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Relationships between Radiophosphorus Accumulation by Bacteria, Algae, and Tubificid Worms in Laboratory Food Chains

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RELATIONSHIPS BETWEEN RADIOPHOSPHORUS ACCUMULATION BY BACTERIA, ALGAE, AND TUBIFICID WORMS IN LABORATORY FOOD CHAINS

by

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Warren L. Stromberg

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INTRODUCTION

Phosphorus is known to be an essential element for all life forms. The concentrations of phosphorus in natural bodies of water may, therefore, play a significant role in limiting the abundance and the distribution of aquatic life forms in many lakes and ponds. The dependence of aquatic organisms on the phosphorus concentrations of water has led limnologists to study the mechanisms of phosphorus movement through producer and consumer organisms in aquatic ecosystems. The transfer of phosphorus in food chains has been measured directly in these investigations by the use of radiophosphorus - ^{32}P as a tracer.

Phillips (1963) investigated the uptake of radiophosphorus by Gammarus sp. which were grown in cultures containing ^{32}P in the culture media. This crustacean was found to accumulate radiophosphorus indirectly by the ingestion of radioactive bacteria in these media, as in bacteria-free cultures uptake of radiophosphorus by Gammarus was negligible. Phillips concluded from these results that micro-organisms may play an essential role in the biological distribution of phosphorus in lakes by removing this element from the bottom sediment and thus making it available to consumer organisms.

Whittaker (1961) investigated the movement of radiophosphorus in food chains in aquaria and in an artificial pond. Daphnia sp. grown in aquaria were found to accumulate ^{32}P by adsorption from the water during the first hour of uptake. Ingestion of bacteria

accounted for subsequent internal radiophosphorus accumulation. More than 95% of the total radiophosphorus accumulated by Daphnia in these cultures was accumulated by ingesting and assimilating radioactive bacteria. Guppies fed upon these Daphnia and 24.0 hours after feeding these fish retained 53% to 64% of the ingested radiophosphorus. Whittaker concluded that the measurement of radiophosphorus concentrations in these consumer organisms reflected (1) the radioactivity levels of the food organisms, and (2) the turnover rate of the isotope in the consumer organisms.

Davis and Foster (1958) measured the distribution of radioisotopes in a river contaminated by radiophosphorus from an industrial source. These authors found that fish collected downstream from the radioactive effluent contained one hundred times as much radioisotope as fish exposed to the effluent in the laboratory but fed only non-radioactive food. These authors concluded that ingestion and assimilation of radiophosphorus in food sources accounted for radiophosphorus concentrations observed in these fish.

Ball and Hooper (1963) investigated radiophosphorus translocation in a Michigan trout stream after the isotope was added to stream water. Patterns of concentration of radiophosphorus were found to be similar in closely related invertebrates, such as nymphs of mayflies in the genus Ephemerella, which occupied different ecological niches. These authors concluded that some patterns of radiophosphorus accumulation observed in aquatic invertebrates may be characteristic of taxonomic groups of these organisms.

Whitten and Goodnight (1969) investigated the accumulation of radiophosphorus by tubificid worms. These worms were found to accumulate radiophosphorus by the ingestion and assimilation of bacteria containing this isotope. Bluegills and bluntnose minnows feeding upon these worms assimilated radiophosphorus and incorporated this isotope into liver, gills, scales, and intestine. These authors concluded that tubificid worms may act as components of the aquatic biota which transfer radiophosphate from bacteria to consumer organisms at higher trophic levels. Gunnison and Goodnight (1971) subsequently demonstrated the ability of Escherichia coli cells to accumulate and retain radiophosphorus in a modified Knop's medium. These bacteria were shown to be a food organism for tubificid worms in laboratory cultures by Coler and Gunner (1967).

However, some planktonic organisms may not be food sources of radiophosphorus for invertebrate consumer organisms in aquatic food chains. Rice (1963), for instance, found that radiophosphorus in Chlorella pyrenoidosa was not absorbed by oysters feeding on these algae. Rice attributed the lack of radiophosphorus uptake by oysters in these experiments to an inability of these organisms to digest Chlorella cells. Davis and Foster (1958) noted that radioisotopes may accumulate in aquatic organisms as a result of (1) adsorption to exposed surface areas, (2) absorption into cells or tissues, or (3) assimilation of ingested compounds.

The purpose of the present study was to determine which of these mechanisms of radioisotope accumulation accounted for radiophosphorus

concentrations in tubificid worms and Chlorella pyrenoidosa cells in laboratory food chains. Tubificid accumulation of radiophosphorus from Chlorella pyrenoidosa was to be compared with accumulation of this radioisotope from bacteria and organic detritus in order to determine whether Chlorella cells may serve as a food source of radiophosphorus for these worms.

LITERATURE REVIEW

Phosphorus Requirements of Algae

Ketchum and Redfield (1949) demonstrated that the normal phosphorus content of green algae was approximately 2% of the dry weight of the algal cell. Al Kholy (1956) found a range of from 10^{-7} g P/cell to 1.5×10^{-6} g P/cell in Chlorella pyrenoidosa.

Intracellular concentrations of phosphorus above and below these values have been reported for algal cells in the laboratory. Ketchum (1939) demonstrated that phosphorus deficiency could be produced in Chlorella cells by incubating these cells in a phosphorus-free medium. Intracellular concentrations of phosphorus in excess of normal concentrations were reported by Lund (1950). This "luxury consumption" was observed in cells grown in artificial media containing phosphate ions.

The concentrations of phosphorus in lake water which are required to support growth of the algae are not known. The diatom, Asterionella formosa, did not grow in artificial media with phosphate concentrations less than ten milligrams per cubic meter (Rodhe, 1948). However, this diatom was found to grow in lake water with less than ten milligrams of phosphate per cubic meter. It has been hypothesized that an unknown ingredient of lake water might have permitted algal growth in lakes with low concentrations of phosphate (Fogg and Westlake, 1955). Although this hypothesized component of lake water

has not been identified, it is referred to as the "phosphate sparing factor".

Physiology of Radiophosphate Uptake by Chlorella

Gest and Kamen (1948) demonstrated that most of the radiophosphate accumulated by C. pyrenoidosa in artificial media was readily removed by rinsing, and hence represented radiophosphate associated with the cell in loosely bound or adsorbed form. Such adsorption of radiophosphate ions may mask metabolic uptake of these ions by this alga. However, using the trichloroacetic acid insoluble portion of cell phosphates as an index of uptake these authors found no direct relationship between radiophosphate absorption by C. pyrenoidosa and inhibition of respiratory activity by cyanide ion.

Light has been reported to influence radiophosphate uptake by the green algae. Radiophosphate accumulation by Chlorella spp. is greater in light than in darkness, and this effect is most pronounced in cells grown under conditions of phosphorus deficiency (Kuhl, 1962; Kylin, 1964). Ketchum (1939) reported similar results in a study of algal accumulation of the stable isotope of phosphorus. Spiegelman and Kamen (1946) found that orthophosphate enters the trichloroacetic acid soluble portion of cell phosphates in the photosynthetic cell as a phosphoester. Organic phosphate compounds were synthesized from a phosphate reservoir in this TCA soluble portion. Gest and Kamen reported that ester phosphates were formed upon absorption of light by the algal cell, but found no evidence of direct

coupling of esterification with light absorption.

Phosphate need not be available as orthophosphate in the extracellular medium for accumulation to occur. Galloway and Krauss (1963) found that Chlorella were capable of accumulating high molecular weight polyphosphates at the same rate as the smaller orthophosphate. An enzyme associated with the cell wall rapidly split the polyphosphate molecule up into orthophosphate subunits, which were accumulated by the cells. Kuenzler (1965) reported that glucose phosphate was degraded by an extracellular enzyme produced by the algal cell. Orthophosphate liberated from glucose phosphate was then readily accumulated by the cell.

Role of Phytoplankton in the Ecology of Radiophosphate

That phytoplankton are capable of rapid accumulation of inorganic radiophosphate has been established (Krumholz and Foster, 1957; Phillips, 1963; Whittaker, 1961). However, the role of phytoplankton in radiophosphorus movement through aquatic food chains is unclear.

Whittaker (1961) demonstrated that rapid initial radiophosphate removal from aquarium water after tracer addition was due to plankton (predominantly unicellular green algae) accumulation of the isotope. However, he was unable to segregate the phytoplankton from other fractions of the plankton to determine their relative importance in the removal of this isotope from the water. Krumholz and Foster (1957) found radiophosphate concentration factors of approximately 200,000

in phytoplankton in field studies. This concentration factor is higher than those recorded for other aquatic community fractions as reviewed by Polikarpov (1966).

The relative importance of phytoplankton as a source of radiophosphate for consumer organisms was investigated by Rice (1963). This author found that radiophosphate was not assimilated by oysters which ingested radioactive Chlorella pyrenoidosa. The failure of these mollusks to assimilate radiophosphorus was attributed to the resistance of this alga to digestion. Whittaker (1961) reported a slow movement of radiophosphorus from mixed plankton to snails in aquaria, but this study did not measure the contribution of phytoplankton to the total movement. Rigler (1956), however, found that more that 60% of the radiophosphate accumulated by mixed plankton in a bog lake was accumulated by bacteria. Rigler concluded that bacteria competed with algae for this isotope. The presence of aquatic bacteria, therefore, may reduce the importance of phytoplankton in radiophosphate accumulation by consumer organisms in aquatic food chains.

Algae may play a role in the conversion of inorganic radiophosphate to organic phosphates in some lakes. Hutchinson and Bowen (1947, 1950), in studies of inorganic radiophosphate circulation in a stratified pond, reported that algae accumulated organic radiophosphorus compounds less rapidly than they accumulated the inorganic ion. Organic phosphate compounds were synthesized by phytoplankton and subsequently were released into the extracellular medium. These

studies did not identify the organic phosphorus compounds.

Watt and Hayes (1963) and Phillips (1963) found that the release of organically bound radiophosphorus was characteristic of senescent phytoplankton cultures, but that comparatively little release occurred in cultures during the growth phase. The dissolved organic radiophosphates released in senescent cultures primarily of nucleotides and phosphorylated carbohydrates.

Hutchinson and Bowen (1947, 1950) found that seston at the mud-water interface rapidly released radiophosphate after initial accumulation. Einsele (1941), using nonradioisotopic methods, found an insignificant loss of phosphate from sedimenting seston, however. Hasler and Einsele (1948) found that the high concentrations of inorganic phosphate in deep water sediments resulted in part from the solubilization of ferric phosphate. Hutchinson (1957) estimated the contribution of sedimenting seston to the hypolimnion phosphorus to be less than that resulting from such solubilization.

Radiophosphate Accumulation by Tubificid Worms

The accumulation of radiophosphate by tubificid worms has been investigated by Whitten and Goodnight (1969) and by Whitten (1966). Whitten reported that the accumulation of radiophosphorus by tubificids was upon addition of the stable isotope of phosphorus to the solution. Radioautographs indicated that the radiophosphate accumulated by tubificid worms from solution was absorbed in the tissues. Whitten and Goodnight (1969) investigated the role of tubificids in radiophosphate movement in an aquatic food chain in the

laboratory. These worms were found to accumulate radiophosphate from water and from bacteria. Accumulation of radiophosphate adsorbed on inorganic sediment, however, was minimal. Fish feeding upon these radioactive worms were found to incorporate radiophosphate into their tissues. Equilibrium values of radiophosphorus accumulation in these fish were observed after two weeks of feeding upon radioactive tubificid worms. Whitten and Goodnight concluded from these results that tubificid worms may act as components of the aquatic biota which transfer radiophosphate from bacteria and from organic detritus to consumer organisms at higher trophic levels.

METHODS AND MATERIALS

Preparation of Algal Cultures

Unialgal cultures of Chlorella pyrenoidosa were obtained from the Carolina Biological Supply Company. Cells from these cultures were inoculated on Trypticase Soy Agar (BBL) slants in the laboratory. These slants were then incubated at 23°C. in a Sherer Model 4-4 environmental chamber. Constant illumination was provided during incubation by a bank of seven 20 Watt KenRad fluorescent lamps. New stock cultures were inoculated in Trypticase Soy Agar slants monthly. Samples of these cultures were plated on this same agar for evidence of bacterial or fungal contamination. All algae used in experiments were obtained from uncontaminated stock cultures.

Bellco 250 ml. culture vessels were coated with "Desicote", a polymethyl siloxane oil, to prevent adsorption of ions onto glass surfaces. 100.0 ml. of a modified Knop's solution (see Appendix I for composition) was placed in each culture vessel. These vessels were then capped with stainless steel lids to prevent microbial contamination, and to inhibit evaporation. Vessels and contents were then autoclaved at 15 psi, 250° C. for 25 minutes. Evaporation in the autoclave and at subsequent stages of these experiments was negligible.

An inoculum of C. pyrenoidosa from agar slants was introduced into each vessel with a sterile loop. Culture vessels were then incubated at 23° C. for further algal growth.

Measurement of Radiophosphorus Accumulation by *Chlorella pyrenoidosa*

To measure accumulation of radiophosphorus by *C. pyrenoidosa* in modified Knop's solution, one microcurie of radiophosphorus as $Na_2H^{32}PO_4$ was introduced into culture vessels inoculated with *C. pyrenoidosa* in a negligible volume. This radioisotope preparation was supplied by Abbott Radiopharmaceuticals as a sterile carrier-free solution. Magnetic stirrers (Thermolyne Model S-7225) and sterile stirring bars provided mixing and aeration throughout the experiment. Temperature was held constant in a Sherer Model 4-4 environmental chamber at 19°C. and constant illumination was provided by a bank of seven 20 Watt KenRad fluorescent lamps.

Six minutes after radiophosphorus inoculation into these cultures a 0.1 ml. aliquot was removed from the contents of the culture vessel and was plated on Trypticase Soy Agar. These petri plates were incubated at 35°C. as a test for microbial contamination of algal cultures. Simultaneously the contents of each culture vessel were filtered through a 0.45 micron pore size membrane filter (Sartorius). The filters and algae were then rinsed with 100 ml. of modified Knop's solution. After this sterile rinse, the membrane filters and algae were dried for a minimum of 72 hours in a desiccation chamber containing Drierite. Filters and algae were then weighed on a Mettler Type H16 balance.

After weighing, the algae on the filter were resuspended in 30 ml. tap water by the use of a magnetic stirrer. Five 1.0 ml. samples of each resulting suspension were placed in individual

1.25 inch diameter aluminum planchettes (Sigma). These planchettes were then dried at 80°C. in a Labline oven for subsequent Geiger-Mueller beta assay. The membrane filters remaining after resuspension of algae were then re-dried and re-weighed. The weight of the membrane filter and algal cells prior to resuspension of the algae, minus the weight of the membrane filter after resuspension of the algae, was the dry weight of algae in the culture vessel.

Additional cultures were incubated for 0.5, 1.0, 3.0, 9.0, and 27.0 hours after radiophosphorus introduction. These cultures were subsequently treated in the same way as those incubated for 0.1 hour.

Measurement of Radiophosphorus Retention by *Chlorella pyrenoidosa*

To measure retention of radiophosphorus by *Chlorella pyrenoidosa*, algal cells suspended in modified Knop's medium were permitted to accumulate radiophosphorus as described above for 27.0 hours. The contents of each culture vessel were then filtered through a sterile 0.45 micron pore size membrane filter in an autoclaved Millipore filter apparatus and rinsed with 100 ml. sterile modified Knop's solution. The cells on each filter were then resuspended in 100 ml. modified Knop's solution in Bellco culture vessels. Each culture was incubated at 19°C. under constant illumination for 1.0 hours. Additional cultures were incubated for 3.0, 9.0, or 27.0 hours. The contents of each culture vessel were then filtered and prepared for counting as described previously for experiments measuring radiophosphorus accumulation.

Radiophosphorus Accumulation by Algae in Strophanthin - G Solutions

In order to determine the effect of Strophanthin - G (a specific inhibitor of cation-dependent adenosinetriphosphatases) on radiophosphorus accumulation by C. pyrenoidosa cells, the following experiment was performed.

A 0.2 ml. aliquot of a CO_2-absorbing KOH solution was placed on a filter paper wick in the center well of each of 20 Gilson respirometer reaction flasks. Each flask then received 1.8 mg. dry weight C. pyrenoidosa cells suspended in 4.0 ml. modified Knop's solution. These twenty flasks were then divided into five groups as follows: Group A received 5×10^{-5} M Strophanthin - G (Sigma) in a 1.0 ml. volume. Group B received 5×10^{-4} M Strophanthin - G in 1.0 ml., and Group C received 5×10^{-3} M. Strophanthin - G in 1.0 ml. Group D received 1.0 ml. modified Knop's solution, and Group E received 2.0 ml. modified Knop's solution. A - D then received .06 microcurie radiophosphorus in 1.0 ml. modified Knop's solution, bringing their volume to 6.0 ml. and their radiophosphorus concentration to ten microcuries per liter. All reaction vessels were equilibrated for 20 minutes before recording of respirometer readout values began. The temperature was held constant at $24^{\circ}C$. and all reaction flasks were rotated at 84 rpm. The above preparations and the experiment were carried out in the dark. Readout values were recorded for a total of 9.0 hours. These values were subsequently adjusted for temperature, barometric pressure, and vapor pressure to reflect microliters of oxygen consumed per milligram dry weight

of algae per hour.

At time intervals of 1.0, 3.0, 9.0, and 27.0 hours after radiophosphorus inoculation the contents of one reaction vessel in each group were filtered through a 0.45 micron pore size membrane filter and were rinsed with 100 ml. modified Knop's solution. These algae were then desiccated and placed on planchettes for beta assay as described previously for experiments measuring algal accumulation of radiophosphate.

Radiophosphorus Accumulation by Tubificid Worms

To determine the extent of radiophosphorus accumulation from bacteria and from algae by tubificid worms at two different temperatures, the following experiments were performed.

Approximately 5000 mature (2 - 3 cm.) Limnodrilus spp. obtained from Turtox were distributed about equally among 24 culture dishes (Carolina Biological Supply Company). Each culture dish contained 100 ml. of modified Knop's solution and 2.0 mg. Chloramphenicol (Sigma), a bacteriostatic antibiotic. These culture dishes were covered with sterile aluminum foil to reduce evaporation and microbial contamination. Twelve of these culture dishes were placed in a Sherer environmental chamber at 10°C. and twelve were placed in a Sherer environmental chamber at 20°C. Each of these groups of tubificid worms were then treated as follows. After five days of acclimation at those temperatures with daily changes of sterile Knop's solution and Chloramphenicol, the tubificids were placed in previously sterilized culture dishes. Chlorella pyrenoidosa

cells (4.6×10^7) in modified Knop's medium containing 2.0 mg. Chloramphenicol were placed in each of four of the culture dishes maintained at 10°C. and in each of four of the culture dishes maintained at 20°C. A second series of our culture dishes maintained at 10°C. and four culture dishes maintained at 20°C. received 5.0 ml. of an Escherichia coli (Strain B) culture. This culture had been inoculated in 2% glucose in modified Knop's solution and incubated at 37°C. for 36 hours. At the end of this incubation period the bacterial suspension contained approximately 10^7 viable cells per ml.

The liquid volume of all culture dishes was then brought to 100 ml. by the addition of sterile modified Knop's solution. Each culture dish then received 1.0 microcurie of radiophosphate in a negligible volume. The culture dishes were then stirred to assure mixing and were covered tightly with sterile aluminum foil to inhibit evaporation and contamination. Constant illumination was provided by a bank of fluorescent lamps as described for experiments measuring accumulation of radiophosphorus by algae.

At present time intervals (24.0, 43.0, 67.0, and 91.0 hours) after radiophosphorus introduction the tubificid worms were removed from one culture dish of the four in each treatment described above. These worms were rinsed in a carrier phosphate solution and were blotted on paper towelling. At the same time, algae or bacteria adhering to the glass culture dishes were carefully removed with a spatula and added to the total plankton and detritus in the culture dishes. The suspended material in the culture dishes was then removed from solution by filtration with 0.35 micron pore size

membrane filters. Membrane filters and contents were then rinsed with 100 ml. of modified Knop's solution. The membrane filters and their contents were retained for later Geiger-Mueller beta assay. The blotted tubificid worms were weighed on a Mettler P120 balance and placed in aluminum planchettes. The planchette contents were digested with 1.0 ml. Hyamine Hydroxide at room temperature for 24 hours for subsequent Geiger-Mueller beta counting.

Origin of Cellulases in the Tubificid Gut

In order to determine if tubificids produce an endogenous cellulase of possible importance in the assimilation of algal cell wall material and cell contents the following experiment was performed. Tubificids collected 12/15/70 from Portage Creek, Michigan were maintained in creek sediment and water at room temperature for one day. At that time a sample of approximately 500 of these worms was removed and was placed in 100 ml. modified Knop's solution containing 2.0 mg. Chloramphenicol. These worms were refrigerated at $4^{o}C$. The Knop's solution and antibiotic were replaced every 24 hours for one week. At that time the worms were homogenized with sterile hand homogenizers and the homogenate, with 0.3 mg. Chloramphenicol/ml. added, was filtered through a 0.45 micron pore size sterile membrane filter. The following procedure was used as an assay for cellulolytic activity in worm homogenate. Eighteen Pyrex test tubes of 8.0 ml. capacity were divided into six triplicate groups and were then treated as follows: Group 1 contained 3.5 mg. pure cellulose powder (Nutritional Biochemical Corporation) buffered

with Tris buffer (Sigma) to a pH of 6.8. Group 2 contained 3.5 mg. cellulose powder buffered with Tris buffer to a pH of 8.5. (The use of a slightly acid pH and a mildly alkaline pH was thought to increase the likelihood of detecting cellulases with different pH optima). Group 3 contained 0.5 ml. filtered worm homogenate buffered at pH 6.8. Group 4 contained 0.5 ml. filtered worm homogenate buffered at pH 8.5. Group 5 contained 3.5 mg. cellulose powder and 0.5 ml. filtered homogenate buffered at pH 6.8. Group 6 contained 3.5 mg. cellulose powder and 0.5 ml. filtered homogenate buffered at pH 8.5. The volume of each of these test tubes was brought to 3.5 ml. with sterile distilled water. Sterile cotton capped each tube. The above protocol was designed to separate glucose appearance resulting from (1) the spontaneous hydrolysis of cellulose, (2) glycogenolysis, and (3) cellulolytic activity.

These test tubes were placed in an Eberbach shaker bath rotating at 92 rpm. The temperature was maintained at 50°C.. Incubation continued for 24 hours, after which 0.1 ml. of the contents of each tube was plated on Nutrient Agar (Difco) and incubated at 16°C. for two weeks to detect possible microbial contamination. For measurement of possible enzymatic activity, a 0.1 ml. aliquot was removed from each tube and from two 3.5 ml. standard solutions containing 0.1 ml. of a 100 mg% glucose solution. This aliquot was mixed in 1.9 ml. distilled water. 1.0 ml. barium hydroxide (Somogyi solution, Fisher Scientific Company) was added to each resulting 2.0 ml. preparation. The preparations then received 1.0 ml. of a 2% solution of $ZnSO_4.7H_2O$ (Fisher). The resulting precipitate was

removed by centrifugation at 6000 rpm for six minutes in a Sorvall GLC - 1 centrifuge. This deproteinizing step was used to stop any enzyme activity present prior to a Glucostat assay.

A 2.0 ml. aliquot of the supernatant was added to 2.0 ml. Glucostat reagent (Worthington Biochemical Corporation), buffered with M/15 buffer (Fisher) to pH 7.0. This mixture was incubated at room temperature for ten minutes, when four drops of 4 M HCL were added to prevent further production of oxidized chromogen. The unknowns and standards were then read on a Bausch and Lomb Spectronic 20 spectrophotometer at 400 millimicrons wavelength for optimum measurement of oxidized chromogen.

The following procedure was used to determine if a cellulolytic microflora could be isolated from the tubificid gut. Tubificid worms (Tubifex sp. and Limnodrilus sp.) were collected from Portage Creek, Michigan, 12/15/70. These worms were rinsed briefly in 95% ethanol to sterilize the exterior integument. Pools of ten worms each were then ground in a volume of 2.0 ml. sterile modified Knop's solution in a sterile hand homogenizer until no visible tissue remained. A 0.2 ml. aliquot of each of our homogenates was then plated on cellulose agar (see Appendix II for composition) in replicate Bray dishes and replicate petri plates. Pyrogallol in alkaline solution was used to absorb oxygen in the Bray dishes. Petri plates and Bray dishes were incubated at 16°C. and at 35°C. for 14 days. The presence of a translucent halo in the cellulose agar at the end of that time, accompanied by a concavity in the agar and abundant colony growth, was considered to be indicative of

production of cellulase. This procedure is similar to methods discussed in Siu (1951).

Radiation Counting Methods

All planchettes were counted with a Geiger - Mueller counting apparatus. The models used were a Picker Nuclear Model 610485 end-window Geiger tube and a Nuclear-Chicago Model NC 000104 end-window Geiger tube. Voltage was adjusted as pre-determined for each Geiger tube. A RIDL Model 49-25 scaler was used in all counting procedures.

Counts per minute were converted to disintegrations per minute by correcting for radioactive half-life, counting geometry, sample absorption, resolution time, background, and sample dilution. When measurements of weight were available results were expressed as disintegrations per minute per gram, or as disintegrations per minutes per milligram. All counts were of sufficient duration to produce repeatable accuracy within 5%.

Statistical Methods

The values obtained for radiophosphorus concentrations in C. pyrenoidosa in experiments designed to measure accumulation and retention were examined with an Analysis of Covariance (Snedecor and Cochran, 1969). This method permitted the identification of equilibrium levels of radiophosphorus concentrations in these algae.

The values for radiophosphorus concentrations in Chlorella pyrenoidosa incubated in solutions of Strophanthin - G were examined with a Model I Analysis of Variance (Sokal and Rohlf, 1969). This

test permitted an evaluation of the variance in cellular phosphorus-32 concentrations which was attributable to specific inhibition of cation-dependent adenosinetriphosphatases by Strophanthin - G.

The mean values obtained for radiophosphorus concentrations in tubificid worms and plankton in culture dishes were examined with a Model I 2x3x4 non-replicated factorial Analysis of Variance (Sokal and Rohlf, 1969). This test permitted an evaluation of the variance in radiophosphorus concentrations which was attributable to different temperature regimens and to the presence in the culture medium of different microbial organisms.

All regressions in these experiments were obtained by the method of least squares (Sokal and Rohlf, 1969).

RESULTS

Accumulation of Radiophosphorus by Chlorella pyrenoidosa

Mean values for the accumulation of radiophosphorus by Chlorella pyrenoidosa cells in carrier-free modified Knop's medium are presented in Table I. In all culture vessels, radiophosphorus was concentrated by these cells.

This data is examined with an Analysis of Covariance in Table 2. This test indicates that data obtained after 3.0 hours of incubation may be pooled and treated as a single sample. No net accumulation or loss of radiophosphorus was measured after 3.0 hours of incubation. Radiophosphorus concentrations measured after 3.0 hours, therefore, may be considered equilibrium values.

The following least squares regression was obtained for this pooled data.

$$Y = 6.27353 - .88625X$$

where:

Y = log dpm/mg. dry weight of algae

X = log mg. dry weight of algae

This relationship between algal biomass and radiophosphorus concentrations in C. pyrenoidosa is shown in Figure 1. The concentrations of radiophosphorus in these algae were affected by the biomass of algae in the culture vessels. Lower radiophosphorus concentrations were measured in cells as the number of cells in the culture media was increased. The significance of these results to possible

accumulation of radiophosphorus from C. pyrenoidosa by consumer organisms in a food chain will be discussed in a different section of this paper.

TABLE 1. Accumulation of Radiophosphorus by C. pyrenoidosa. Values given are mean dpm/mg. dry weight of algae obtained from five samples. S.E. is the standard error of the mean.

Hours after ^{32}P Introduction	Mg. algae/100 ml. Knop's Medium	DPM/Mg x 1000	S.E. x 1000
0.2	2.3	110	4.9
	2.1	180	3.7
	2.0	130	4.0
0.5	3.5	540	18.0
	1.8	720	28.0
	1.9	850	8.9
1.0	2.8	550	19.0
	2.4	810	33.0
	2.6	690	19.0
	5.4	380	8.0
3.0	3.6	600	8.0
	2.4	860	11.0
	3.4	650	4.9
9.0	3.2	580	4.9
	3.2	700	9.2
	1.4	1400	20.0
27.0	1.9	1100	58.0
	5.5	440	11.0
	8.1	290	6.8

TABLE 1

TABLE 2. Analysis of Covariance of Radiophosphorus Concentrations Measured in Chlorella pyrenoidosa after 3.0 Hours of Incubation in Modified Knop's Medium Containing 1.0 uC ^{32}P/100 ml. An asterisk (*) identifies those tests which gave a nonsignificant result at the 95% level of confidence.

Source	d.f.	SS	MS	F
Slopes	2	.000082	.000041	.024*
Residual error	3	.005123	.001708	
Elevations	1	.000121	.000121	.116*
Residual error	5	.005205	.001041	

TABLE 2

FIGURE 1. The Effect of Algal Cell Biomass on Radiophosphorus Concentrations in Chlorella pyrenoidosa in Accumulation Experiments. Each point represents the mean of five experimentally determined values for radiophosphorus concentrations. These concentrations were measured after three or more hours of incubation of Chlorella pyrenoidosa cells in modified Knop's medium containing 1.0 uC ^{32}P/100 ml. Regression equation:

$$Y = 6.2735 - .8863(X)$$

where

Y = Log dpm/mg. dry weight of algae

X = Log mg. dry weight of algae.

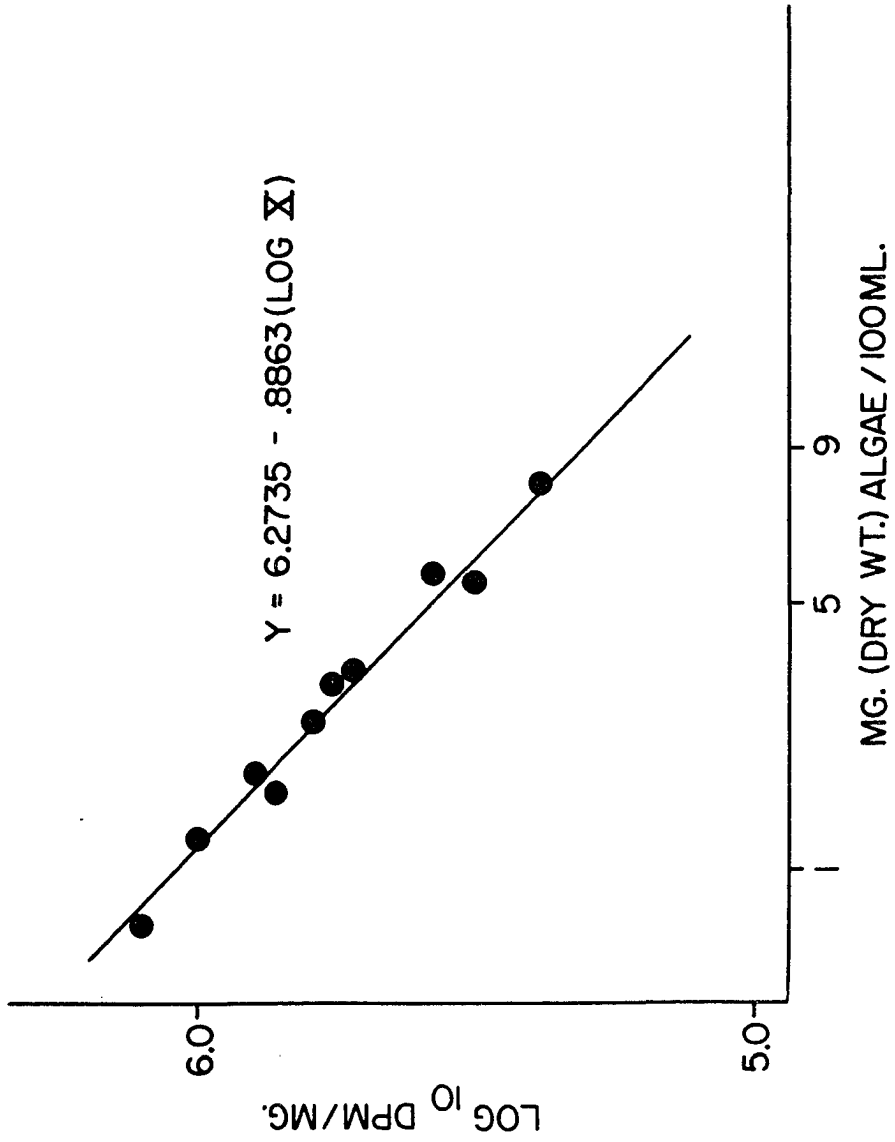

FIGURE 1

Retention of Radiophosphorous by Chlorella pyrenoidosa

Mean values for the retention of radiophosphorus by C. pyrenoidosa in carrier-free modified Knop's medium are presented in Table 3. In all culture vessels, a portion of accumulated radiophosphorus was retained by these algal cells.

This data was examined with an Analysis of Covariance in Table 4. This test indicates that data obtained after 1.0 hours of incubation may be pooled and treated as a single sample. No net accumulation or loss of radiophosphorus was measured after 1.0 hours of incubation of irradiated cells in a phosphorus-free culture medium. Radiophosphorus concentrations measured after 1.0 hours, therefore, may be considered equilibrium values.

The following least squares regression was obtained for this pooled data.

$$Y = 6.06282 - .94127X$$

where the values "Y" and "X" are defined as in the previous regression of radiophosphorus concentrations in algae during uptake experiments.

The relationship between algal biomass and radiophosphorus concentrations in C. pyrenoidosa is shown in Figure 2. The concentrations of radiophosphorus in these algae were affected by the biomass of algae in the culture vessels. Lower radiophosphorus concentrations were measured in cells as the number of cells in the culture media was increased. The significance of these results to possible accumulation of radiophosphorus from C. pyrenoidosa by consumer organisms in aquatic food chains will be examined later in this paper.

TABLE 3. Retention of Radiophosphorus by Chlorella pyrenoidosa.

Values given are mean dpm/mg. dry weight of algae x 1000.

S.E. is the standard error of the mean (N = 5).

Hours after ^{32}P Introduction	Mg. Algae/100 ml. Knop's Medium	DPM/Mg x 1000	S.E. x 1000
1.0	3.8	450	45.0
	1.4	790	20.0
	2.9	370	11.0
	0.9	1200	44.0
3.0	4.7	340	100.0
	2.1	670	75.0
	2.6	570	38.0
	2.0	570	12.0
9.0	3.4	510	21.0
	4.0	330	16.0
	2.5	340	4.3
	4.8	260	17.0
27.0	6.5	160	7.1
	2.6	380	8.3
	3.2	430	23.0
	7.1	140	2.6

TABLE 3

TABLE 4. Analysis of Covariance of Radiophosphorus Concentrations Measured in Chlorella pyrenoidosa after 1.0 Hours of Incubation in Phosphorus-free Modified Knop's Medium. An asterisk (*) identifies those tests which gave a nonsignificant result at the 95% level of confidence.

Source	d.f.	SS	MS	F
Slopes	3	.0158	.0053	.582*
Residual error	8	.0725	.0091	
Elevations	3	.0358	.0120	1.500*
Residual error	11	.0883	.0080	

FIGURE 2. The Effect of Algal Cell Biomass on Radiophosphorus Concentrations in Chlorella pyrenoidosa in Retention Experiments. Each point represents the mean of five experimentally determined values for radiophosphorus concentrations. These concentrations were measured after irradiated Chlorella pyrenoidosa cells were incubated in modified Knop's medium without a radiophosphorus addition. Regression equation:

$$Y = 6.063 - 0.9413(X)$$

where

Y = Log dpm/mg. dry weight of algae

X = Log mg. dry weight of algae

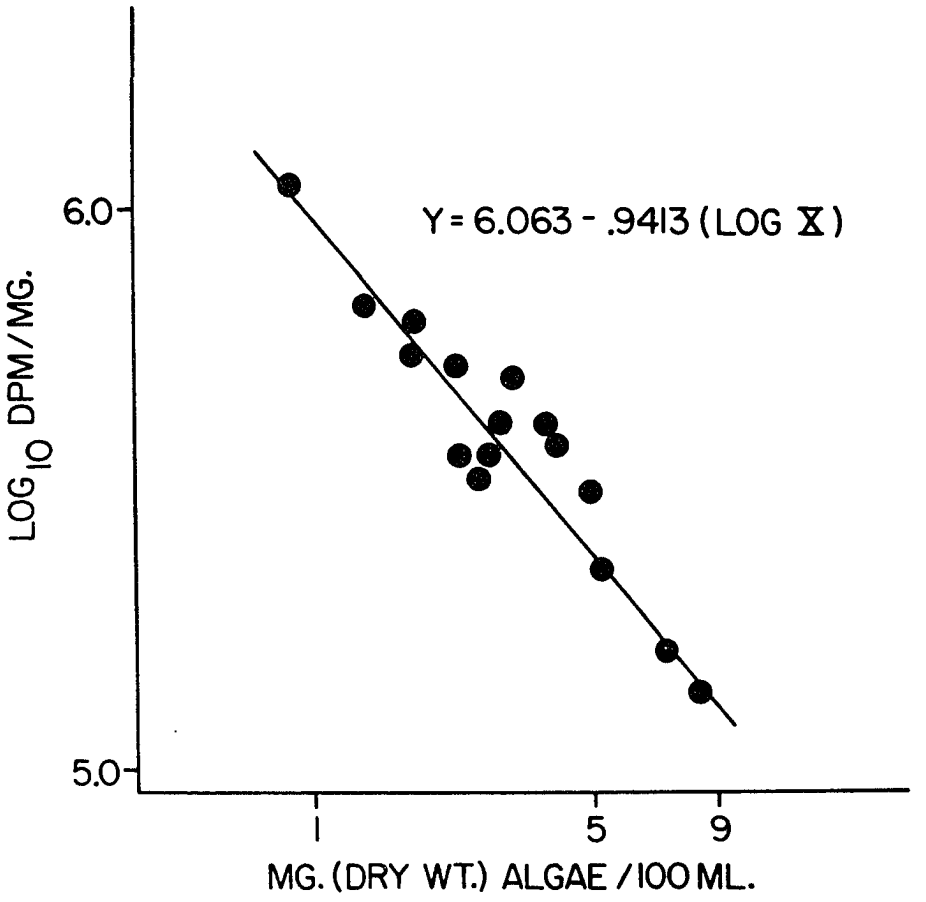

FIGURE 2

Accumulation of Radiophosphorus by Chlorella pyrenoidosa in Solutions of Strophanthin - G

Mean values for the accumulation of radiophosphorus by C. pyrenoidosa in different concentrations of Strophanthin - G are presented in Table 5. An Analysis of Variance of these data is presented in Table 6. This test indicates that concentrations of Strophanthin - G as high as 5.0 mM did not affect measured concentrations of radiophosphorus in these algal cells.

Oxygen consumption by Chlorella pyrenoidosa cells, however, was inhibited by 0.5 mM to 5.0 mM Strophanthin - G in this experiment (Figure 3). Oxygen consumption by algal cells in reaction vessels containing radiophosphorus without Strophanthin - G was identical to oxygen consumption by cells in reaction flasks without radiophosphorus or Strophanthin - G in the extracellular medium. The lower oxygen consumption observed in reaction vessels containing Strophanthin - G, therefore, may be attributed to inhibition of cation-activated ATPases rather than to beta irradiation of algal cells.

These results indicate that, although cation-activated ATPases were present in these algal cells, inhibition of these enzymes did not affect radiophosphorus accumulation by these cells. Furthermore, radiophosphorus accumulation in these cells was not affected by depression of ATP synthesis. These results do not suggest an active transport mechanism for phosphorus-32 accumulation by Chlorella pyrenoidosa as measured in these experiments.

TABLE 5. Concentrations of Radiophosphorus in Chlorella pyrenoidosa Incubated in Strophanthin - G Solutions. Values given are mean dpm/mg. dry weight of algae x 1000. S.E. is the standard error of the mean (N = 5).

Hours after ^{32}P Introduction	1.6	3.0	9.0	27.0
Concentration of Strophanthin - G				
0.0 mM	430 S.E. 0.8	430 S.E. 2.1	430 S.E. 0.85	420 S.E. 1.5
5.0 mM	390 S.E. 2.4	440 S.E. 2.4	430 S.E. 1.4	340 S.E. 0.5
0.5 mM	470 S.E. 2.2	420 S.E. 1.8	420 S.E. 0.9	400 S.E. 1.5
0.05 mM	480 S.E. 0.7	380 S.E. 1.2	340 S.E. 1.0	360 S.E. 1.2

TABLE 5

TABLE 6. Analysis of Variance of Radiophosphorus Concentrations in Chlorella pyrenoidosa in Strophanthin - G Solutions. An asterisk (*) identifies those tests which gave a nonsignificant result at the 95% level of confidence.

Source of Variation	d.f.	SS	MS	F
Time of Sampling	3	83.75	27.92	2.4*
Concentration of Strophanthin - G	3	44.25	14.75	1.3

TABLE 6

FIGURE 3. Oxygen Consumption by Chlorella pyrenoidosa Cells in Solutions of Strophanthin - G. Each horizontal line represents the mean of experimentally determined values for Oxygen consumption by Chlorella pyrenoidosa cells in stipulated concentrations of Strophanthin - G. Vertical lines represent $\pm$ 1.0 standard error of the mean. Each reaction flask, with the exception of the control, contained 1.0 uC ^{32}P/100 ml. modified Knop's solution.

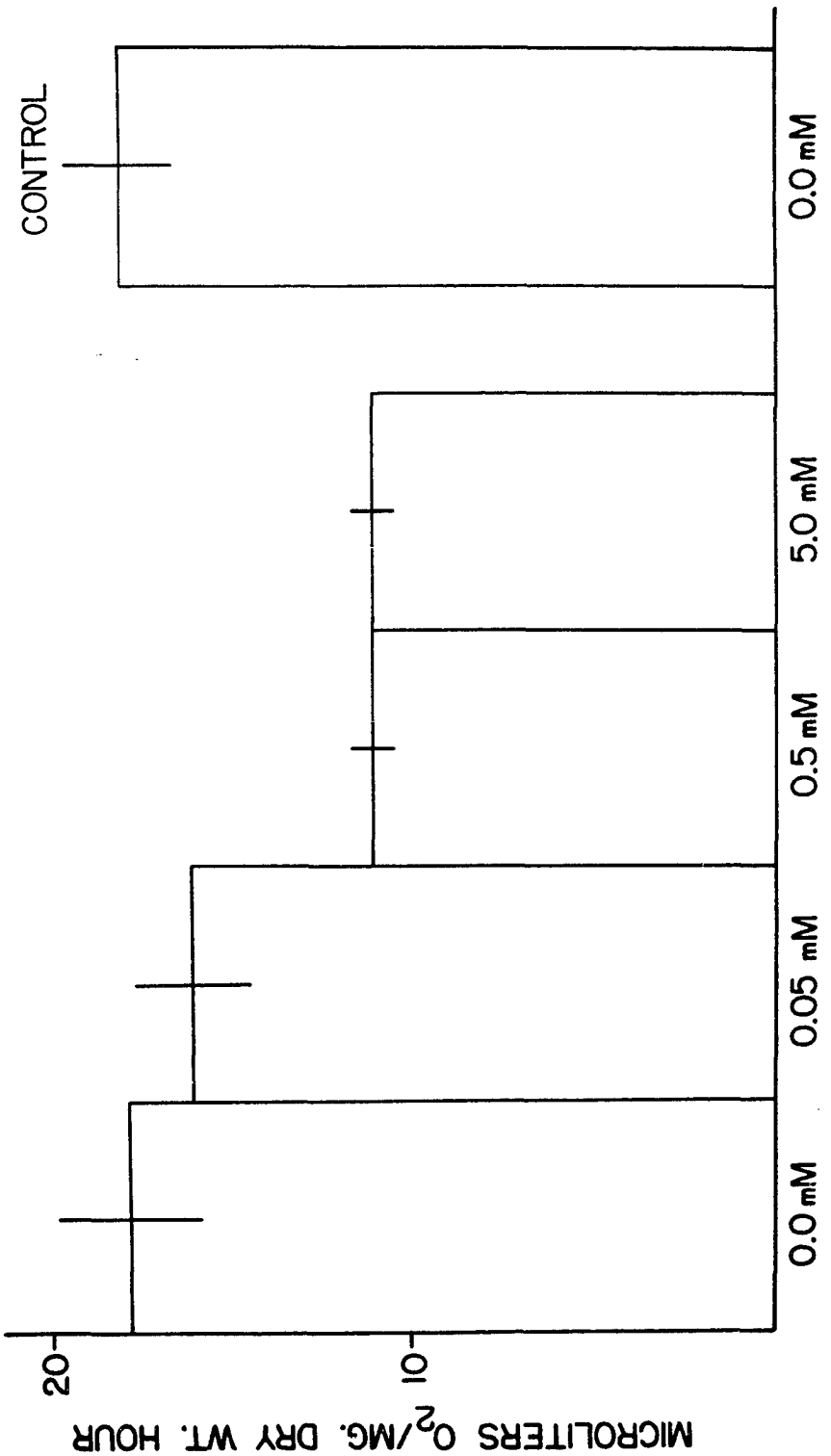

FIGURE 3

Radiophosphorus Accumulation by Algae, Bacteria, and Tubificid Worms in Laboratory Food Chains

The mean values for radiophosphorus concentrations in tubificid worms are presented in Table 7. These values are presented graphically in Figure 4 and in Figure 5. The mean values for radiophosphorus concentrations in the food sources (Chlorella pyrenoidosa, Escherichia coli, and detritus) of these worms are presented in Table 8. These data are shown graphically in Figure 6 and in Figure 7. Standard errors of the values for radiophosphorus concentrations in tubificid worms and in the food sources have not been provided, since each value is the concentration of radiophosphorus in organisms from only one culture dish.

An Analysis of Variance (Table 9) indicates that radiophosphorus concentrations in C. pyrenoidosa, E. coli, and in detritus were unequal. The lowest concentrations of radiophosphorus, as shown in Figure 6 and in Figure 7, were those measured in detritus (tubificid secretions and excretions) in culture dishes containing Chloramphenicol without a plankton addition.

Table 10 is an Analysis of Variance of the mean concentrations of radiophosphorus in the tubificid worms in these culture dishes. The significant treatment main effect indicates that these worms did not concentrate radiophosphorus at the same rate in the different food chains. Concentrations of radiophosphorus in tubificid worms were highest in culture dishes containing bacteria (Figure 4, Figure 5). Radiophosphorus concentrations in these worms were lowest in culture dishes containing C. pyrenoidosa and in culture dishes

containing Chloramphenicol without a microbial inoculum. Microscopic observations of tubificid worms and of their fecal material, however, confirmed that these worms did ingest C. pyrenoidosa cells.

TABLE 7. Accumulation of Radiophosphorus by Tubificids in Laboratory Food Chains. Values given are dpm/gram x 1000.

Hours after ^{32}P Introduction		24	43	67	91
Temperature	Plankton Addition				
10°C.	none	7.2	34.0	54.0	100.0
	C. pyrenoidosa	35.0	38.0	67.0	93.0
	E. coli	26.0	100.0	120.0	190.0
20°C.	none	44.0	87.0	84.0	86.0
	C. pyrenoidosa	140.0	61.0	100.0	110.0
	E. coli	150.0	140.0	120.0	190.0

TABLE 7

FIGURE 4. Accumulation of Radiophosphorus by Tubificid Worms in Laboratory Food Chains at 10°C. Each point represents the radiophosphorus concentrations measured in tubificid worms feeding upon Escherichia coli, Chlorella pyrenoidosa, or detritus (antibiotic added to inhibit microbial growth) in culture dishes. Curve 1: Limnodrilus sp. feeding on Chlorella pyrenoidosa. Curve 2: Limnodrilus sp. feeding on detritus. Curve 3: Limnodrilus sp. feeding on Escherichia coli. Regression equations for these graphs are:

Curve 1 $Y = 913.6(t) + 6858.0$

Curve 2 $Y = 1328(t) - 25922$

Curve 3 $Y = 2256(t) + 17923$

where Y = dpm/gram and t = time of incubation in hours.

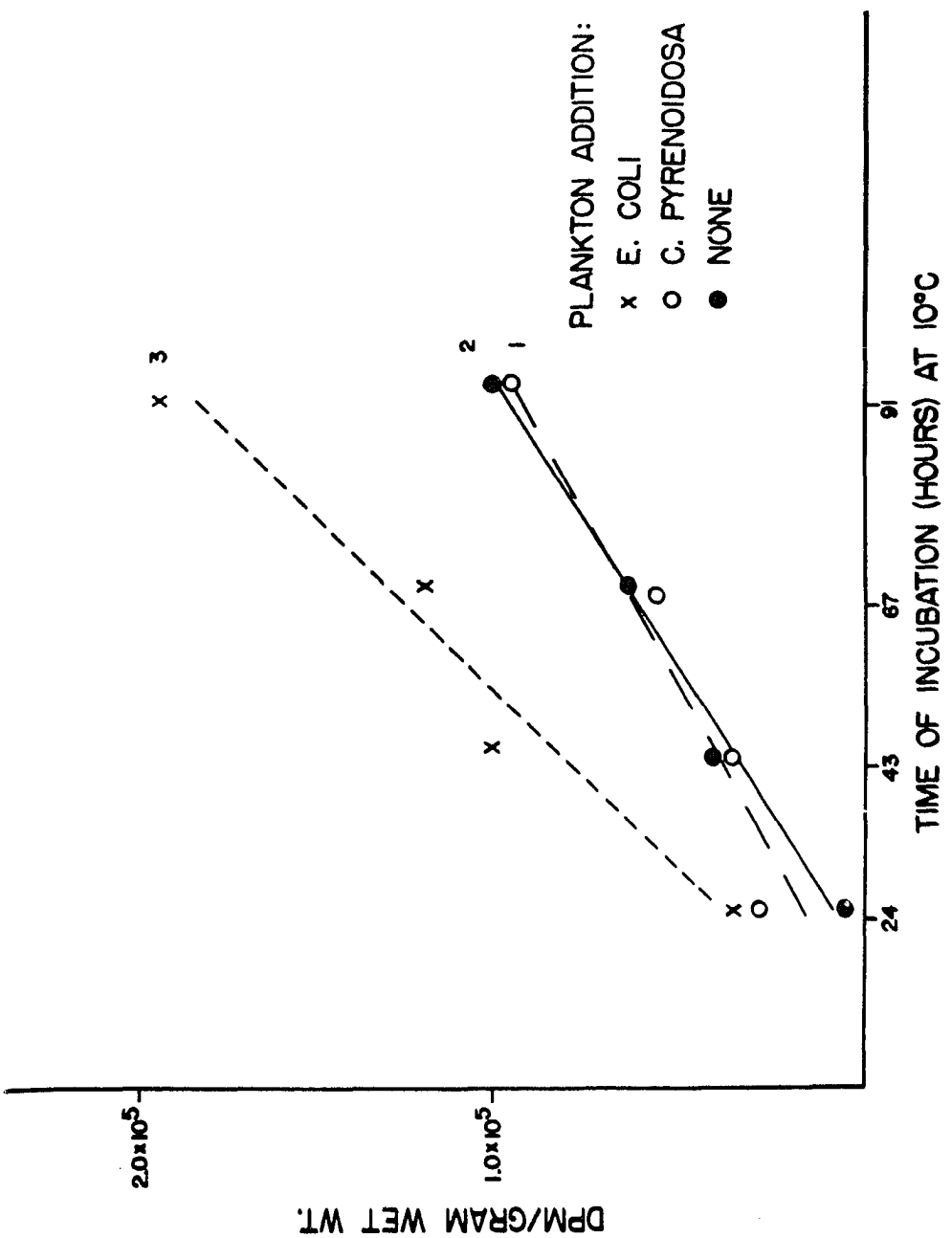

FIGURE 4

FIGURE 5. Accumulation of Radiophosphorus by Tubificid Worms in Laboratory Food Chains at 20°C. Each point represents the radiophosphorus concentrations measured in tubificid worms feeding upon Escherichia coli, Chlorella pyrenoidosa, or detritus (antibiotic added to inhibit microbial growth) in culture dishes. Curve 1: Limnodrilus sp. feeding on Chlorella pyrenoidosa. Curve 2: Limnodrilus sp. feeding on detritus. Curve 3: Limnodrilus sp. feeding on Escherichia coli. Regression equations are not available for these curves.

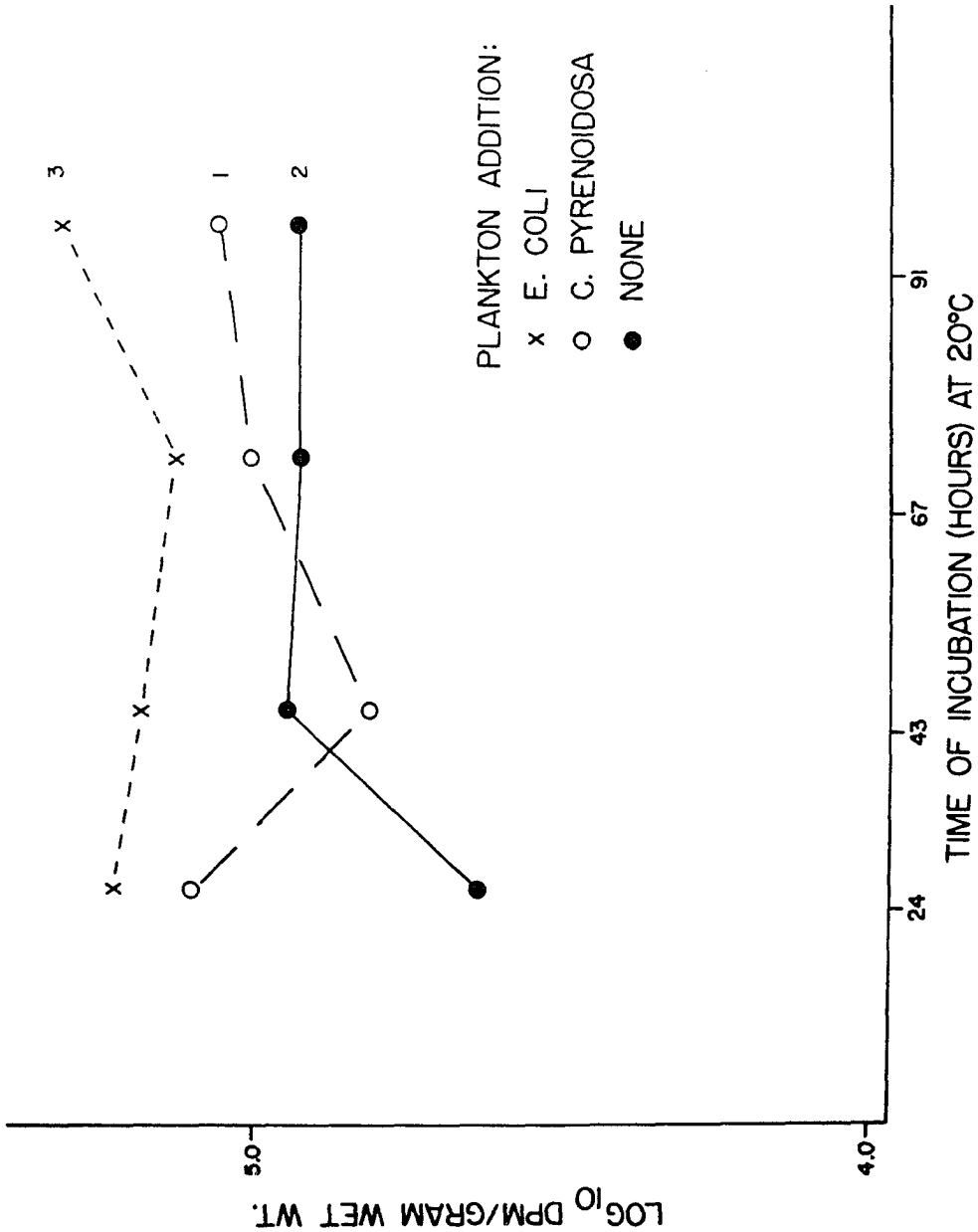

FIGURE 5

TABLE 8. Accumulation of Radiophosphorus by Plankton in Three Food Chains. Values given are dpm x 1000.

Hours after ^{32}P Introduction		24	43	67	91
Temperature	Plankton Addition				
10°C.	none	41.0	130.0	120.0	240.0
	C. pyrenoidosa	450.0	680.0	540.0	510.0
	E. coli	15.0	530.0	560.0	370.0
20°C.	none	45.0	140.0	340.0	160.0
	C. pyrenoidosa	510.0	460.0	580.0	580.0
	E. coli	360.0	390.0	470.0	330.0

TABLE 8

FIGURE 6. Radiophosphorus Accumulation by Algae, Bacteria, and Detritus in Laboratory Food Chains at 10°C. Each point represents the radiophosphorus level measured in Escherichia coli, Chlorella pyrenoidosa, or in detritus (antibiotic added to inhibit microbial growth) in culture dishes containing tubificid worms.

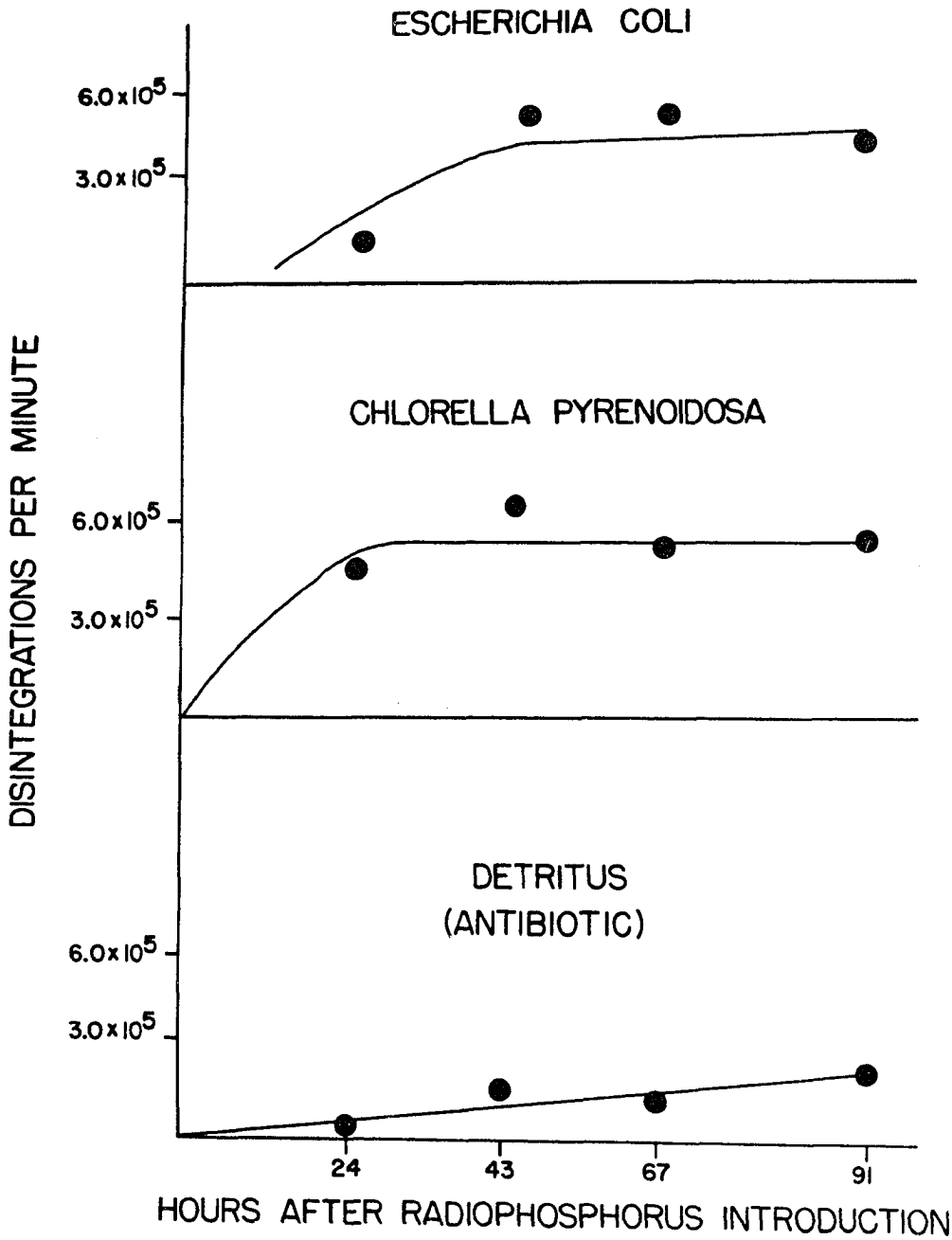

FIGURE 6

FIGURE 7. Radiophosphorus Accumulation by Algae, Bacteria, and Detritus in Laboratory Food Chains at 20°C. Each point represents the radiophosphorus level measured in Escherichia coli, Chlorella pyrenoidosa, or in detritus (antibiotic added to inhibit microbial growth) in culture dishes containing tubificid worms.

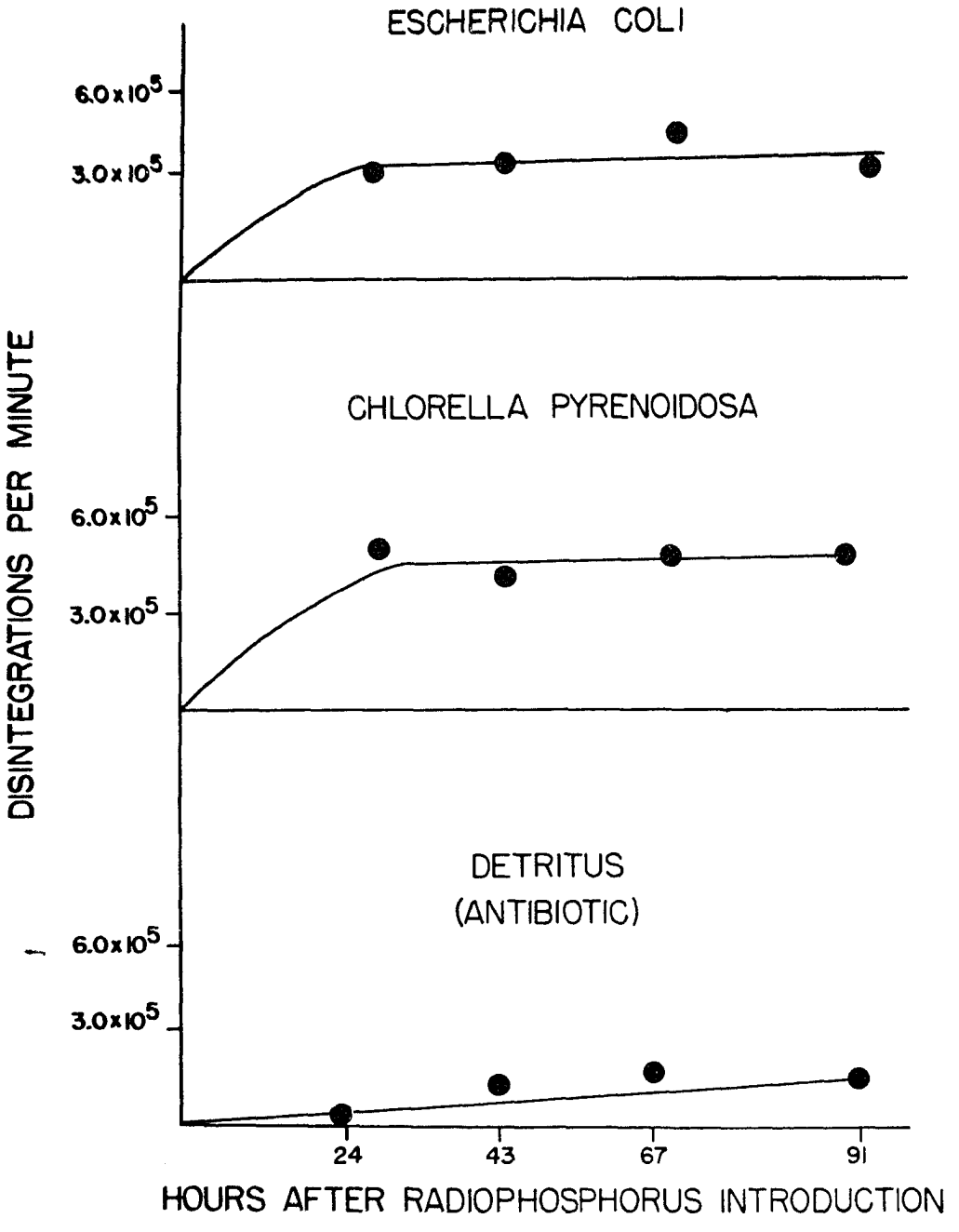

FIGURE 7

TABLE 9. Analysis of Variance of Radiophosphorus Concentrations in Plankton. An asterisk (*) identifies those tests which gave nonsignificant results at the 95% confidence level.

Source of Variation	d.f.	M.S.	F	Significance level
Main effects				
Temperatures	1	.0714	0.761	*
Food Chains	2	.8735	9.311	.025
Time of Sampling	3	.3394	3.617	*
Interactions				
Temperatures x Times	3	.1059	1.128	*
Temperatures x Food Chains	2	.0453	0.483	*
Food Chains x Times	6	.0747	0.796	*
Times x Food Chains x Temperatures	6	.0938		

TABLE 9

TABLE 10. Analysis of Variance of Radiophosphorus Concentrations in Tubificid Worms. An asterisk (*) identifies those tests which gave nonsignificant results at the 95% confidence level.

Source of Variation	d.f.	M.S.	F	Significance level
Main effects				
Temperatures	1	.4484	79.65	.001
Food Chains	2	.2616	46.47	.001
Time of Sampling	3	.2192	38.93	.001
Interactions				
Temperatures x Times	3	.1465	26.02	.001
Temperatures x Food Chains	2	.0055	0.97	*
Food Chains x Times	6	.0454	8.07	.025
Times x Food Chains x Temperatures	6	.0056		

TABLE 10

As shown in Table 10, a ten degree Centigrade difference in temperatures resulted in significantly different concentrations of radiophosphorus in tubificid worms. Radiophosphorus concentrations in tubificid worms in all food chains were highest at 20°C.

Cellulases in Tubificid Worms

Results of the Glucostat assay for cellulolytic activity in the filtered homogenate of tubificid worms were negative. This assay provided no evidence for the presence of cellulolytic enzymes in tubificid worm homogenates.

Cellulolytic bacteria, however, were isolated from tubificid worm homogenates. These bacteria were Gram negative rods. On cellulose agar these bacteria produced yellow pigmented colonies in the center of a translucent zone of one centimeter diameter when incubated at 16°C. for two weeks. No bacterial growth was observed on cellulose agar incubated at 35°C., and no growth was observed in anaerobic Bray dishes.

DISCUSSION

Davis and Foster (1958) noted that radioisotopes may accumulate in aquatic organisms as a result of (1) adsorption to exposed surface areas, (2) absorption into cells or tissues, or (3) assimilation of ingested compounds. Rice (1963) has further suggested that concentrations of a radioisotope in a consumer organism may reflect the ability of the consumer organism to digest phytoplankton food sources of the radioisotope, as well as the number of phytoplankton cells available to the consumer and the cellular concentrations of the radioisotope in phytoplankton. Rice found that radiophosphorus in a green alga may not be available to some invertebrate consumer organisms. Accordingly, it was of interest to determine if a green alga might serve as a food source of radiophosphorus to tubificid worms in laboratory food chains. Previous investigations indicated that these worms may concentrate this radioisotope from bacteria in bottom sediments and serve as a food source of radiophosphorus for consumer organisms at higher trophic levels (see Literature Review).

The regressions of radiophosphorus concentrations in Chlorella pyrenoidosa on algal biomass which were obtained in this investigation indicate that radiophosphorus concentrations in these cells were influenced by the concentration of cells in the culture medium. Cells grown in dense algal cultures were found to contain fewer concentrations of radiophosphorus than cells grown in cultures containing fewer algae. These differences in radiophosphorus

concentrations may result from competition. This explanation is supported by the observation that each culture contained only 1.1×10^{-10} millimoles of radiophosphorus, and more than 85% of this amount was taken up during the first three hours of incubation. Competition, therefore, may act to depress radiophosphorus concentrations in these algae, decreasing their value to consumer organisms as a potential food source of this radioisotope.

The values for retention of radiophosphorus by Chlorella pyrenoidosa also indicate an inverse relationship between the algal biomass of the cultures and the concentrations of radiophosphorus in these algae. Equilibrium values of radiophosphorus retention in this experiment were highest in those algae grown in cultures containing relatively few algal cells. These equilibrium values of radiophosphorus concentrations were observed within one hour after introduction of the cells into a phosphorus-free culture medium.

The rapid loss of radiophosphorus from these cells suggests that a portion of the total radiophosphorus pool in these algae was bound weakly to cellular constituents, or was associated with the cell as a pool of soluble ions. This result is similar to results obtained by Gest and Kamen (1948; see Literature Review).

The site of such adsorption, presumably, would be the algal cell membrane or the cell wall. Cation-dependent adenosinetriphosphatases, localized on the cell membrane of many eukaryotic cells, are known to play a role in the transport of some elements and compounds across cell membranes (Glynn, 1964). It was of interest, therefore, to investigate the possible importance of these enzymes in the

accumulation of radiophosphate by Chlorella pyrenoidosa.

The depression of oxygen consumption by Strophanthin - G observed in these experiments indicates that cation-dependent ATPases were inhibited by this cardiac glycoside. Strophanthin - G is a specific inhibitor of cation-dependent ATPases which is known to depress oxygen consumption by producing elevated ATP/ADP ratios in the cell.

Concentrations of radiophosphorus measured in algal cells in this study were not related to cation-dependent ATPase inhibition as measured by depressed oxygen consumption. These results do not suggest a role for cation-dependent ATPases in radiophosphorus accumulation by Chlorella pyrenoidosa cells as measured in these experiments. Gest and Kamen (1948) found that radiophosphorus concentrations measured in the trichloroacetic acid-insoluble phosphate pool of these algal cells were not directly dependent on cellular oxygen consumption. The results of Gest and Kamen and the results obtained in the present investigation suggest that cellular radiophosphorus concentrations as measured in this study result from adsorption rather than from active transport processes at the cell membrane. The high surface-to-volume ratio of Chlorella pyrenoidosa cells, which have a cell diameter of approximately four microns, provides a large surface area for effective adsorption to occur.

The high concentrations of radiophosphorus measured in Chlorella pyrenoidosa in these experiments suggested that this alga may be a potential food source of radiophosphorus for tubificid worms in aquatic food chains. This possibility was examined with a factorial

design in which tubificid accumulation of radiophosphorus from Escherichia coli and ^{32}P accumulation by these worms in a control without a plankton inoculum was compared with radiophosphorus accumulation from Chlorella pyrenoidosa.

If bacteria and other suspended particulate material could be eliminated completely from the culture media, then the concentrations of radiophosphorus measured in tubificid worms in culture dishes without a plankton inoculum would reflect direct absorption or adsorption of this radioisotope from the culture medium. Chloramphenicol is known to be bacteriostatic in the concentrations used in experiments measuring the accumulation of radiophosphorus by tubificid worms. The tubificid worms, however, added suspended particulate material to the culture media in the form of waste products and secretions. These particles provided a surface area for the adsorption of radiophosphorus. Radiophosphorus concentrations in tubificid worms in culture dishes which did not receive a plankton inoculum, therefore, may represent direct uptake of this isotope from the water and also uptake by assimilation of these suspended detritus particles.

The concentrations of radiophosphorus measured in filtered plankton and in organic detritus in these laboratory food chains were significantly different. Concentrations of particulate radiophosphorus in culture dishes which did not receive a microbial inoculum were lower than concentrations of particulate radiophosphorus measured in culture dishes which received an inoculum of algal or bacterial cells, as shown in Figure 6 and in Figure 7. This result indicates

that concentrations of radiophosphorus in tubificid worms ingesting Chlorella pyrenoidosa or Escherichia coli cells may be compared with radiophosphorus concentrations in tubificids grown in culture dishes in which microbial growth was inhibited by Chloramphenicol.

The concentrations of radiophosphorus in tubificid worms which had fed upon E. coli for 91.0 hours (the duration of the experiment) in culture media at 10°C. were found to be 90% higher than the concentrations found in tubificids grown in culture media without a microbial inoculum for the same period of time. The concentrations of radiophosphorus in worms which had fed upton this bacteria for 91.0 hours at 20°C. were found to be 61% higher than radiophosphorus concentrations measured in tubificids grown in culture media without a microbial inoculum for the same period of time. Tubificid worms are known to ingest E. coli and to assimilate compounds concentrated in these bacterial cells (Coler and Gunner, 1967). Assimilation of radiophosphorus from ingested bacteria by these worms has been demonstrated by autoradiographic methods (Whitten, 1966). The higher concentrations of radiophosphorus measured in tubif icid worms grown in culture media containing E. coli, therefore, may be attributed to the ingestion and assimilation of these bacterial cells.

Radiophosphorus concentrations in tubificid worms grown in culture dishes containing Chlorella cells at 10°C. or at 20°C. for 91.0 hours (the duration of the experiment) were found to be within 13% of the concentrations of this radioisotope in tubificid worms grown in culture media without a microbial inoculum for the same period of time. However, tubificid worms in these experiments

were found to ingest algal cells. These cells were identified intact in the foregut, midgut, and hindgut, and in the fecal material of tubificid worms. Algal cells were identified by the presence of a cell wall, a cell diameter of approximately four microns, and green pigmentation.

The low levels of radiophosphorus accumulation from Chlorella cells by tubificid worms which was observed in these experiments may result from the resistance of this alga to digestion. Northcote, et al. (1958) have shown that the cell wall of this alga is approximately 15% cellulose and 31% hemicellulose by weight. Cellulose was found to occur in the multilayered cell wall of Chlorella pyrenoidosa as fibrous strands contained in a hemicellulose and protein matrix. The negative results of the Glucostat assay for cellulolytic activity in tubificid worm homogenates suggests that the full complement of digestive enzymes required for degradation of this cell wall is not present in the tubificid intestinal tract. It should be noted that the enzyme cellulase is comprised of three distinct fractions: (1) C', an affinity protein; (2) endo-splitting and exo-splitting enzymes; and (3) cellobiase. The Glucostat assay for cellulolytic activity requires that all three of these fractions be present, and hence may be a conservative assay for cellulolytic activity.

The isolation of cellulolytic bacteria from tubificid worm homogenates suggests that further research is required before the importance of algal cell wall celluloses and hemicelluloses as a barrier to assimilation of algae by tubificid worms in field

conditions may be evaluated.

The above experimental studies are of use in identifying some of the factors involved in the accumulation of radiophosphorus by tubificid worms in laboratory food chains. However, because of the complex biological and chemical constituents of natural bodies of water, these results may not reflect patterns of radiophosphorus movement in food chains under field conditions.

CONCLUSIONS

1. Chlorella pyrenoidosa cells were found to concentrate and retain radiophosphorus in a carrier-free modified Knop's medium.
2. Concentrations of radiophosphorus in Chlorella pyrenoidosa cells during accumulation and retention experiments were found to be inversely related to the algal cell biomass in the culture medium. This relationship is attributed to competition by algal cells for this radioisotope.
3. Inhibition of oxygen consumption in Chlorella pyrenoidosa cells by Strophanthin - G is attributed to the specific action of this compound on cation-dependent adenosinetriphosphatases.
4. Radiophosphorus concentrations in Chlorella pyrenoidosa cells were found to be unrelated to specific inhibition of cation-dependent ATPases by 0.5 to 5.0 mM Strophanthin - G.
5. Tubificid worms in the genus Limnodrilus were found to accumulate radiophosphate from Escherichia coli by ingestion and assimilation of these bacterial cells. Lower radiophosphorus concentrations were measured in these worms when Chlorella pyrenoidosa cells were the only microbial source of radiophosphorus available to these worms, and when chloramphenicol was added to the culture medium to inhibit prokaryotic cell growth.
6. Chlorella pyrenoidosa cells ingested by tubificid worms were identified intact in the gut and in the feces of these worms. Glucostat assays for cellulolytic activity in tubificid worm

homogenates indicated that these worms may not possess the digestive enzymes required to degrade the cell wall of Chlorella pyrenoidosa.

7. Cellulolytic bacteria were isolated from the homogenate of tubificid worms collected in the effluent from a paper mill. These bacteria were Gram negative rods, one micron in length, which produced yellow pigmented colonies and a translucent halo on cellulose agar.
8. Radiophosphorus concentrations in tubificid worms in laboratory food chains were found to be significantly higher at 20°C. than was the case of 10°C.

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Appendix I

Composition of Modified Knop's Solution

KNO_3	300 mg./liter HOH
$MgSO_4$	300 mg./liter HOH
$Ca(NO_3)_2$	900 mg./liter HOH

pH: 6.1

Appendix II

Composition of Cellulose Agar

NaCl	5.0 g./liter HOH
$MgSO_4$	0.2 g./liter HOH
$(NH_4)_2SO_4$	1.0 g./liter HOH
K_2HPO_4	1.0 g./liter HOH
Cellulose	20.0 g./liter HOH