13,151
24	22,109	96	11,576
24	13,461	96	11,077

Appendix 10

Experimental Data for the Retention of ^{137}Cs by L. hoffmeisteri in 2 ppm Potassium Modified Knops Solution with Chloramphenicol at 20°C (Experiment 5)

Sample Time in Hours	DPM/GM Wet Weight of Worms	Sample Time in Hours	DPM/GM Wet Weight of Worms
0	19,746	12	23,017
0	19,843	12	14,173
0	35,701	12	18,503
0	14,631	12	20,616
0	19,041	24	20,142
0	40,082	24	16,254
0	41,906	24	14,101
0	33,816	24	16,561
0	14,881	24	13,920
0	28,192	24	13,319
1	18,076	24	19,556
1	31,249	24	13,588
1	34,387	24	15,598
1	14,441	24	15,051
1	23,202	48	24,558
1	28,788	48	14,899
1	15,300	48	15,362
1	47,791	48	14,464
1	36,214	48	21,639
1	15,346	48	15,995
3	17,908	48	15,934
3	16,704	48	15,989
3	16,212	48	12,774
3	15,290	48	16,846
3	16,288	72	23,035
3	16,235	72	14,667
3	16,171	72	11,363
3	25,337	72	17,910
3	19,134	72	19,718
3	16,435	72	17,726
6	15,767	72	10,420
6	21,738	72	22,337
6	15,624	72	17,606
6	24,211	72	14,538
6	10,705	96	13,064
6	12,869	96	13,641
6	11,308	96	14,272
6	11,243	96	14,914
6	12,561	96	20,189
6	14,013	96	10,357
12	21,692	96	17,519
12	18,484	96	13,642
12	14,680	96	11,457
12	17,412	96	15,538