MICHIGAN DEPARTMENT OF CONSERVATION
Research and Development Report No. 50*

January 28, 1966

BACTERIAL TRANSPORT OF PHOSPHORUS IN A STREAM ECOSYSTEM[1]

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*Institute for Fisheries Research Report No. 1710.

[1]Paper presented at the Symposium on the Disposal of Radioactive Wastes into Seas, Oceans and Surface Waters, Vienna, Austria, May 16-20, 1966.

Bacterial Transport of Phosphorus in a Stream Ecosystem*

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Tracer quantities of radioactive phosphorus (^{32}P) added to a trout stream equilibrate rapidly with water-borne solids and are assimilated into the food chain. Previous studies [1] have suggested that the rate of entry into the food chain and the pathways taken by phosphorus may be strongly influenced by the amount and kind of suspended solids (e. g. bacteria and algae) present in the water at the time phosphorus is added to the stream. The present experiment, in which bacteria labeled with ^{32}P were added to a stream, was designed to test the efficacy of bacteria as agents of phosphorus transport and to better define their position in the phosphorus cycle of freshwater streams.

Much of the information on the role of bacteria in the cycling of phosphorus comes from laboratory and quasi field experiments. Hayes and Phillips [2] followed the exchange of ^{32}P through 100 or more artificial systems consisting of lake water, mud and in some instances aquatic macrophytes and phytoplankton. The action of bacteria was

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*These studies were supported by the U. S. Atomic Energy Commission AT(11-1)-655 and by Federal Aid to Fisheries Restoration funds (F-27-R-3).

demonstrated by comparing the flow of ^{32}P in systems with and without antibiotics. These experiments demonstrated that bacteria (1) equilibrate rapidly with the added phosphorus, (2) maintain phosphorus in the water phase and delay its incorporation into mud and rooted plants, and (3) convert inorganic phosphorus into soluble organic phosphorus compounds at a relatively rapid rate. Hayes [3] noted a much greater uptake of ^{32}P by Gammarus in normal than in sterilized sea water, suggesting that bacteria may greatly enhance flow of ^{32}P to higher levels of the food chain. Working with sea water systems Pomeroy [4] was able to verify the inhibition of phosphorus release from solids by antibiotics and found that methylene blue and cyanide blocked phosphorus uptake. These findings indicated that metabolic processes were involved. Rigler [5] noted the rapid turnover of phosphorus in the surface waters of lakes treated with ^{32}P. From laboratory experiments and from experiments with polyethylene bags suspended in the lake, he concluded that bacteria were responsible for the rapid turnover of phosphorus and suggested that they might compete with algae for inorganic phosphorus.

The above studies have shown a close association between phosphorus concentration and bacterial activity but they have not identified the chemical state of the phosphorus and its location in or on the bacterial cell. It is clear that phosphorus is adsorbed on cell surfaces and this source would be far more labile than phosphorus within the cell with regard to its use in natural systems. Phosphorus within the bacterial cell exists chiefly as nucleotides. For cultured Escherichia coli Taylor [6] found 2.72% of the dry weight of E. coli cultured in broth was phosphorus of which 85% was in the form of nucleic acid and 12% phospholipids. Phosphorus incorporated into E. coli by culturing this species in nutrient broth has been shown to be fixed within the cell and is not subject to exchange with the medium [7]. Phosphorus in this form may remain relatively unavailable to photosynthetic organisms until the cell dies and decays. Consumers, e.g. filter feeders, could, however, incorporate this source of phosphorus into the food chain. Specifically in this experiment we planned to investigate the translocation of phosphorus within the food chain of a stream when (1) phosphorus was bound intracellularly in a bacteria cell (E. coli) and thus (2) was available to producers only after mineralization, and (3) when the largest input of phosphorus to the higher levels of the food chain would be to filter feeders and to detritus feeders capable of utilizing E. coli after the cells are deposited on the stream bottom.

The authors wish to acknowledge contributions made to this study by Michael Bender, James Bacon, Tom Wojtalik and Naylord Urshel. Dr. Carl Latta and the staff of the Pigeon River Trout Research Station assisted in many ways.

Study area

The study area consisted of a 2.5-mile section of the West Branch of the Sturgeon River, Cheboygan County, Michigan. Descriptions of the area and locations of collecting stations are given elsewhere [1] [8]. It is sufficient to say here that the West Branch is a clear, cool, unpolluted trout stream. Stream flow through the study area averages about 43 cu ft per sec. The flow is very stable and the summer temperature fluctuation is small (52°-58° F). In the experiment discussed in this paper (1961) and in experiments in other years (1958, 1959, 1960, and 1962) a spike of radioactive phosphorus has been added at one location in the stream and activity in the water and in the biota has been studied at a series of stations below the site of release. Data discussed in this report come chiefly from stations 3, 8, 12, and 14 which were located 300, 1,030, 2,580 and 3,280 yd below the release point. At two additional stations (station 5, 550 yd, and station 16, 5,280 yd) we monitored only water activity.

Methods

Preparation of spike of labeled bacteria

Escherichia coli was selected as an experimental organism because (1) its growth requirements are well known, (2) techniques for culturing and enumeration are well established, (3) the phosphorus metabolism of this species has been studied [7], and (4) because cells of this species could be recovered and identified by relatively simple culture techniques.

An initial attempt at preparing a spike of labeled E. coli was made with strain B. However, when cells of this strain lost their activity at a critical stage in the culturing procedure (presumably because of a phage), we substituted strain 0111. To insure maximum uptake of ^{32}P and to minimize the amount of stable phosphorus in the culture, cells were grown on a medium low in phosphorus. This medium consisted of 2.0 g of L-glutamic acid, 7.5 g glycogen, 2.0 g of glucose, 1.0 g glycerol, inorganic salts and 1,000 ml of distilled water. In a preliminary test it was found that there was a maximum uptake of phosphorus when it was used at a concentration of about 0.3 mg per 100 ml of media. A trial, in which 0.3 mg phosphorus (as Na_2HPO_4) and 0.1 mc of ^{32}P were used per 100 ml of media, gave 99% uptake. This ratio of stable phosphorus to culture medium was used in preparation of cells for the stream experiment. Cells were grown in four flasks. Each flask contained 500 ml of broth, 11.1 mc of ^{32}P and enough stable phosphorus to bring the concentration to 0.3 mg per 100 ml. Flasks were inoculated with enough E. coli 0111 cells to bring the optical density to 20

units on the scale of a Klett Summerson colorimeter. During incubation the culture was stirred with a magnetic stirrer. Uptake of ^{32}P was checked at frequent intervals. After two hours at 35° C, the cells showed an uptake of 99.9% of the ^{32}P. Cultures were incubated four additional hours to bring cells to desired density. At this point they were filtered from the medium in 100 ml aliquots using a millipore membrane covered with 10 g of celite. We used 1,050 ml of culture which gave an assayed activity of 25.0 mc. The slurry of cells and celite was then placed in an ice bath, transported to the stream and this mixture was added to 55 gallons of stream water. This cell suspension was fed into the stream at a constant rate for a 33-minute period. The calculated water activity at the release point was similar to that of previous experiments (approximately 1.59×10^{-5} μc per ml).

We cultured samples of water, made enumerations of coliforms, and then compared cell counts of E. coli with the activity filtered from stream water. Serial dilutions were made of water samples which were then filtered through a 0.45 μm filter and cultured on eosine-methylene blue agar. Colonies were counted after 20 hours incubation at 35° C. Methods used were similar to those given in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes 10th edition (Amer. Public Health Assoc., Inc., 1955). Preliminary tests using this culture technique were made on (1) untreated stream water and (2) water collected at various locations below the point of release of test batches of E. coli cells. Samples of untreated water collected on three dates at four collecting stations showed no coliforms, however, the non-coliform flora averaged about 1.27×10^3 cells per ml. Trial releases of E. coli cells in the stream showed that when 1.3×10^{10} cells were added to 55 gallons of water and released over a 30-minute period, the passage of the cells could be easily detected 500 yd or more below the release site. To insure adequate numbers for quantitative evaluation for at least 3,280 yd of the study area, the concentration of cells used was 70 fold greater (9.2×10^{11}) than the number used in the above trial.

Measurement of stream radioactivity

The passage of the activity downstream was monitored by collecting water samples at downstream stations at 10-minute intervals. One-liter samples of water were stored in an ice bath until a 3-ml aliquot could be removed for preparation of bacteriological cultures. Two additional 200-ml aliquots were filtered through a 0.45 μm filter. One of these filtered aliquots was rinsed with 100 ml of distilled water while the second was rinsed with 100 ml of 0.01 N HCl. A two-way analysis of variance on both solids and filtrate aliquots from five stations showed that the activity of the aliquots rinsed in these two ways did not differ significantly. This indicates that there was little or no adsorbed ^{32}P.

Details of methods used in the processing and counting of samples of aquatic macrophytes, periphyton, aquatic insects, and fish have been described earlier [1]. It is only necessary to say here that a wet ashing technique was used and, in general, procedures were those given by Robeck, et al. [9]. Samples were counted on a gas flow proportional counter and corrections were made for self adsorption, back scatter, background and decay. Sampling of periphyton, aquatic macrophytes, insects, and fish was continued at all stations at weekly intervals for approximately 8 weeks after release of the spike. At this time activity was so low as to make counting difficult.

Results

Downstream movement of radioactivity

Data on the passage of particulate and filterable activity downstream is given in Table I. An analysis of variance showed that total activity was significantly different between stations but that filterable activity did not differ significantly. The progressive downstream decrease in total activity appears to be due chiefly to a fallout of suspended solids. The rate of fallout appears to have been greatest between stations 3 and 5 and between stations 8 and 12. Little, if any, fallout of activity occurred between stations 12 and 14.

Because of sample variability, the upstream-downstream differences in the means of filterable activity are not statistically significant. A small decrease in filterable activity with passage of the spike downstream is suggested by the mean values (Table I). Aquatic macrophytes and periphyton had small but significant amounts of activity after passage of the tracer indicating that some soluble radiophosphorus was available and was taken up by plants. The small upstream-downstream differences in filterable activity probably reflect this uptake.

Downstream passage of bacteria cells

Considerable heterogeneity appeared in plate counts made on samples collected at various stations during passage of the spike. This was to be expected since three serial dilutions were made from 3 ml of the original sample and this greatly increased the possibility of aberrant aliquots. However, plate counts of E. coli cells passing each station agreed well with the pulse in activity found on membrane filters with passage of the spike. Poor agreement between plate counts and activity counts was most noticeable at the end of the sampling, a time when there was high variability in the number of cells.

Table I. --Water-borne radioactivity passing sampling stations after release of spike of 9.2×10^{11} E. coli cells labeled with 25 mc of ^{32}P. Filterable activity is that passing a 0.45 μm membrane filter.

Station	Distance from release site (yards)	Activity in millicuries			
		Total		Filterable	
		Mean and standard error [a]	Percentage of released activity	Mean and standard error [a]	Percentage of released activity
3	300	25.30 ± 0.75	100.0	2.48 ± 1.0	9.92
5	550	19.12 ± 0.75	76.5	1.93 ± 1.0 [b]	7.73
8	1,030	16.76 ± 0.75	67.0	2.18 ± 1.0	8.72
12	2,580	15.15 ± 0.75	60.6	2.22 ± 1.0	8.88
14	3,280	16.26 ± 0.75	65.0	2.05 ± 1.0	8.20
16	5,280	13.07 [b]	52.2	...	...

[a] Mean is for 2 aliquots from a single set of water samples. Standard error was calculated from the error term of 2-way analysis of variance of aliquots from 5 stations.

[b] Value calculated from 50 ml samples rather than 200 ml samples.

The activity of cells passing downstream was calculated by dividing plate counts by the activity counted on membranes before incubation. The mean cell activity for all cells at all stations was 0.0218 μc per cell with a 95% confidence limit of 0.0148 μc - 0.0288 μc. An independent estimate of the activity of each cell was obtained by subtracting the average filterable activity (8.69%, c.f. Table I) from the total activity released in the stream (25 mc). This figure (22.8 mc) was then divided by the estimate of the total cells released into the stream (9.2×10^{11} cells). This gave an average of activity per cell of 0.0248 μc per cell--a figure in good agreement with the estimates from plate counts. Agreement of calculations made from measurements of field samples with those from laboratory cultures, suggest that once cells were added to the stream there was little or no isotopic exchange of ^{32}P with phosphorus of the stream water. Further evidence on this point comes from the finding that there was no significant change in the activity of cells during their passage downstream. A two-way analysis of variance made on the calculated activity of cells for the five stations shows that there was no significant difference between stations ($F = 1.429$ for 4 d.f.).

Movement of radioactivity through the food chain in 1961

Following treatment we measured radioactivity in a variety of plants and animals at four stations below the point at which the spike of radioactive bacteria was introduced (stations 3, 8, 12, 14). The most abundant plant life in the stream was the algal incrustation over rocks and other substrata (periphyton). To measure periphyton uptake, we incubated plastic substrates in the stream for a period of 2 weeks before introducing the tracer. During this time periphyton (chiefly diatoms) accumulated on the plastic. Plates were removed at various intervals of time after treatment and the activity of the periphyton measured. Aquatic macrophytes (Chara, Fontinalis and Potamogeton) were much less abundant on a biomass basis than periphyton and accumulated little activity (Table II).

It has been previously demonstrated [1] that after application of ^{32}P each species of the bottom fauna shows a time-activity curve characteristic of the species. The time of maximum activity is related to the trophic position of the organism in the food chain. Filter feeders and animals grazing on living periphyton showed their peak activity a few days after treatment. Detritus-feeders were intermediate in time of their activity peaks while the larger predacious forms (fish) did not show maxima 6 to 8 weeks following application of the tracer. The absolute amount of activity entering any food group following treatment is reflected by the height of the time curve but does not appear to influence the time of maximum activity.

Table II. --Activity ($\mu\mu$c/g) of stream invertebrates and plants and ratio of activity of the species (S) to activity of periphyton (P) after treatment with 23-25 mc of ^{32}P in various years

Species	Period of data (days after treatment)	1958		1959		1960		1961		1962	
		Mean	Ratio $\frac{S}{P}$	Mean	Ratio $\frac{S}{P}$	Mean	Ratio $\frac{S}{P}$	Mean	Ratio $\frac{S}{P}$	Mean	Ratio $\frac{S}{P}$
Periphyton	1- 7	2428	...	674	...	3725	...	194	...	1826[a]	...
Chara sp.	1-14	148	0.061	179	0.266	224	0.060	15	0.077	56	0.031
Fontinalis antipyretica	1-14	375	0.154	577	0.856	285	0.077	26	0.134	90	0.049
Potamogeton sp.	1-14	190	0.078	277	0.411	140	0.038	16	0.083	30	0.016
Simulium sp.	2-21	4789	1.972	2490	3.695	4029	1.082	509	2.624	2579	1.412
Hexagenia limbata	2-21	339	0.139	125	0.186	...	...	53	0.273	94	0.052
Ephemerella needhami	2-28	...	...	...	...	8083	2.170	...	...	8975	4.915
Ephemerella cornuta	2-28	...	...	...	...	6286	1.688	...	...	3871	2.119
Brachycentrus sp.	2-21	1916	0.789	2655	3.940	...	...	408	2.103	1774	0.971
Hydropsyche sp.	2-21	...	...	...	...	4650	1.248	1828	9.423	1703	0.933
Pteronarcys sp.	2-21	872	0.359	1129	1.675	...	...	121	0.624	1311	0.718
Atherix sp.	7-21	2611	1.075	1416	2.101	...	...	231	1.191	1377	0.754
Nigronia sp.	21-35	551	0.227	899	1.334	...	...	190	0.979	1243	0.681
Physa sp.	2-35	1123	0.463	1735	2.574	...	...	365	1.882	378	0.207
Cottus cognatus	14-28	3947	1.626	2350	3.487	5049	1.355	180	0.928	215	0.118
Salmo trutta	7-28	3106	1.279	3981	5.907	...	...	102	0.526	894	0.489
Lampetra lamottei	14-28	...	...	113	0.167	...	...	98	0.505	96	0.052

[a]Data from stations 3 and 8 on date of treatment estimated by extrapolating time-activity curve.

Following the treatment with radioactive bacteria in 1961, activity appeared in all groups in which it was noted following additions of ^{32}P in soluble form. Activity levels in plants were far below previous levels and could be measured for only short periods. Finding very low plant activity was consistent with the small fraction of activity released in filterable form (9.9%) and with the small loss of filterable activity from the spike during its passage downstream (less than 0.5 mc). The observed activity levels in various segments of the food chain therefore must reflect a small input of ^{32}P into the photosynthetic level and a much larger input of activity in the form of bacteria to filter feeders and to forms capable of utilizing the bacterial fallout reaching the stream bottom.

Following the four treatments of the stream with inorganic ^{32}P a delayed downstream movement of phosphorus was noted. This phosphorus was the ^{32}P adsorbed on various substrates and later brought into solution and the ^{32}P released by isotopic exchange with the biota. This activity could not be measured in the water directly but measurable amounts were concentrated by periphyton on substrates introduced after treatment. Substrates introduced after treatment with the bacterial spike in 1961 failed to concentrate a measurable amount of activity, indicating that all fixation in the photosynthetic level occurred with movement of the spike downstream. Thus there was little or no ^{32}P released by isotopic exchange with adsorbed ^{32}P and the amount of ^{32}P released by decay of bacterial cells or through biological recycling must have been insignificant.

Comparison with releases of ^{32}P in soluble form

Further analysis and an interpretation of the results of the bacteria experiment depend upon comparison with treatments made in other years. The validity of such comparisons, of course, depends not only upon year-to-year uniformity in treatment techniques but also upon year-to-year similarity in a host of environmental conditions. The environmental conditions for treatments in 1958, 1959 and 1960 have been discussed previously [1]. Techniques used in 1961 were similar to other years except that the ^{32}P was incorporated into bacteria. In the 1962 experiments phosphorus was introduced in soluble form as in 1958, 1960 and 1961. However, in 1962 a new technique was used in dispersing the radioactive phosphorus within the stream water which caused a greater fraction of activity to be adsorbed by sediments in the upper part of the study area and a smaller fraction to be fixed in periphyton at downstream stations.

There were major differences in the fraction of activity becoming fixed in a particulate form in various years after it was added to the stream (Table III). In 1960 and 1962 the filterable fraction was high compared to 1959 and 1961. Data on the percentage of filterable activity in 1958 are lacking, but the stage of the stream, the degree of water clarity and the previous history of run-off suggest that conditions in 1958 were similar to those in 1960, when a small fraction of ^{32}P went into a particulate form. In 1959 rains preceded addition of ^{32}P. Although stream flow was only slightly above normal stage at the time the isotope was introduced, the water was slightly discolored and carried organic detritus and bacteria reaching the stream as surface drainage from soil and adjacent bogs and marshy areas. In a study of the coliform bacteria of polluted and unpolluted streams, Taylor [10] found that in the case of unpolluted streams counts of coliforms fluctuated in the same way as stream level. The increase in bacteria after rains was believed to be due to detritus and animal feces washed into the stream.

Comparing average activities of various species with stations from year to year, it appears that nearly all activities were in the order of magnitude lower in 1961 (Table IV). Activity of the brook lamprey, Lampetra, was not significantly different in 1961 from other years (1959, 1962) and the activity of the caddisfly (Hydropsyche) and the snail (Physa) did not differ significantly between 1961 and 1962. Certain similarities exist in the activity pattern within the food chain between the 2 years in which there was a high fraction of particulate activity, e. g. 1959 and 1961. In these years there was a relatively low fixation of ^{32}P in periphyton and relatively high levels in Physa, Brachycentrus, Simulium, Hexagenia and Nigronia as compared to 1958, 1960 and 1962, suggesting that these forms utilized a greater fraction of food receiving phosphorus from particulate sources.

Further interpretation of the 1961 data can be made only by obtaining a relative rather than absolute measure of activity within the food chain. Periphyton is the principal source of ^{32}P within the stream environment which is available for incorporation into the food chain after the spike of radioactivity has passed downstream. It is also the chief source for all forms utilizing plant foods directly, e. g. ^{32}P that has not been recycled. It is of interest to compare the ratio of activity of a given species with the periphyton activity (Table IV). This gives a relative rather than absolute basis for comparison and a means of comparing activity in the food chain arising from periphyton with that arising from bacteria and particulate activity. A higher ratio in a given year compared to other years would suggest either (1) that a greater non-periphyton fraction was utilized or (2) that there were

Table III. --Quantity and form of ^{32}P activity after treatments

Year of treatment	Form of ^{32}P added	Activity in spike passing Station 3			
		Quantity (mc)	Forms (percentage)		
			Filterable	Adsorbed	Particulate
1958	Soluble	8	a	a	a
1959	Soluble	16	12	18	70
1960	Soluble	13	65	22	13
1961	Particulate (bacteria)	25	10	0	90
1962	Soluble	4	36	38	26

[a]Data not available but spike believed to have contained a high percentage of filterable activity.

Table IV. --Comparison of actual ratios of species activity to periphyton activity in 1961 (A) with predicted ratios in 1961 (B)

Species	Actual ratio (A)	Predicted ratio [a] (B)	Level of significance of difference in ratios [b]	Estimated fraction of 1961 activity from bacteria $(\frac{A - B}{A})$
Simulium sp.	2.62	1.97	N.S.	..
Hexagenia limbata	0.27	0.19	N.S.	..
Brachycentrus sp.	2.10	0.97	0.67	0.53
Hydropsyche	9.42	1.25	0.80	0.86
Pteronarcys	0.62	0.72	N.S.	..
Atherix variegata	1.19	1.08	N.S.	..
Nigronia sp.	0.98	0.68	N.S.	..
Physa	1.88	0.46	0.90	0.75
Lampetra lamottei	0.51	0.05	0.99	0.90
Cottus cognatus	0.93	1.63	N.S.	..
Salmo trutta	0.53	1.28	N.S.	..

[a] Maximum ratio from 1958, 1960 and 1962.

[b] N.S. = differences in ratios not statistically significant.

year-to-year differences in concentration mechanisms. Choosing the former interpretation, a comparison of ratios can be used to determine the fraction of activity within the food chain in 1961, which can be assigned to the periphyton source and the fraction which arises from particulate activity. An expected ratio of the activity of each species to periphyton activity for 1961 was obtained by selecting the highest ratio among the 3 years 1958, 1960 and 1962. Using the highest ratio among these years gives a ratio which maximizes the expected activity from the particulate sources and minimizes activity from the periphyton source. Thus among the actual ratios of various species in 1961, values which are higher than expected ratios almost certainly arise from the concentration of ^{32}P from the particulate source.

Activity ratios significantly higher than predicted ratios were found for the filter feeder, Hydropsyche, and for ammocoetes of the brook lamprey, Lampetra, which apparently subsist on the ooze and detritus of depositing substrates (Table IV). Larvae of a caddisfly (Brachycentrus) which feed largely on drift material, and snails of the genus Physa which are omnivorous, had fractions of their activity attributable to the bacterial cells. Predicted ratios were lower than actual ratios in the case of filter feeder, Simulium; the mayfly, Hexagenia, which feeds principally on the ooze of depositing substrates; and the micro-predator, Nigronia. However differences in these ratios were not significant at the 0.67% level or above. The actual ratios of the micro-predator, Atherix, and the macro-detritus feeder, Pteronarcys, were below the predicted ratio, indicating that these species were independent of phosphorus from the bacterial source. Two species of fish (Cottus and Salmo) also had smaller amounts of activity than predicted, indicating that the flow of phosphorus through the food chain to fish was chiefly from periphyton.

By dividing the difference between actual and predicted ratios by the actual ratio an estimate has been made of the fraction of the activity which could be assigned to the bacterial source (Table IV). In cases in which the actual ratio was not significantly greater than the predicted ratios and when predicted ratios were greater, no activity could be assigned to bacteria. These calculations indicate that heterotrophic components of energy arising from the bacterial source amounted to as much as 90% in the brook lamprey and 86% in the case of Hydropsyche.

The abundance of a species must be considered in interpreting its role in the transfer of phosphorus through the food chain. A fish's intake of a rare species having a high activity level might well be masked by extensive feeding on abundant species containing little activity. Thus the accumulated activity reflects both the abundance of food species and

their trophic position. Certain of the species preyed upon by fish, at least seasonally, e.g. Hydropsyche and Hexagenia, were so scarce as to constitute small dietary fractions. Brook lampreys are probably seldom eaten by Cottus and trout. Physa was abundant and contained at least a small fraction of activity which could be assigned to bacteria. Failure to find activity in fish greater than might be predicted from periphyton sources indicates that species receiving significant activity from bacteria were of minor importance in the diet of fish.

Discussion

The normal bacteria flora of the stream water was measured before treatment with bacterial cells. On June 27 the bacterial count on EMB agar was 1.27×10^3 cells per ml. None of these bacteria were coliforms. The concentration of E. coli cells introduced was 9.2×10^{11} cells per ml. Of this number 52% traversed the entire 2.5 miles of the study area. Thus, the bacterial spike increased the cell count by a factor of 7.3×10^8. Even with such a large increase in water-borne bacteria, there appeared to be little or no fixation of activity within the filter feeders segment of the community. The activity of Simulium was not above the level that might have been predicted from the amount of activity in periphyton. Hydropsyche had a level higher than predicted but the activity peak was not reached until 3 weeks after the radioactive bacteria were introduced. The activity concentrated by this filter feeder probably came from bacterial fallout that subsequently found its way to substrates of organic detritus which were filtered from the water by the larvae.

The absence of an effective endogenous system for the removal of E. coli cells has certain interesting biological implications. First of all, it suggests a high degree of adaptation and specialization by the filter feeders which perhaps are equipped to handle only cells in the diatom range (5 to 200 μm) and not in the range of E. coli (2 μm). Secondly, the absence of an effective system for removing E. coli cells indicates that self purification of the stream from this type of contamination is apt to be slow and ineffective.

The lack of biological uptake permitted wide dispersion of cells. Such wide dispersion reduced transfer through segments of the food web leading to items of interest to man, e.g. fish. Radiophosphorus dispersed in this way in stream systems would perhaps not accumulate at any point in the environment and biological accumulation might in large part be delayed until decay had taken place. Such a dispersion

system might be practical for disposal of ^{32}P wastes and might prevent build-up such as has been observed in the food fish of the Columbia River by Foster [11].

The apparent lack of biological system for removal of cells is also significant in regard to disposal of domestic wastes in unpolluted trout streams. Very likely organisms capable of purification must be recruited from outside sources or must be built up by population increases from the rare forms within the system. In any case the capability for removal of coliform cells must be developed and does not appear to be present in this unpolluted environment under steady state conditions and this ecological system was not well adapted to cope with sudden short-term input of coliforms.

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