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Utilization of Nucleoside Monophosphates Per Se for Intraperiplasmic Growth of *Bdellovibrio bacteriovorus*

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During growth of *Bdellovibrio bacteriovorus* on *Escherichia coli*, there was a marked preferential use of *E. coli* phosphorus over exogenous orthophosphate even though the latter permeated into the intraperiplasmic space where the bdellovibrio was growing. This preferential use occurred to an equal extent for lipid phosphorus and nucleic acid phosphorus. Exogenous thymidine-5'-monophosphate competed effectively with [^{3}H]thymine residues of *E. coli* as a precursor for bdellovibrio deoxyribonucleic acid; exogenous thymidine competed less effectively and thymine and uridine not at all. A mixture of exogenous nucleoside-5'-monophosphates equilibrated effectively with *E. coli* phosphorus as a phosphorus source for *B. bacteriovorus*; the nucleotide phosphorus entered preferentially into bdellovibrio nucleic acids. A comparable mixture of exogenous nucleosides plus orthophosphate had only a small effect on utilization of *E. coli* phosphorus by *B. bacteriovorus*, as did orthophosphate alone. A mixture of exogenous deoxyriboside monophosphates equilibrated effectively with *E. coli* phosphorus as a phosphorus source for bdellovibrio growth; the phosphorus from this source entered preferentially into deoxyribonucleic acid. These data show that nucleoside monophosphates derived from the substrate organism are utilized directly for nucleic acid biosynthesis by *B. bacteriovorus* growing intraperiplasmically. As a consequence, the phosphate ester bonds preexisting in the nucleic acids of the substrate organism are conserved by the bdellovibrio, presumably lessening its energy requirement for intraperiplasmic growth. The data also suggest, but do not prove, that the phosphate ester bonds of phospholipids are also conserved.

We have reported that the energy efficiency (Y_{ATP}) (3) for the intraperiplasmic growth of *Bdellovibrio bacteriovorus* on *Escherichia coli* as the substrate organism (20) is about 26. A hypothetical bacterium, whose only energy expenditure for growth is the adenosine triphosphate required for polymerization of preformed monomeric units into cell polymers, would, according to the calculations of Gunsalus and Shuster (10), have a Y_{ATP} of 33. More recent calculations by Forrest and Walker (8) lower this value to 26 to 27, which is about the same as our observed value for *B. bacteriovorus*.

If our reported value of 26 is valid, it must be assumed that *B. bacteriovorus* growing intraperiplasmically does little biosynthesis of essential monomers. It follows that its intraperiplasmic growth should not be affected by inhibitors of such biosynthetic reactions. This prediction proved valid for the inhibitor methotrexate, which strongly inhibits the axenic growth of a mutant *B. bacteriovorus* 109Ja in complex medium while exerting little effect on the intraperiplasmic growth of the wild-type strain (17).

A second, less obvious prediction follows from our reported Y_{ATP}: phosphate ester bonds existing in the substrate organism are conserved during conversion of substrate organism cell material to bdellovibrio cell material. If nucleic acids of the substrate organism were degraded only to nucleoside monophosphates that were assimilated as such, and if the phosphate ester bonds of phospholipids were similarly conserved, then the energy expended by the bdellovibrio in polymerization reactions would be significantly reduced as compared with the hypothetical organisms of Gunsalus and Shuster (10) and Forrest and Walker (8), and a large Y_{ATP} would be reasonable. Two additional experimental findings lent support to this prediction: thymidine-5'-monophosphate is more effective than thymidine or thymine in reversing methotrexate inhibition of axenic growth of a mutant of *B. bacteriovorus* (17); and in contrast to most aerobic bacteria, which show a

multifold increase in respiration rate over the endogenous rate when provided with readily oxidizable substrates, the respiration rate of *B. bacteriovorus* increases by only 25 to 50% during intraperiplasmic growth (12). The latter finding suggests that energy required for growth is little more than energy required for maintenance. This paper is concerned with the testing of this prediction.

MATERIALS AND METHODS

Organisms and culture conditions. *B. bacteriovorus* 109J (19) was used in all experiments. Cell suspensions of this organism were prepared in 10^{-3} M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.5) from cultures grown on *E. coli* growing in dilute nutrient broth (25) as previously described (12).

Pregrown *E. coli* ML35 (*lacI*, *lacY*) served as substrate for bdellovibrio growth experiments. HEPES buffer suspensions of the following types of cells were used.

(i) Nonradioactive *E. coli*; cultures were grown in nutrient broth (Difco) supplemented with 0.5% yeast extract.

(ii) Uniformly labeled [U-^{32}P]*E. coli*; cultures were grown in a glucose-mineral salts medium (12) supplemented with 0.1% Casamino Acids (Difco). The medium contained 10^{-2} M potassium [^{32}P]phosphate with a specific activity of approximately 7.5×10^3 disintegrations per min per μg of phosphorus.

(iii) [^{3}H]thymine-labeled *E. coli*; cells with a ^{3}H label in the thymine moiety of their deoxyribonucleic acid (DNA) were obtained by a modification of the method of Boyce and Setlow (5) from cultures grown in glucose-mineral salts-Casamino Acids medium as previously described (16). Over 95% of the radioactivity in the *E. coli* cells thus labeled was extractable by 10% hot trichloroacetic acid.

All *E. coli* cultures were grown at 37 C with constant shaking. Cells were harvested in late exponential phase and suspensions were prepared in HEPES buffer containing $CaCl_2$ (2 mM) and $MgCl_2$ (3 mM). Numbers of cells in suspension were determined by reference to standard curves relating optical density (OD) at 420 nm to colony counts.

Growth experiments. *E. coli* cells served as the major or exclusive source of all nutrients for growth of the bdellovibrios. The growth medium consisted of HEPES buffer suspension of one of the three types of *E. coli* cells described above supplemented, as appropriate, with potassium orthophosphate (pH 7.4), nucleic acid bases, nucleosides, or nucleoside-5'-monophosphates. About 5×10^9 *E. coli* cells per ml of medium was used in all experiments; the precise quantity is specified in the legends to the tables as total micrograms of *E. coli* phosphorus per culture. Sixty micrograms of *E. coli* phosphorus is equivalent to about 10^{10} cells.

Multicycle and single-cycle growth experiments were done. In the former, an initial input ratio of 1:100 or less of *B. bacteriovorus* to *E. coli* was used. In this situation, the amount of bdellovibrio phosphorus initially added to the culture was negligible as compared with the starting *E. coli* phosphorus. At least four cycles of bdellovibrio development occurred before complete utilization of all *E. coli*, and the final bdellovibrio population was at least 300 to 400 times the initial. In the latter type of experiment, an input ratio of two or more bdellovibrio per *E. coli* was used. A fairly synchronous attack on all *E. coli* present occurred (21), and only a single cycle of bdellovibrio development took place. The amount of bdellovibrio phosphorus added to such cultures amounted to 0.5 to 1 times the *E. coli* phosphorus present, and the final bdellovibrio population was some 2 to 2.5 times the original. Suspensions of *E. coli* and *B. bacteriovorus* and solutions of supplements were mixed to start the experiments. Cultures were shaken at 30 C until lysis of all *E. coli*. This required overnight incubation for the multicycle experiments and 3 to 4 h for the single-cycle experiments.

When growth of the bdellovibrios was complete, the total culture (usually 10 ml) was centrifuged at 1,000 $\times$ *g* for 5 min and the small pellet which consisted of a mixture of *E. coli* debris and clumped bdellovibrio cells was discarded. Since knowledge of the total yield of bdellovibrios or the total transfer of phosphorus from various sources to the bdellovibrios during growth was not important in these experiments, the discarding of a considerable number of bdellovibrios at this step was of no significance. The remaining suspension was then centrifuged at 7,800 $\times$ *g* for 20 min. The pellet was washed twice by resuspension in HEPES buffer (1 volume per initial volume of culture) and recentrifuging the suspension as above. The washed bdellovibrios were usually suspended in 4.2 ml of HEPES buffer, and the analytical data presented are based on the total cells present in the final suspension. Control experiments described elsewhere (13, 20) proved that the final suspensions contained negligible amount of *E. coli* debris.

Fractionation of bdellovibrio cells and determination of phosphorus and radioactivity. Two 0.1-ml samples were removed from the 4.2-ml suspension of harvested bdellovibrios and ashed for determination of total phosphorus. Samples of the original *E. coli* suspension, the original bdellovibrio suspension, and the zero-time culture were similarly treated. The remaining 4.0 ml of harvested bdellovibrios were fractionated by following in general the procedures of Roberts and co-workers (23). One milliliter of cold 50% trichloroacetic acid was added and the suspension was held in ice for 30 min and then centrifuged. The pellet was washed by suspending it in 5 ml of cold 10% trichloroacetic acid; the suspension was centrifuged and the supernatant was discarded. The pellet was then extracted at 45 C with 5 ml of 75% ethanol for 30 min and then with 5 ml of a 1:1 (vol/vol) mixture of ethyl ether-75% ethanol for 15 min. The supernatant fluid from the two extractions was combined to give the lipid fraction. The pellet was extracted with 10% trichloroacetic acid at 100 C for 30 min to give the nucleic acid fraction. The residue from

this extraction was washed first with 5 ml of 4% HCl in ethanol and then with 5 ml of ethyl ether, and the washings were discarded. The residue was resuspended and partially dissolved in 0.2 ml of 10% NaOH to give the protein fraction.

In those experiments in which DNA and ribonucleic acid (RNA) were analyzed separately, harvested bdellovibrios were treated as described above through the lipid extraction steps. The pellet was then suspended in 2 ml of 1 M KOH and hydrolyzed at 37 C for 20 h. Two milliliters of 1.2 N HCl-5.6% trichloroacetic acid was added, and the mixture was held in ice for 5 min and then centrifuged. The pellet was washed in 4 ml of cold 5% trichloroacetic acid and centrifuged. The wash contained negligible amounts of RNA and was discarded. The first supernatant was analyzed for RNA by the orcinol procedure (24) and for phosphorus as described below. The pellet was extracted with 4 ml of hot 5% trichloroacetic acid, and the extract was assayed for DNA by the diphenylamine method (6) and for phosphorus.

All phosphorus determinations were done on samples ashed by the method of Ames and Dubin (1). Usually, 0.1-ml samples of the starting cell suspensions, original cultures, harvested bdellovibrios, trichloroacetic acid extracts, and final residues were ashed. However, the combined lipid extracts were evaporated to dryness and the total material was ashed. In all cases, 0.3 ml of 1 N HCl was added to the ash and the resulting solution was heated in a boiling water bath for 15 min. The final solution was made up to 4.0 ml with distilled water. Appropriate portions of the 4 ml were used for phosphorus assays by the method of Chen et al. (7) and for determination of radioactivity. Acid-cleaned glassware and glass double-distilled water were used throughout to minimize phosphorus contamination.

Radioactivity from ^{32}P was measured by scintillation counting using a mixture of 750 ml of toluene, 250 ml of Triton X-100, 0.25 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, and 4 g of 2.5-diphenyloxazole as the scintillation fluid. All samples from an experiment were counted at the same time and all samples counted were of a very similar overall composition and concentration. Thus, corrections for decay and quenching were unnecessary.

In those experiments involving [^{3}H]thymine-labeled *E. coli*, bdellovibrios were harvested as described above and subsamples of the suspension were spotted on 3-mm Whatman filter disks and placed into cold 5% trichloroacetic acid. Filters were washed twice in cold 5% trichloroacetic acid, twice in cold 95% ethanol, and once in cold ethyl ether. They were air dried overnight and then added to the scintillation fluid for determination of radioactivity. The DNA content of the suspension was determined by the diphenylamine method (6), and the specific activities reported are the ratios of the two values. We have already shown that over 90% of the thymine label incorporated by *B. bacteriovorus* under the conditions of these experiments is in the DNA fraction (16).

Phosphate permeation. A modification of a technique described by Roberts and co-workers (23) was used to determine whether phosphate could permeate into the periplasmic space of the substrate organism after bdellovibrio attack and penetration. Single-cycle cultures of *B. bacteriovorus* growing on *E. coli* were initiated as described. By 1 h, essentially all *E. coli* had been converted to "bdelloplasts." (Shortly after attack and penetration by *B. bacteriovorus*, rods and spirilli alter to spherical forms, which have been referred to in the bdellovibrio literature as "spheroplasts." Since the term spheroplast [18] is usually used to designate a gram-negative, osmotically unstable cell essentially devoid of peptidoglycan, and since the spherical body produced by bdellovibrio attack is osmotically stable and initially contains most of the peptidoglycan [S. K. Nishimoto and S. C. Rittenberg, unpublished data], we prefer to designate it by the more noncommittal term of "bdelloplast.") The bdelloplasts were harvested by centrifugation at 23,500 × *g* for 10 min and washed twice in HEPES buffer. The pellet was then suspended in 2 ml of 1.25×10^{-2} M potassium phosphate solution, pH 7.5, containing 0.08% high-molecular-weight (about 2×10^{6}) blue dextran (OD at 625 nm [OD_{625}] = 0.89) and 0.5 μCi of [^{32}P]phosphate. The phosphate concentration used in these experiments was equal to the highest concentration of phosphate used in evaluating the incorporation of exogenous phosphate (see Table 2). After 10 min at 30 C, the suspensions were centrifuged and the OD_{625} and radioactivity of the supernatant fluid were determined. The total volume of "extracellular" water (i.e., the initial 2 ml of buffer plus the interstitial water in the starting bdellovibrio pellets) in the suspensions was determined from the decrease in OD_{625}. The calculation assumes that the blue dextran does not permeate into the bdelloplast. A comparison of the decrease in radioactivity of the supernatant fluid with the decrease in OD, then, indicates whether the phosphate equilibrates with an additional volume (i.e., the bdelloplasts) not accessible to the blue dextran.

RESULTS

Phosphorus content and distribution in B. bacteriovorus. Table 1 (I) shows the phosphorus content and distribution for *B. bacteriovorus* grown on *E. coli* as the sole source of phosphorus and all other nutrients. Based on an average of seven experiments, total phosphorus amounted to 20 μg per 10^{10} bdellovibrios. The distribution of phosphorus among the four major cell fractions (Table 1) corresponded closely to its distribution in *E. coli* (23). The percentage of total phosphorus in the cold acid-soluble fraction was somewhat smaller and may indicate lower pool levels of phosphorylated intermediates in *B. bacteriovorus* than in *E. coli*. Otherwise, the differences were not great and do not suggest a unique phosphorus composition for the former organism. Neither the total phosphorus content nor its distribution

TABLE 1. *Phosphorus content and distribution in B. bacteriovorus 109J*[a]

Cell fraction	Phosphorus content (μg/10^{10} cells)		Total P (%)		
	I	II	I	II	*E. coli*[b]
1. Cells (total)	20.9	23.8	100	100	100
2. Cold acid soluble	1.9	2.6	10.8	10.9	17.6
3. Lipids	2.9	3.1	14.0	12.9	13.7
4. Nucleic acids	14.6	16.1	70.0	67.5	64.9
5. Proteins	0.5	0.8	2.2	3.4	2.3
6. Sum of 2-5	19.9	22.6	97.0	94.7	98.5

[a] *B. bacteriovorus* was grown on *E. coli* cells suspended in HEPES buffer. Potassium orthophosphate (1.25 mM) was present in II and absent in I.

[b] Data from Roberts et al. (23).

changed appreciably when inorganic phosphate was present in the medium (Table 1 [II]).

Effect of exogenous phosphate on uptake of E. coli phosphorus. *B. bacteriovorus* was grown on [U-^{32}P]*E. coli* without and with added orthophosphate in the medium. Analyses of the total phosphorus of the bdellovibrios harvested at the end of growth showed that the specific activity of bdellovibrio phosphorus was not greatly altered by exogenous inorganic phosphate. This result was obtained even when the exogenous inorganic phosphorus was present at up to 10 times the quantity of phosphorus in the substrate *E. coli* (Table 2). At a starting ratio of *B. bacteriovorus* to *E. coli* of about 1:100 (series A, Table 2), there was a 16 to 24% decrease in the specific activity of bdellovibrio phosphorus as compared with the starting *E. coli* phosphorus, depending on the concentration of unlabeled inorganic phosphate in the medium. Thus, in these experiments over 75% of the bdellovibrio phosphorus was derived from *E. coli*.

Since most of the *E. coli* originally added to the series A cultures were not attacked by bdellovibrios until many hours after the start of the experiment, the possibility existed that some unlabeled phosphate phosphorus was incorporated by the *E. coli* either as a result of endogenous metabolism or cannibalism on wastes from lysing *E. coli*. If so, some part of the phosphate phosphorus apparently incorporated by the bdellovibrios could have been derived via *E. coli* phosphorus. To test this possibility, the experiment was repeated at a large input ratio of bdellovibrios to *E. coli* (about 3.3:1). This high multiplicity would tend to minimize possible incorporation of exogenous phosphate into *E. coli* phosphorus. Under these conditions

TABLE 2. *Utilization of exogenous inorganic phosphate during intraperiplasmic growth of B. bacteriovorus on E. coli*[a]

Culture	Added PO_4 phosphorus (μg)	Sp act (dpm/μg of P) of total phosphorus in:		Bdellovibrio phosphorus from PO_4 (%)
		Starting culture	Harvested bdellovibrios	
A				
1	None	1,790	1,705	
2	1,284	400	1,490	16.7
3	2,568	226	1,380	22.8
4	3,752	161	1,360	24.0
B				
1	None	864	1,000	
2	1,284	315	788	8.8
3	2,568	198	746	13.5
4	3,752	146	680	21.3

[a] *B. bacteriovorus* was grown on [U-^{32}P] *E. coli* cells (1,800 disintegrations/min [dpm] per μg of P) suspended in 10 ml HEPES buffer containing indicated amounts of potassium orthophosphate. At the start of the experiments, the series A cultures (multicycle growth experiment) contained 369 μg of *E. coli* phosphorus and 2 μg of *B. bacteriovorus* phosphorus; and the B series (single growth cycle experiment) contained 369 and 396 μg of *E. coli* and *B. bacteriovorus* phosphorus, respectively.

(series B, Table 2) the utilization of exogenous inorganic phosphate by the bdellovibrios was reduced somewhat and amounted to about 20% of the total at the highest phosphate concentration tested.

It is obvious from the above results that *B. bacteriovorus* utilizes *E. coli* phosphorus in preference to exogenous phosphate phosphorus. This preference holds equally for all of the major phosphorus fractions of the cell. Analyses of bdellovibrios grown on [U-^{32}P]*E. coli* in the presence of unlabeled inorganic phosphate showed that the specific activities of total phosphorus and of phosphorus in cold acid-soluble material, in lipids, and in nucleic acids were all reduced by the same amount as compared with the control bdellovibrios grown without phosphate (Table 3). The specific activity of the protein fraction (residue) was much lower, but the quantity of phosphorus in this fraction was so low that trace impurities in the analytical reagents or glassware could greatly influence the result and the low value is believed to be an experimental artifact.

Permeation of phosphate into the periplasm of the substrate organism. One possi-

TABLE 3. *Utilization of exogenous inorganic phosphate during intraperiplasmic growth of B. bacteriovorus on E. coli and its incorporation into the major phosphorus fractions of B. bacteriovorus*[a]

Constituent	I			II		
	Phosphorus (μg)	10^{-6} dpm	Sp act (dpm/μg of P)	Phosphorus (μg)	10^{-6} dpm	Sp act (dpm/μg of P)
Starting culture						
E. coli	1,826	5.74	3,140	1,826	5.86	3,140
Potassium orthophosphate	0			8,524		
Total phosphorus	1,826	5.74	3,140	10,350	5.86	566
Harvested bdellovibrios						
Total phosphorus	640	1.97	3,080	594	1.30	2,190
Cold acid soluble	65	0.19	2,910	43	0.091	2,120
Lipids	103	0.32	3,060	88	0.19	2,200
Nucleic acids	481	1.45	3,010	406	0.89	2,190
Proteins	5	0.012	2,400	6	0.058	970

[a] *B. bacteriovorus* was grown on [U-^{32}P]*E. coli* cells (3,140 disintegration/min (dpm)/μg of P) suspended in 10 ml of HEPES buffer. The indicated amount of orthophosphate phosphorus was added in experiment II; none was added in I. The input ratio of *B. bacteriovorus* to *E. coli* in both experiments was less than 1:100, and the initial quantity of *B. bacteriovorus* phosphorus was negligible.

ble explanation for the preferential utilization of *E. coli* phosphorus over exogenous inorganic phosphate by the bdellovibrios is that orthophosphate does not permeate into the periplasmic space of *E. coli* where the bdellovibrios are growing. Direct measurements of phosphate permeation indicate, however, that this is not the reason. Table 4 shows the results of an experiment in which sedimented bdelloplasts were suspended in buffer solution containing high-molecular-weight blue dextran and [^{32}P]phosphate and then resedimented by centrifugation. Analyses of the resulting supernatant fluid showed that the decrease in phosphate concentration was proportionately greater than the decrease in blue dextran concentration. Furthermore, the difference was proportional to the number of bdelloplasts used. Accepting that the bdelloplasts are not permeable to the blue dextran, the decrease in its concentration is due to its equilibration with the interstitial water of the bdelloplast pellet. The additional decrease in phosphate concentration indicates that it equilibrates with an additional water space, i.e., the free water of the bdelloplast per se.

TABLE 4. *Permeability of bdelloplasts to inorganic phosphate*[a]

Bdelloplasts	OD_{625}	Calculated extracellular water (ml)	Radioactivity (10^{-3} dpm)[b]		
			Calculated	Observed	Difference
None	0.890	2.0		1,073	
5×10^{10}	0.791	2.25	954	900	−54
1×10^{11}	0.765	2.33	917	837	−80
1.5×10^{11}	0.751	2.37	905	747	−158

[a] *B. bacteriovorus* and *E. coli* were suspended in HEPES buffer at a ratio of 2:1 and incubated at 30 C for 1 h. At this time, the bdelloplasts (the rounded *E. coli* cells containing a bdellovibrio) were sedimented by centrifugation and resuspended in 2 ml of 1.25×10^{-2} M PO_4 solution containing 0.08% high-molecular-weight (about 2×10^6) blue dextran (OD_{625} = 0.89) and 0.5 μCi of [^{32}P]phosphate. After 10 min the bdelloplasts were sedimented and the supernatant fluid was analyzed. The amount of extracellular water was calculated from the decrease in OD_{625}. The calculated radioactivity was obtained by assuming the [^{32}P]phosphate equilibrated only with extracellular water.

[b] dpm, Disintegrations per minute.

Utilization of exogenous nucleoside-5'-monophosphates during intraperiplasmic growth. An alternate explanation for the preferential use of *E. coli* phosphorus over exogenous inorganic phosphate by *B. bacteriovorus* is that phosphate esters derived from the *E. coli* are used per se. To explore this possibility, the uptake of exogenous thymidine monophosphate was examined. *B. bacteriovorus* was grown on [^{3}H]thymine-labeled *E. coli*. Potential precursors of this DNA component were added to these cultures, and the extent of incorporation of the ^{3}H label into the bdellovibrios was measured (Table 5). The presence of thymine or uridine in the medium had no appreciable effect on the extent of incorporation by the bdellovibrios of labeled thymine residues from the *E. coli*. In contrast, exogenous thymidine re-

TABLE 5. *Effects of potential thymine precursors on incorporation of [^{3}H]thymine from E. coli DNA during intraperiplasmic growth of B. bacteriovorus*[a]

Culture medium	Sp act of harvested bdellovibrios[b]		
	I	II	III
[^{3}H]thymine-labeled *E. coli*	1.93 (100)	11.3 (100)	1.35 (100)
+ Thymine	1.69 (88)	11.2 (99)	
+ Uridine	1.82 (94)	10.7 (95)	
+ Thymidine	0.82 (42)	6.5 (58)	0.83 (62)
+ Thymidine 5′-phosphate	0.46 (24)		0.38 (28)

[a] Initial cultures contained 5×10^9 [^{3}H]thymine-labeled *E. coli*, 10^{10} *B. bacteriovorus*, and 1 μmol of indicated compound per ml of HEPES buffer (10^{-3} M), pH 7.5.

[b] Disintegrations per minute per microgram of DNA × 10^{-3}. Numbers in parentheses are percentage of control. Initial specific activities of [^{3}H]thymine-labeled *E. coli* were 5.7×10^3 (experiment I), 2.2×10^4 (experiment II), and 4.1×10^3 (experiment III).

duced the incorporated radioactivity by 40 to 60% and exogenous thymidine monophosphate by some 75%. The results clearly demonstrate that although the thymine moiety of exogenous thymidine competed with thymine residues derived from the *E. coli* DNA, thymidine monophosphate competed to an even greater extent. The data suggest that the bdellovibrios were incorporating thymidine monophosphate per se.

The above inference was confirmed by measuring the effects of exogenous nucleoside monophosphates on the incorporation by *B. bacteriovorus* of labeled phosphorus from [U-^{32}P]*E. coli* and comparing these effects with those resulting from the presence of the corresponding nucleosides plus the equivalent amount of phosphate phosphorus or from the presence of phosphate alone (Table 6). The addition of exogenous nucleoside monophosphates to the culture resulted in a 60% reduction in the specific activity of the total bdellovibrio phosphorus as compared with the specific activity of the control bdellovibrios grown on *E. coli* alone. Growth of bdellovibrios in the presence of nucleosides plus phosphate resulted in only an 18% drop in the specific activity of total bdellovibrio phosphorus, a decrease comparable to that caused by exogenous phosphate alone (Table 6; see also Table 2).

The bdellovibrios harvested from the experiment described above were fractionated, and the specific activities of the lipid phosphorus and nucleic acid phosphorus of each culture were determined (Table 6). The specific activities of the bdellovibrio lipid phosphorus and nucleic acid phosphorus were the same in the cultures containing nucleosides plus phosphate or phosphate alone and were only 18% less than the controls. However, for bdellovibrios grown in the presence of nucleoside monophosphates, the specific activity of the nucleic acid phosphorus was about half that of the lipid phosphorus and 30% that of the nucleic acid phosphorus of the control cultures. The data show that the phosphorus from exogenous nucleoside monophosphates moved preferentially into the nucleic acids of the bdellovibrios. In contrast, phosphorus from inorganic phosphate was incorporated equally into both fractions independent of the presence or absence of nucleosides.

In a similar experiment in which a mixture of the four natural deoxyriboside monophosphates was added to the experimental flask, the specific activity of phosphorus in the bdellovibrio DNA was approximately one-half that of the RNA phosphorus and one-third that of the lipid phosphorus (Table 7). Actually the specific activity of the *B. bacteriovorus* DNA phosphorus was less than that of the total initial phosphorus in the culture, indicating that at the concentrations used the exogenous deoxyriboside monophosphates were being used in preference to *E. coli* phosphorus by the bdellovibrios for synthesis of DNA.

DISCUSSION

The data presented show unequivocally that during intraperiplasmic growth *B. bacteriovorus* uses the phosphorus of its prey in prefer-

TABLE 6. *Utilization of exogenous nucleoside monophosphates during intraperiplasmic growth of B. bacteriovorus on [U-^{32}P]E. coli*[a]

Culture medium	Sp act (dpm/μg of P)			
	Initial total P	*B. bacteriovorus*		
		Total P	Lipid P	Nucleic acid P
[U-^{32}P]*E. coli*	1,529	1,506	1,522	1,438
+ Nucleoside monophosphates	314	605	752	384
+ Nucleosides + phosphate	300	1,247	1,263	1,282
+ Phosphate	306	1,233	1,235	1,240

[a] Initial cultures contained [U-^{32}P]*E. coli* (298 μg of P, 2,164 disintegrations/min [dpm] per μg of P) + *B. bacteriovorus* (134 μg of P) and, as indicated, adenosine, uridine, guanosine, cytidine, and thymidine monophosphates (10 μmol each) or the corresponding nucleosides + 50 μmol of potassium orthophosphate or potassium orthophosphate (50 μmol) in 10 ml of HEPES buffer (10^{-3} M), pH 7.5.

TABLE 7. *Effects of exogenous deoxynucleoside monophosphates on the utilization of E. coli phosphorus during intraperiplasmic growth of B. bacteriovorus*

Culture medium	Sp act (dpm/μg of P)				
	Initial total P	*B. bacteriovorus*			
		Total P	DNA P	RNA P	Lipid P
[U-^{32}P]*E. coli*	1,250	1,247	1,084	1,218	1,400
+ Deoxynucleoside monophosphates ..	305	371	209	403	613

[a] Cultures contained 317 μg of *E. coli* phosphorus (specific activity, 2,020 disintegrations/min [dpm] per μg of P) and 194 μg of *B. bacteriovorus* phosphorus and, as indicated, 12 μmol each of deoxyadenine, -guanosine, -cytidine, and -thymidine monophosphates in 12 ml of HEPES buffer (0.001 M), pH 7.5.

ence to phosphate phosphorus in the environment even when the latter is present in large excess over the former. As direct measurements have shown, this preference is not due to the exclusion of phosphate from the periplasmic space of the prey in which the bdellovibrio is growing. Nor can the preferential use of *E. coli* phosphorus be explained by impermeability of the bdellovibrio to orthophosphate. The strongest evidence for the latter statement is the observation that 20% or so of the phosphorus of bdellovibrios growing intraperiplasmically is derived from exogenous phosphate when the latter is added to the medium in high concentration. Instead, the preferential use of the phosphorus from the prey is a consequence of the preferential utilization of organic phosphorus compounds by *B. bacteriovorus*.

The above conclusion is supported by the experiments showing a greater uptake of thymine residues from exogenous thymidine monophosphate than from thymidine or thymine per se. More direct and conclusive are the data showing greater uptake of phosphorus from nucleoside monophosphate than from nucleosides plus inorganic phosphate or inorganic phosphate alone. These results can only be explained if thymidine monophosphate and other nucleoside monophosphates are taken up by the bdellovibrio as a unit. One might argue that the thymidine monophosphate permeates more readily than orthophosphate into the periplasmic space of the substrate organism and is dephosphorylated there to thymidine and phosphate, which then enter the bdellovibrio. This argument, however, is negated by the observation that exogenous phosphate flows equally into the nucleic acids and lipids of the bdellovibrio whereas exogenous nucleoside monophosphates enter preferentially into its nucleic acid fraction. Likewise, exogenous deoxyriboside monophosphates enter preferentially into bdellovibrio DNA.

It was observed that, during intraperiplasmic growth of *B. bacteriovorus*, phosphorus from exogenous nucleoside monophosphates also enters into the lipids of the bdellovibrio and that phosphorus from exogenous deoxyribotides is found in the RNA of the bdellovibrio. It is of interest that in both situations the amount of phosphorus incorporated into these cell fractions is much greater than that incorporated from an equivalent concentration of exogenous phosphate. This result strongly indicates that the nucleoside monophosphates are more permeable than orthophosphate. Apparently, after entry into the bdellovibrio the nucleoside monophosphates are not only used as such but are in part dephosphorylated and the phosphate formed intracellularly is used in de novo synthesis of phospholipids and ribonucleotides. This aspect of phosphorus metabolism may be of no physiological significance to the bdellovibrio in the natural environment and may result only from the rapid and excessive availability of nucleoside monophosphates under the artificial conditions of these experiments. We have previously shown (16) that during intraperiplasmic growth of *B. bacteriovorus* there is a carefully regulated breakdown of the DNA of the substrate organism, with precursor production paralleling synthesis. Under these conditions of limited supply, a dephosphorylation of nucleoside monophosphates after their entry into the bdellovibrio may not occur to any significant extent. We have no direct evidence on this point, however.

Although the uptake of α-glycerolphosphate (15) and hexose phosphates (9, 11, 22) by bacteria are well-documented phenomena, we know of no previous data indicating that bacterial cells are permeable to nucleoside 5'-monophosphates. To the contrary, the few reported investigations of this question (2, 4, 14) show quite conclusively that *E. coli* does not take up nucleoside monophosphates under typical growth conditions.

As yet we have no information on the mechanism of permeation of the nucleoside monophosphates. It would be particularly interesting to determine whether orthophosphate and nucleoside monophosphates use the same or different transport systems and the k_s (the half-saturating concentration) of the systems employed. From the data presented here, one

would predict a much lower k_s for the nucleoside monophosphates.

Regardless of the nature of the transport mechanism, the physiological significance of this ability is readily surmised. Each nucleoside monophosphate taken up and incorporated by the bdellovibrio represents a savings of at least one adenosine triphosphate in energy expenditure for growth as compared with the energy required to assimilate ribosides or earlier precursors of DNA. The conservation of phosphate ester bonds preexisting in the substrate organism by the bdellovibrio during intraperiplasmic growth may also encompass those of the phospholipids. We have shown elsewhere that *B. bacteriovorus* derives a significant quantity of its fatty acids directly from the substrate organism (13), and the data in this paper show that exogenous orthophosphate does not contribute greatly to the lipid phosphorus of the bdellovibrio growing intraperiplasmically. These findings suggest that the glycerol phosphate moiety of phospholipids is utilized as a unit by the bdellovibrio. We are now testing this possibility. If, indeed, the bdellovibrio is conserving available high-energy phosphate bonds from its substrate organism, then its energy expenditure for growth should be significantly less than that of the usual bacterium. Experiments related to this question are reported in a following paper (20).

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