Polychlorinated Biphenyls: Transfer from Microparticulates to Marine Phytoplankton and the Effects on Photosynthesis

Abstract. *Polychlorinated biphenyls (PCB's) initially associated with microparticulates are incorporated into marine diatom cells. The time course of transfer is rapid; equilibrium is attained within several hours. Assays with chlorophyll a fluorescence in vivo indicate that the transferred PCB's reach sites in the photosynthetic machinery that are sensitive to the effects of these compounds.*

Polychlorinated biphenyls (PCB's) are widespread contaminants of the marine environment (*1, 2*). They are virtually insoluble in seawater and, because of their hydrophobic nature, are readily removed from solution by adsorption to particulate matter (*3-5*). The extent of PCB adsorption to particles depends on the area, organic content, and nature of the adsorbent surface (*4, 6*). Phytoplankton accumulate substantial amounts of PCB's from seawater; this uptake is rapid and occurs at the very low concentrations (parts per trillion) that have been detected in seawater (*7-10*). The low concentration of PCB's in seawater is therefore related to both their low solubility and their high affinity for particulate matter.

The routes of PCB transport into the marine environment include the introduction of sewage and industrial effluents and atmospheric fallout of particles, rainwater, and runoff (*2, 11*). Regardless of the mode of introduction, most PCB's probably enter with, and remain associated with, particulate matter.

Several recent studies have focused on the effects of PCB's on the photosynthesis and cell division of marine phytoplankton (*12, 13*). The concentrations required to elicit detrimental effects on these processes are higher than those reported in seawater in the open ocean but similar to the concentrations measured in some coastal, estuarine, and freshwater systems (*8-10, 14*). Earlier investigators, however, have not considered the availability of particle-bound PCB's for uptake by phytoplankton. This potential source of PCB's may provide significant quantities of these compounds to algal cells. We report here the transfer of PCB's from microparticulates to marine phytoplankton, the effects of transferred PCB's on photosynthesis, and probable mechanisms for the transfer process.

The three types of particulate matter used in these studies were (i) Nuchar-Attaclay, (ii) sewage microparticulates (SM) collected from the Monterey, California, secondary treatment facility just prior to chlorination, and (iii) natural particulate matter (NPM) collected with cartridge-type filters from the seawater system of Hopkins Marine Station (*15*). Particles were resuspended in filtered seawater (0.45-μm Millipore and Whatman GFA filters) and gravity-filtered once through Nitex screens (80-μm pore size) and eight to ten times through 26-μm Nitex screens. The Coulter counter model T was used to determine the particle sizes present in these suspensions (*16*).

The phytoplankton species studied were *Guinardia flaccida* strain 58, *Lithodesmium undulatum* strain 25, *Lauderia borealis* strain 14, and *Ditylum brightwelli* strain 64, which were obtained from the algal culture collection of the Food Chain Research Group, Scripps Institution of Oceanography. We used these species of centric diatoms because of their large size and extreme sensitivity to PCB's in terms of photosynthesis and growth (*13*). The medium, incubation conditions, and other procedures used in algal culture have been described elsewhere (*13, 17*).

Freundlich adsorption isotherms (*18*) for the four phytoplankton species and three microparticulate types were determined with methods based on the use of 2,4,5,2′,5′-pentachlorobiphenyl (Mallinckrodt; specific activity = 9.87 mCi/mmole) (*7*). One establishes these isotherms by measuring the amounts of PCB per mass of adsorbent, that is, associated with phytoplankton or particulate matter, and the amounts remaining in seawater at equilibrium over a range of PCB concentrations introduced in solution. The use of Freundlich adsorption isotherms for the description of PCB uptake by various adsorbents has been reported (*4, 5*).

Figure 1A presents the results for *D. brightwelli*, *G. flaccida*, and NPM. The other diatoms and particle types had similar adsorption isotherms, with the exception of the SM; these data for SM did not fit the Freundlich adsorption isotherm, perhaps because of the inclusion of a nonsedimentable particle fraction in the SM. Freundlich constants for the phytoplankton species and microparticulates are given in Table 1. Figure 1B presents log-log plots of cell or particle dry weight (D) as a function of the equilibrium concentration of PCB's associated with the phytoplankton cells or particles (X/m). The relative affinities of the marine phytoplankton species and microparticulates for the PCB's in solution are similar and may be assessed from these plots and the regression equations (Table 1) for all algal species and particle types studied. In addition, concentration factors are also similar for the particles and diatom species (Table 1).

Table 1. Freundlich adsorption isotherm constants $\log K$ and $1/n$; regression equations for $\log D$ versus $\log X/m$ plots, where $\log D$ is the abscissa in micrograms of dry weight per liter and $\log X/m$ is the ordinate in micrograms of PCB per gram of dry weight; and concentration factors in units of micrograms of PCB per kilogram (dry weight) of adsorbent divided by the micrograms of PCB per liter. The standard errors are indicated; ND, not determined.

Phytoplankton or microparticulate	Freundlich constants		Regression equations for $\log D$ versus $\log X/m$	Concentration factors
	$\log K$	$1/n$		
		Phytoplankton		
Guinardia flaccida	−3.41 ± 2.45	2.45 ± 1.21	$y = 4.13(\pm 0.13) - 0.70(\pm 0.04)x$	$4.56 \pm 0.93 \times 10^5$
Lithodesmium undulatum	−2.29 ± 1.76	1.90 ± 0.91	$y = 3.29(\pm 0.24) - 0.56(\pm 0.07)x$	$3.55 \pm 0.96 \times 10^5$
Ditylum brightwelli	−0.19 ± 0.30	1.05 ± 0.19	$y = 4.03(\pm 0.13) - 0.65(\pm 0.03)x$	$8.65 \pm 1.07 \times 10^5$
Lauderia borealis	−0.71 ± 0.88	1.48 ± 0.58	$y = 4.14(\pm 0.15) - 0.69(\pm 0.04)x$	$1.42 \pm 0.39 \times 10^6$
		Microparticulates		
Nuchar-Attaclay	0.35 ± 0.36	0.91 ± 0.27	$y = 4.79(\pm 0.18) - 0.85(\pm 0.04)x$	$4.60 \pm 0.76 \times 10^5$
Sewage microparticulates	ND	ND	$y = 4.06(\pm 0.15) - 0.69(\pm 0.04)x$	$4.60 \pm 1.62 \times 10^5$
Natural particulate matter	−3.00 ± 1.24	2.13 ± 0.62	$y = 3.12(\pm 0.20) - 0.53(\pm 0.06)x$	$3.04 \pm 0.41 \times 10^5$

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We measured the transfer of ^{14}C-labeled PCB's from microparticulates to diatoms, using separation techniques similar to those of Malone (*19*). The adsorption of ^{14}C-labeled PCB's to particles was conducted in Corex centrifuge tubes. Labeled PCB was added in nanograde acetone (Matheson Coleman and Bell). After an initial 24-hour incubation period, the tubes were centrifuged at 18,000 rev/min (39,100*g*) for 20 minutes in a Sorvall RC2B. The supernatants were carefully removed and the pellets resuspended in filtered seawater. After gentle mixing with a Vortex, Jr., these samples were recentrifuged, the supernatants removed, and the pellets resuspended. These procedures were repeated until the activity in the supernatant was near background. Four to five washings were required. We mixed a 3.0-ml portion of the supernatant with 10 ml of Aquasol scintillation fluor (New England Nuclear) and determined the activity with a liquid scintillation spectrometer (Nuclear-Chicago Unilux II) using the channel ratio method of efficiency determination. The initial activity associated with the microparticulate pellet was similarly determined.

The time course of PCB transfer from microparticulates to phytoplankton was examined for the three types of particulate matter and the four algal species. Suspensions of labeled particles were prepared and brought to 12°C. Phytoplankton that had been collected on 26-μm Nitex screens and resuspended in sterile filtered seawater were added to the suspension of labeled particles (*20*). The experimental flasks were placed in a constant-temperature, shaking water bath. Samples were periodically withdrawn from the flasks, and the phytoplankton were separated from the micro-

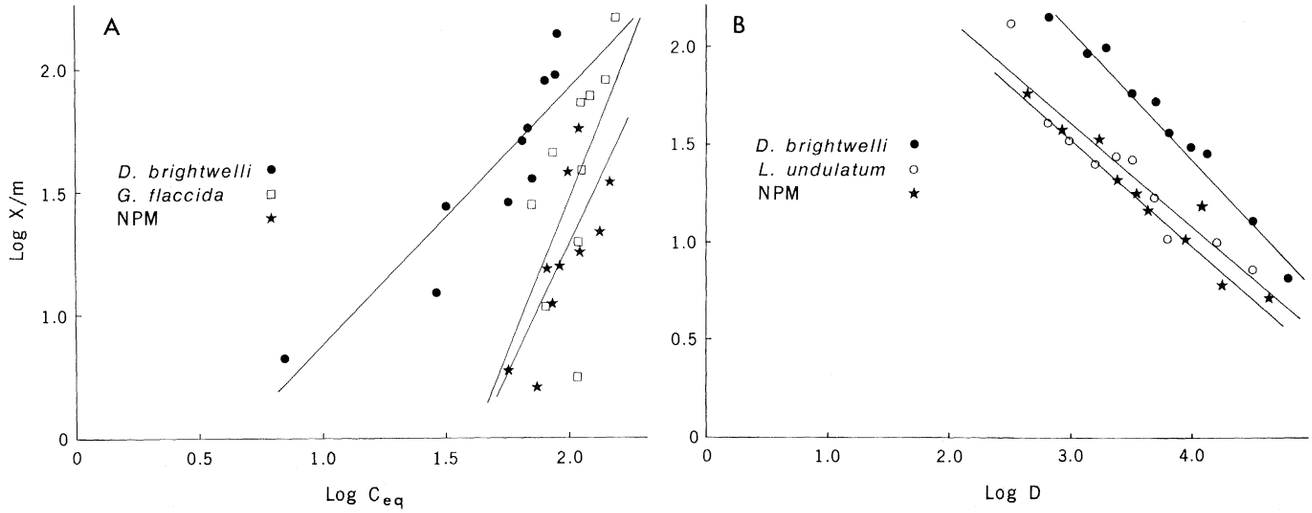

Fig. 1. (A) Freundlich adsorption isotherms (12°C) for *D. brightwelli, G. flaccida*, and natural particulate matter (*NPM*). The amounts of PCB associated with phytoplankton or natural particulate matter are plotted against the amounts remaining in seawater at equilibrium over a range of PCB concentrations introduced in solution; C_{eq} = equilibrium concentration of PCB's in seawater (nanograms of PCB per liter); X/m = equilibrium concentration of PCB's associated with phytoplankton or microparticulates (micrograms of PCB per gram of dry weight). These data were analyzed with least-squares linear regression; the coefficients and their standard errors are presented in Table 1. (B) Logarithmic plots of cell or particulate dry weight D (micrograms of dry weight per liter) versus X/m. These data were also analyzed with least-squares linear regression, and the coefficients are presented in Table 1.

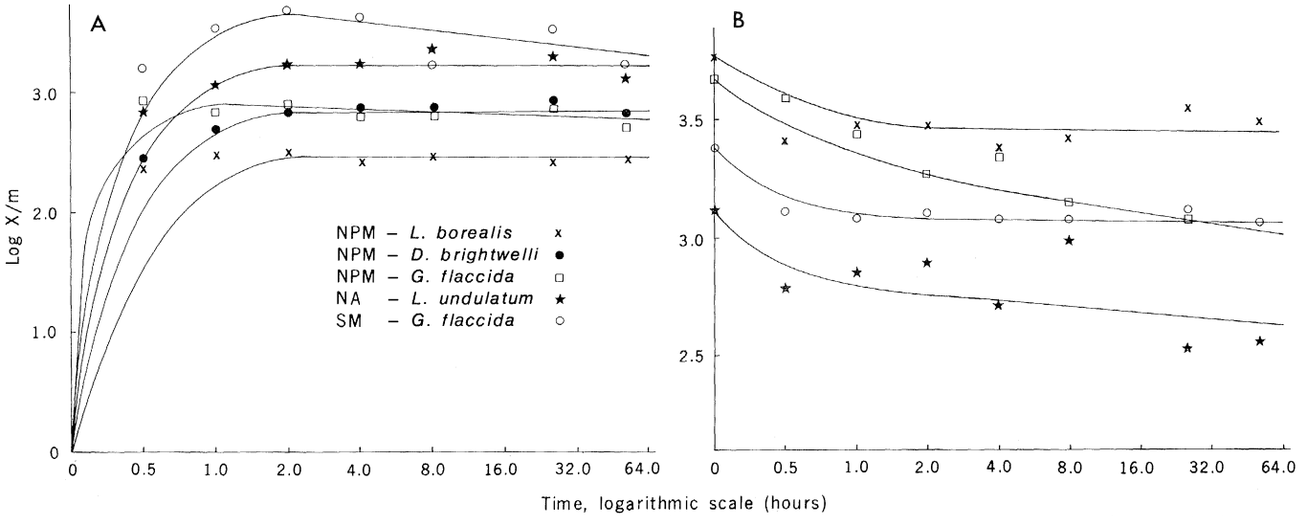

Fig. 2 (A) The time course of ^{14}C-labeled PCB transfer from microparticulates to phytoplankton. The amount of labeled PCB's associated with the algal fraction at the end of each time interval is represented by X/m (nanograms of PCB per gram of dry weight). (B) The time course of ^{14}C-labeled PCB loss from microparticulates. The amount of labeled PCB associated with the microparticulate pellet at the end of each time interval is represented by X/m.

particulates with 26-μm Nitex screens. The filtrate was collected in a Corex centrifuge tube and centrifuged as above. The Nitex screen, supernatant, and microparticulate pellet were all placed in scintillation vials with Aquasol, and their activities were determined. Phytoplankton activity was corrected for the amount of labeled PCB retained on the 26-μm Nitex screens in blanks. This correction amounted to approximately 1 to 2 percent of the total activity associated with the algae throughout the course of each experiment.

Figure 2, A and B, presents the results of these transfer studies. All four species of phytoplankton acquired significant amounts of ^{14}C-labeled PCB's from each microparticulate type. The time courses were similar among species and were also similar to the time courses of the PCB uptake from seawater reported elsewhere (*7*). Possible mechanisms for the transfer process include (i) loss of adsorbed PCB's from particles to solution and their subsequent uptake by phytoplankton, (ii) adsorption of the suspended microparticulates to the surface of phytoplankton cells, and (iii) the transfer of PCB's from particles to phytoplankton during a transient association. We examined microscopically numerous phytoplankton cells throughout the time course of the transfer and found no evidence of particles associated with cell surfaces. The data presented in Fig. 1 and Table 1 indicate that microparticulates and phytoplankton have similar affinities for PCB's. From these observations and experimental evidence, we conclude that either a transient association of particles with algae, or a loss of adsorbed PCB's to solution and their rapid uptake by phytoplankton cells, or both, is responsible for the transfer. Both processes may occur in the marine environment with the net result that PCB is transferred into algal cells from the particle-bound state.

The effects of PCB's on both in vivo chlorophyll a fluorescence and ^{14}C-bicarbonate assimilation may provide information on the movement of transferred PCB's into algal cells. These methods have been used in examining the effects of various toxic agents, including PCB's on photosynthesis (*12, 13, 21, 22*). Either reduction or enhancement of chlorophyll a fluorescence may reflect inhibition of photosynthesis, depending upon the site of action of the particular inhibitor (*22*). We assessed the impact or effects of PCB's transferred from microparticulates to phytoplankton cells by using these techniques. Suspensions of Nuchar-Attaclay and SM were prepared as described above with nonlabeled PCB (Aroclor 1254 in ethanol; Monsanto).

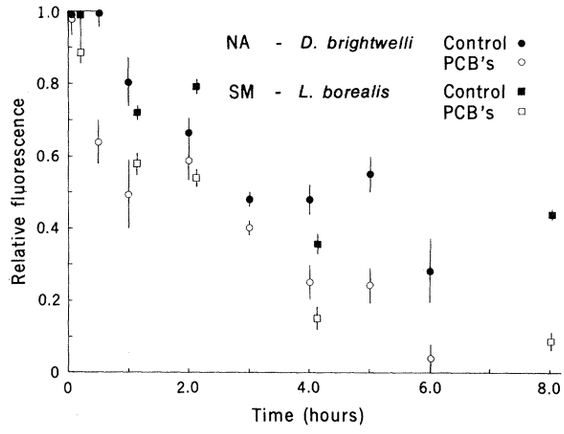

Fig. 3. In vivo chlorophyll a fluorescence assays for the effect of PCB's transferred from microparticulates to phytoplankton. Control and experimental chlorophyll a fluorescence values were significantly different for both *D. brightwelli* and *L. borealis* (Student's t-test, $P < .05$) with the exception of the 2-hour values for *D. brightwelli*. Each point represents the mean of fluorescence values for three or four separate cultures with standard errors indicated as vertical bars.

Algal cultures were incubated with these particle suspensions. Controls received equivalent amounts of particles without adsorbed PCB (*23*). Portions were withdrawn periodically for measurements of chlorophyll a fluorescence with a fluorometer (Turner model 111 equipped with Corning 5-60 and 2-64 excitation and emission filters, respectively, and modified with a red-sensitive R446 photomultiplier). Labeled bicarbonate (^{14}C, 3.0 μCi/100 ml) was added to each flask and to dark controls for the measurement of photosynthetic carbon assimilation. The methods used have been described elsewhere (*13*). Both *D. brightwelli* and *L. borealis* showed substantial inhibition of in vivo chlorophyll a fluorescence (Fig. 3). These results indicate an effective transfer of PCB's from microparticulates to algal cells. However, there were no significant differences (Student's t-test, $P > .05$) in ^{14}C-bicarbonate assimilation between control cultures and those receiving particle-bound PCB's at the concentrations of PCB transferred in these studies. It has been demonstrated that, at PCB concentrations that have been shown to inhibit phytoplankton photosynthesis and cell division (*12, 13*), in vivo chlorophyll a fluorescence is significantly reduced (*21*). That study indicated that PCB-induced reduction of chlorophyll a fluorescence provides a sensitive assay of the effects of these compounds on phytoplankton photosynthesis. Therefore, these results indicate that transferred PCB's reach sensitive sites in the photosynthetic apparatus and do not merely remain associated with the cell surface.

The transfer of PCB's from suspended microparticulates to phytoplankton cells provides another mechanism for the movement of these compounds into marine food webs, and one of paramount significance. Because of the low solubility of PCB's in seawater and the rapid uptake by particles, the amounts of PCB's actually present in solution represent equilibrium concentrations and not the quantities of PCB's available for uptake and transport. The ability of phytoplankton to acquire particle-bound PCB's indicates that the measurement of dissolved PCB concentrations alone provides little insight into the relationship between phytoplankton and these inhibitory compounds.

In addition, the residence time of PCB's in the euphotic zone and the availability of these compounds for uptake by marine organisms are limited because of the rapid removal of PCB's by adsorption to particles and subsequent sedimentation (*9, 24*). The rapidity with which phytoplankton cells can obtain PCB's from particles greatly decreases the significance of this removal process and demonstrates that particle-bound PCB's are of great biological importance. Coastal and estuarine areas represent the most productive of marine systems and also have higher concentrations of suspended particulate matter than the open ocean. This spatial coincidence of high particle loads with high phytoplankton production makes PCB transfer from particles to algae of particular significance in these systems.

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15. Nuchar-Attaclay (NA) is a mixture of finely divided charcoal and clay particles (attapulgite, Kensington Scientific). The natural particulate matter from Monterey Bay was collected with two cartridge filters connected in series to the Hopkins Marine Station seawater system (Culligan Gard Series, with Filterite Foto Flow cartridges).
16. The 15-channel Coulter counter model T was calibrated with Dow-Corning polystyrene beads of known diameters. Stock microparticulate suspensions were diluted 1:20 with filtered seawater, and their size spectra were determined. The results were as follows: NA, 97.1 percent < 5.49 μm in diameter, 99.9 percent < 10.98 μm; SM, 99.5 percent < 5.49 μm, 99.9 percent < 10.98 μm; NPM, 99.9 percent < 5.49 μm. The modal particle diameters were as follows: NA, 2.74 μm; SM, 2.18 μm; and NPM, 0.67 μm.

We determined the dry weights of microparticulates by filtering 100-ml portions of stock particle suspensions at low vacuum (125 mm-Hg) onto predried and tared Millipore filters. These filters were dried in a vacuum drying oven at 60°C to constant weight as determined with an analytical balance (Mettler H20T). Corrections were made for the dry weight of salt retained by the filters. The dry weights of the four diatom species were estimated from measurements of cell dimensions, calculation of cell volumes, and conversion to dry weight units according to data presented in T. R. Parsons, K. Stephens, and J. D. H. Strickland [*J. Fish. Res. Board Can.* **18**, 1001 (1961)] and R. R. Strathman [*Limnol. Oceanogr.* **12**, 411 (1967)]. Cell numbers were determined with an improved Neubauer hemacytometer or Coulter counter model T equipped with a 400-μm aperture.
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18. Freundlich adsorption isotherms describe the relationship between the concentration of solute, X or PCB, per unit of adsorbent, m, as phytoplankton or particle dry weight, and the equilibrium concentration of solute in the solvent seawater, C_{eq}, as

$$X/m = KC_{eq}^{1/n}$$

where K and $1/n$ are constants. The usual plot of log C_{eq} as a function of log X/m results in a linear relationship with log K as the y-intercept and $1/n$ as the slope. The empirical relationship described by the Freundlich adsorption isotherm is related to the multilayered nature of the adsorption mechanism.
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20. The range of diatom concentrations used in these transfer studies was approximately 5 to 40 μg of dry weight per milliliter. Microparticulate concentrations (in micrograms of dry weight per milliliter) were as follows: NA, 263; SM, 14.7; and NPM, 10.9.
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23. Approximately 76.2 μg of PCB per gram (dry weight) of particles was used. Since about 50 percent of the particle-bound PCB is transferred (see Fig. 2), the dose at equilibrium would be about 0.068 μg of PCB per microgram of phytoplankton carbon. This value is similar in magnitude to the 50 percent effect dose (ED_{50}) for photosynthetic inhibition when PCB is present in solution (*13*).
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25. We thank G. Baxter for technical assistance, J. Jordan for providing inocula of the algal cultures, S. Tucker for the use of the Coulter counter model T, and D. P. Abbott and R. D. Burnett for reviewing the manuscript. This work was supported in part by a graduate fellowship, Department of Biological Sciences, Stanford University, to L.W.H.
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Phanerozoic Peridotitic and Pyroxenitic Komatiites from Newfoundland

Abstract. *Peridotitic and highly magnesian pyroxenitic komatiites, thus far known to occur almost exclusively in the Archean (before 2.5 × 10^9 years ago) terranes, are reported from an Ordovician (0.5 × 10^9 years) ophiolite suite in Newfoundland. Their occurrence as pillow lavas or as chilled dikes, their possession of quench textures and geochemical parameters such as high contents of magnesium oxide, nickel, and chromium and low contents of titanium dioxide and potassium monoxide, low ratios of iron to iron plus magnesium, and values of the ratio of calcium oxide to aluminum oxide of close to unity demonstrate that they were formed through the rapid cooling of a highly mobile komatiitic melt. These features resemble those of many Archean peridotitic-pyroxenitic komatiites and indicate that the Archean-type magmatism did prevail in the younger segments of the earth's history although perhaps in a more erratic manner.*

I know of no report of definitive peridotitic and associated pyroxenitic komatiites (*1*) from Phanerozoic rocks. With the exception of two alleged Proterozoic occurrences in Manitoba and Quebec (*2, 3*), all such komatiites are confined to various shield areas of Archean age (*3–9*). This observation has led some scientists (*10*) to postulate that certain unique geotectonic conditions must have prevailed during the early part of the earth's history. I present here evidence for the existence of peridotitic and high-Mg pyroxenitic komatiites from an Ordovician ophiolite suite exposed at Betts Cove (55°47′46″W, 49°49′11″N) in the Newfoundland Appalachians and suggest that the komatiite-based argument for the geotectonic uniqueness of the Archean may no longer be tenable.

The komatiites reported herein occur as thin dikes and pillow lavas within the Betts Cove ophiolite suite, which consists of four well-developed members: ultramafite, gabbro, sheeted dike, and pillow lava. This suite has been described and interpreted as a remnant Appalachian oceanic crust-mantle sequence (*11*). The sheeted dikes consist of diabase and less commonly clinopyroxenite and peridotite. Field and petrochemical evidence shows that the dikes acted as feeders to the overlying pillow lavas. The ophiolite is known to be of Early Ordovician age (*12*). The entire suite has undergone greenschist metamorphism without any significant obliteration of igneous textures. The komatiites of Betts Cove represent a complete compositional spectrum ranging from peridotitic through pyroxenitic to basaltic varieties. The compositions of four peridotitic and three high-Mg pyroxenitic types are presented in Table 1 (columns 2 through 8); three selected Archean peridotitic komatiites are also listed for comparison (columns 9 through 11) (*13*). The peridotitic and pyroxenitic komatiites consist chiefly of clinopyroxene with variable amounts of serpentinized olivine. Chromite occurs in all specimens although the content varies. The five pillowed samples possess sparse globules consisting chiefly of high-Mg, devitrified glass with or without minor amounts of quartz and other silicic material; similar glass and silicic material also occur in the matrix of these pillow lavas (*14*). Crystalline plagioclase is extremely rare or absent in these samples.

All the Betts Cove komatiites discussed here contain subequant or bladed skeletal crystals of olivine or clinopyroxene, or both. The two most magnesian samples (samples 70S168 and 70S215) possess very sparse subequant skeletal

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