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Effects of Polychlorinated Biphenyls on Macromolecular Synthesis by a Heterotrophic Marine Bacterium†

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Growth rates and final cell yields of a polychlorinated biphenyl (PCB)-sensitive pseudomonad isolated from the open ocean were reduced in a dose-dependent manner by 10 to 100 μg of Aroclor 1254 per liter, a commercial mixture of PCB isomers added to its culture medium. Effects on growth rates were detected within 1 h (approximately one doubling time) of treatment. By 4 h posttreatment, the amounts of deoxyribonucleic acid and ribonucleic acid per cell in exponentially growing populations treated with sublethal doses of Aroclor were detectably lower than in appropriate controls. Corresponding cell protein values were slightly higher than in controls. Selective degradation of cell proteins or nucleic acids was not detected in cells whose growth was totally suppressed for 4 h by PCBs. Cells whose growth rate was inhibited 20 to 50% by Aroclor synthesized protein at normal rates for periods in excess of 5 h from the time the chlorinated hydrocarbons were added. In contrast, rates per cell of adenine uptake and adenine incorporation into deoxyribonucleic acid and total nucleic acids by the cells treated with PCBs were significantly lower than in control cells. Intracellular adenine pools of cells whose growth was inhibited to 20% of the control rate by PCBs were 30% smaller and appeared to require a longer interval to equilibrate than those of untreated cells. This may indicate impaired transport and/or efflux of this nucleic acid precursor through the membrane of affected cells. Inhibition of nucleic acid synthesis in this sensitive bacterium by PCBs could explain the observed inhibitory effects of the chlorinated hydrocarbons on its growth.

It was previously reported that Aroclor 1254, a mixture of isomers of polychlorinated biphenyls (PCBs), when added to cultures at a concentration of 10 to 100 μg/liter (ppb), inhibited growth of two marine heterotrophic bacterial isolates but did not impair, on a per-cell basis, their ability to take up and oxidize amino acids or carbohydrates tested (1). With 10 μg of Aroclor per liter in the medium, cells of one of these, a pseudomonad, had subnormal carotenoid levels and grew with altered, filamentous morphology. The results were consistent with the possibility that PCBs inhibited its growth by impairing biosynthesis.

This paper describes the effects of Aroclor 1254 on macromolecular composition and rates of protein and nucleic acid synthesis in this PCB-sensitive pseudomonad. The results indicate that growth inhibition resulting from exposure to Aroclor is accompanied by significant reduction in this organism's rate of synthesis of nucleic acids but not of proteins.

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MATERIALS AND METHODS

Organism and culture conditions. A pure culture of isolate 41, a pseudomonad obtained from the open ocean and used previously in studies of growth and respiration, was used. Methods of its culture, its respiratory activity, and its growth characteristics have been described (1). In preliminary measurements of cell chemical composition and rates of protein synthesis, the organism was cultured in dilute nutrient broth-salts (DNS) medium (1). In this medium without Aroclor it grew in chains of from 2 to 10 cells, and, with 10 to 50 μg of PCBs per liter present, it grew in long filaments. In seawater-Casamino Acids-yeast extract medium (SWCAA) (1), it grew as single, motile cells in the absence of PCBs and in short chains of cells when Aroclor was present at 10 to 100 μg/liter. SWCAA medium was developed in response to the need for accurate estimates of cell numbers in the work to be described.

A commercial mixture of PCB isomers, Aroclor 1254, was used throughout this study. Because of its extreme hydrophobicity, it was dissolved in methanol. Concentrations were adjusted such that methanol was not added to culture medium in excess of 0.01% (vol/vol), a level at which no effects on cell growth, morphology, or physiology could be detected. Aroclor 1254, methods of handling it, and methods of cleaning culture glassware have been described (1).

Estimates of cell numbers. Direct microscopic cell counts were made at the time of each assay and, when necessary, were corrected for cell chaining. Whenever possible, data for cell composition and synthetic rates were expressed on a per-cell basis. Growth of cultures was monitored by measuring optical density changes at 540 nm.

Chemical analyses. For chemical analyses, a single culture (400 ml) was grown to late exponential phase, and equal portions (50 or 100 ml) were transferred to each of three flasks containing 500 ml of fresh medium (see Fig. 1). The subcultures were incubated for 30 to 45 min, and Aroclor was added to one. A comparable volume of methanol was added to the second subculture, and the third served as an untreated control. Each subculture was incubated for an additional 3 to 5 h, and the cells were harvested by centrifugation and washed, using procedures already described (1). Cells were analyzed for their chemical composition immediately after harvesting.

Nucleic acids were extracted from cells by the modified Schneider method described by Herbert et al. (5). A 2-ml sample of washed-cell suspension (ca. 1.4×10^{10} cells per ml) was combined with an equal volume of cold 0.25 N $HClO_4$ and incubated for 15 min with vigorous intermittent mixing. This mixture was centrifuged at $5,000 \times g$ for 10 min at 4°C. The supernatant fluid was decanted, and the sediment was washed by resuspending it in 2 ml of cold 0.25 N $HClO_4$. The suspended-cell material was extracted and centrifuged as before. The supernatant fluids contained no detectable protein or DNA and were discarded. A very small amount of material present in the supernatant fraction reacted with orcinol and was presumed to consist of pentoses unrelated to ribonucleic acid (RNA). The sediment from the cold perchloric acid extraction was suspended in 1 ml of 0.5 N $HClO_4$, and the reaction mixture was incubated for 30 min at 70°C with intermittent vigorous shaking. The mixtures were cooled and centrifuged at $5,000 \times g$ for 10 min. The supernatant fluid was carefully decanted and saved. The pellet fraction was again extracted with 1 ml of 0.5 N $HClO_4$ at 70°C for 30 min and centrifuged as before. The supernatant fluids from the two extractions were combined and stored on ice for immediate analysis of RNA with orcinol (5), using RNA as the standard. The deoxyribonucleic acid (DNA) content of extracts was also immediately determined, using the diphenylamine assay of Burton (2) with calf thymus DNA as the standard. The pellet fraction was suspended in 0.25 N $HClO_4$, adjusted to a known volume, and analyzed for cell protein by the method of Lowry et al. (7), using crystalline bovine serum albumin as the standard. In a preliminary experiment, no RNA or DNA was detected in the supernatant fluid from a third 30-min extraction of the cell pellet fraction with 1 ml of 0.5 N $HClO_4$ at 70°C.

Rates of protein synthesis. Rates of protein synthesis were determined by measuring incorporation of L-[U-^{14}C]leucine or a mixture of 15 ^{14}C-uniformly labeled L-amino acids into cell material precipitated by 10% (wt/vol) trichloroacetic acid.

L-[U-^{14}C]leucine (5 μmol/ml; 80 nCi/μmol) in basal salts was used to measure the rate of synthesis by growing cells. The basal salts mixture consisted of (grams per liter of distilled water): NaCl, 29; $MgCl_2$, 12.1; KCl, 1.5. Appropriate volumes of [^{14}C]leucine (up to 200 μl) and basal salts were added to 1.5-ml fractions of cells growing exponentially in DNS medium to give a final reaction volume of 2 ml. Assays were performed in triplicate for intervals of 60 min.

The mixture of ^{14}C-labeled L-amino acids was used to measure the rate of protein synthesis by cells growing exponentially in SWCAA medium and by concentrated-cell suspensions. It consisted of 0.1 ml of a commercial mixture (New England Nuclear, Boston, Mass., NEC-445 L-amino acids mixture, 0.1 mCi/ml) in 0.8 ml of distilled water to which 0.1 ml of 1% (wt/vol) Casamino Acids (Difco) was added. This stock solution contained approximately 1 μg of total amino acids per ml and 2×10^5 cpm/μl. Each reaction mixture received 5 or 10 μl of this or a 1/10 dilution of this stock. Reaction mixtures each consisted of a 0.2-ml fraction of concentrated-cell suspension or of a 1- to 2-ml fraction of exponentially growing culture.

Each reaction mixture was contained in a test tube (12 by 75 mm). Radiolabeled compounds were added to start reactions, and the vigorously mixed samples were incubated at the approximate temperature of growth. Appropriate reaction times (usually about 9 min) were determined from time-course experiments conducted at saturating-substrate concentrations. Reactions were stopped, and cell proteins were precipitated by the addition of 2 ml of 10% trichloroacetic acid to each tube after a precisely timed interval. One hour later, each sample was filtered through a 0.3-μm-diameter (pore size) membrane filter and rinsed twice with 2 to 5 ml of basal salts. The membrane filters were dried at 60°C, and their radioactivity was determined as described below. Corrections were made for nonspecific binding of radiolabeled amino acids to the filters.

Rates of nucleic acid synthesis. Thymine was incorporated into cells of strain 41 at very low rates compared with their growth rate. To circumvent this problem, a method also used by others (3) was employed. Incorporation of [^{14}C]adenine into total nucleic acids was measured, and the extent of incorporation into DNA versus RNA was differentiated by alkali treatment of cells that had incorporated this precursor (see below).

To measure rates of adenine incorporation into cell macromolecules, each portion of exponentially growing cells, or concentrated-cell suspension, was incubated in a glass tube (12 by 75 mm) at the growth temperature. Uptake was initiated by adding [8-^{14}C]-adenine to each sample. To measure incorporation into growing cells, a 2-ml sample of culture in exponential growth was incubated with 5 μl (5.1×10^3 cpm) of [8-^{14}C]adenine (specific activity, 4.6×10^4 cpm/nmol; no carrier added). Rates of [^{14}C]adenine uptake by growing cells were constant between 1 and 12 min under these conditions. Adenine incorporation by cell suspensions was studied, using reaction mixtures each consisting of: filter-sterilized, aged Sargasso Sea seawater, 1 ml; cell suspension, 0.2 ml; [8-^{14}C]-adenine (specific activity, 2.3×10^3 cpm/nmol), 5 to 100 nmol/ml in a total reaction mixture volume of 1.21 ml. Mixtures were agitated vigorously during the assay, and the uptake was stopped after not more than

10 min by forceful injection of one of the reagents described below.

To determine [^{14}C]adenine incorporation into total nucleic acids (RNA + DNA), each reaction was stopped with 2 ml of cold 0.5 N $HClO_4$. Tubes containing the mixtures were stored for 30 min to 1 h in an ice bath, and the contents of each were filtered through a 0.3- or 0.45-μm membrane filter and rinsed twice with 2 to 5 ml of cold 0.25 N $HClO_4$. The filters were dried at 60°C, and their radioactivities were determined (see below).

To determine [^{14}C]adenine incorporation into DNA, the reactions described above were stopped by the addition of 0.2 ml of 5.5 N NaOH to each tube. The tubes were then incubated for 18 h at 37°C to allow RNA to be degraded to mononucleotides. The reaction mixtures were then neutralized by the addition of 0.2 ml of 6 N HCl to each tube in an ice bath. DNA was then precipitated by the addition of 2.5 ml of cold 10% trichloroacetic acid and 0.05 ml of a 0.2% (wt/vol) solution of bovine serum albumin. After 1 h at 4°C, the contents of each tube were filtered on a membrane filter and rinsed twice with 4 ml of cold 5% trichloroacetic acid. Filters were dried and their radioactivity determined as described below.

To determine total uptake rates of [^{14}C]adenine into cells, 2 ml of exponentially growing cultures was incubated with radiolabeled adenine for a precisely measured interval of from 1 to 10 min. Uptake was stopped by immediately filtering each fraction of culture on a 0.45-μm membrane filter. Filters were immediately washed with 5 ml of filter-sterilized, 75% (vol/vol) glass-fiber-filtered, aged seawater in distilled water and quickly immersed in 10 ml of liquid scintillation cocktail (Aquasol) to stop further activity of the cells. Filtration of each sample was accomplished in less than 60 s. Radioactivity on filters was then measured.

Radioactivity in internal cellular pools of adenine and, presumably, of adenine nucleotides and nucleosides was determined by subtracting the measured values of incorporated adenine from values for total adenine uptake.

Radioactivity measurements. Radioactivities of membrane filters and of labeling mixtures were determined by liquid scintillation spectrometry. Each sample or filter was added to 10 ml of Aquasol liquid scintillation cocktail mixture in a glass vial. Radioactivity was measured to within an error of 1.5%, using a Beckman LS-100 C liquid scintillation spectrometer. Counting efficiencies were never less than 86%.

Chemicals. Trichloroacetic acid, perchloric acid, and Nanograde methanol were obtained from Mallinckrodt Chemical Works (St. Louis, Mo.). Type IV RNA was from Sigma Chemical Co. (St. Louis, Mo.). Calf thymus DNA was from Calbiochem (San Diego, Calif). Orcinol and diphenylamine were from Fisher Scientific Co. (Fairlawn, N.J.). Bovine serum albumin was from K & K Laboratories (Plainview, N.Y.). All radiochemicals and Aquasol liquid scintillation cocktail mixture were from New England Nuclear (Boston, Mass.). Cellulose ester membrane filters (type HA or PF) were from Millipore Corp. (Bedford, Mass.). Aroclor 1254 (lot no. AA-1), a commercial mixture of PCBs, was obtained from Monsanto Chemical Co. (St. Louis, Mo.).

RESULTS

Cell chemical composition. Preliminary experiments were conducted with cells grown in DNS medium. The total cell fraction from a culture treated with 10 μg of Aroclor per liter was harvested in late exponential growth. It contained 66% of the cell protein, 55% of the RNA, and 43% of the DNA present in the total cell material harvested from an identical volume of untreated or methanol-treated control culture (data not shown). These results provided preliminary evidence for alterations in the macromolecular composition of cells growing suboptimally with PCBs in the medium.

Growth of cells in DNS was completely arrested by 100 μg of Aroclor per liter in the medium. By 4 h posttreatment, RNA/protein, DNA/protein, and DNA/RNA ratios were similar in the total cell fraction from an untreated control culture and one in which growth was completely inhibited. Furthermore, cells whose growth was stopped by PCBs grew at normal rates without a lag when transferred to PCB-free medium (1). These findings indicated that Aroclor-treated cells did not selectively degrade their nucleic acids or proteins during the period their growth was arrested.

Although somewhat less sensitive to Aroclor when growing in SWCAA medium, cells did not form filaments and could be counted microscopically. With 100 μg of Aroclor per liter added to this medium when cells were in exponential growth, the cells' rate of growth decreased to approximately one-half that of control cells within 1 h (Fig. 1). Table 1 shows the macromolecular composition of PCB-treated and control cells harvested during exponential growth in SWCAA medium. By 4 h posttreatment, PCB-treated cells contained 68% of the DNA, 60% of the RNA, and 113% of the protein found in control cells grown with methanol in the medium. These results suggested a specific inhibition of nucleic acid synthesis and stimulation of protein synthesis by Aroclor 1254. To further clarify these possible effects on the cell, measurements were made of rates of protein and nucleic acid synthesis by cells under various conditions of growth.

Rates of protein synthesis. Exponentially growing cells, with or without Aroclor in the medium, incorporated L-[^{14}C]leucine into protein at constant rates of 440 pmol/μg of cell protein per h for intervals in excess of 80 min under the assay conditions used (Fig. 2). The total amount incorporated per hour by the population exposed to Aroclor was less than that of controls. However, when corrected for the amount of cell material (cell protein) used in

each assay mixture, no significant differences were observed in the rates of L-[^{14}C]leucine incorporation by cells of either population (Fig. 2). Rates of L-[^{14}C]leucine incorporation by cells growing suboptimally with PCBs present or at optimal rates without it were similarly saturated at substrate concentrations of about 0.15 mM. The apparent K_m for the reaction, which appeared similar for cells from either population, was 7.5 mM (Fig. 2, inset). L-[^{14}C]leucine incorporation by cells of either population was inhibited by concentrations of leucine in the medium higher than 0.2 mM (Fig. 2), in a manner typical of substrate inhibition (4). Inhibition by excess substrate was nearly complete at 0.66 mM leucine. Radioactivity in $^{14}CO_2$ recovered from cells supplied with L-[U-^{14}C]leucine over the range of substrate concentrations 0.03 to 0.66 mM was measured. The same pattern of inhibition was observed for rates of leucine catabolism as for leucine incorporation. Thus, some aspect of cell physiology common to both the catabolic and anabolic fates of leucine, probably its transport into the cell, was inhibited at high substrate concentrations. The inhibition was not related to an effect of PCBs on the cell. Inhibition of leucine transport in cells of other gram-negative organisms at high external leucine concentrations has been reported (8).

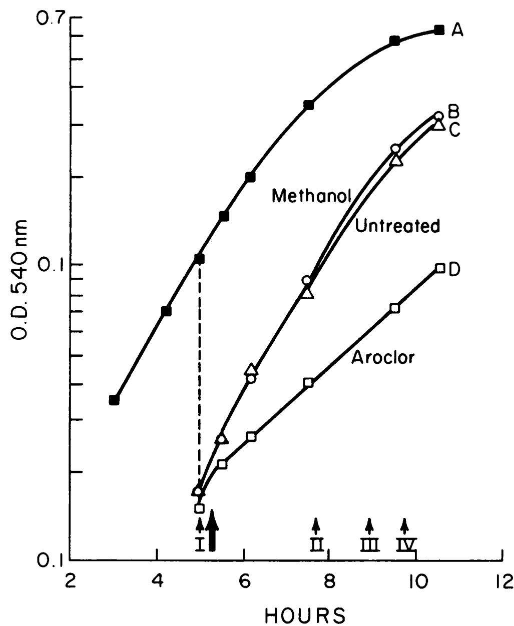

FIG. 1. *Effect of Aroclor 1254 on growth of strain 41 in SWCAA medium. Three 50-ml portions of an exponentially growing culture (A) were diluted into 500 ml of fresh medium (dashed line) and reincubated. At the time indicated by the heavy vertical arrow, 100 μg of Aroclor per liter was added to one subculture (D) and 0.01% (vol/vol) methanol to another (B). The third (C) served as an untreated control. Roman numerals indicate times at which 2-ml fractions of each culture were assayed for rates of synthesis of protein and nucleic acids; these values are presented in Table 2.*

Rates of incorporation of a mixture of ^{14}C-labeled L-amino acids by concentrated-cell suspensions were measured. The organism was cultured in SWCAA medium to obtain information on cell numbers. Suspensions were prepared from each of three replicate cultures in exponential growth 2 h after treating one with Aroclor 1254 (100 μg/liter) and another with methanol. The third was an untreated control (see, for instance, Fig. 1). The rate of growth of the culture treated with PCBs was one-half that of the control populations at the time cells were harvested, although each was in exponential growth. Rates of protein synthesis and rates of nucleic acid synthesis (discussed below) by cells in each population were determined immediately after harvesting the cells. Measured rates of amino acid incorporation achieved, within 3 min, a constant value (8.3×10^{-7} cpm/cell per min) for periods in excess of 10 min at nonlimiting substrate concentrations (Fig. 3I). No significant differences were detected in the rates of amino acid incorporation per cell by samples from each population (Fig. 3I, curves A, B, C).

As mentioned, chemical analyses had indicated possible stimulatory effects of PCBs on protein synthesis. Moreover, the effects of PCBs on growth and pigmentation in this bacterium were reversed by diluting cells into fresh me-

TABLE 1. *Effect of PCBs on chemical composition of strain 41*[a]

Treatment	Final cell yield (cell/ml)	Amount detected (μg/cell)			Ratio × 10² of:	
		Protein	RNA	DNA	RNA/protein	DNA/protein
None	1.62×10^8	9.50×10^{-8}	2.90×10^{-8}	5.24×10^{-9}	30.5	5.5
Methanol	1.66×10^8	9.44×10^{-8}	3.60×10^{-8}	5.57×10^{-9}	38.1	5.9
PCBs (100 μg/liter)	6.40×10^7	10.90×10^{-8}	2.15×10^{-8}	3.79×10^{-9}	19.7	3.5

[a] Treatments shown were made 4 h before cell harvest. All cultures were harvested in exponential growth in SWCAA medium.

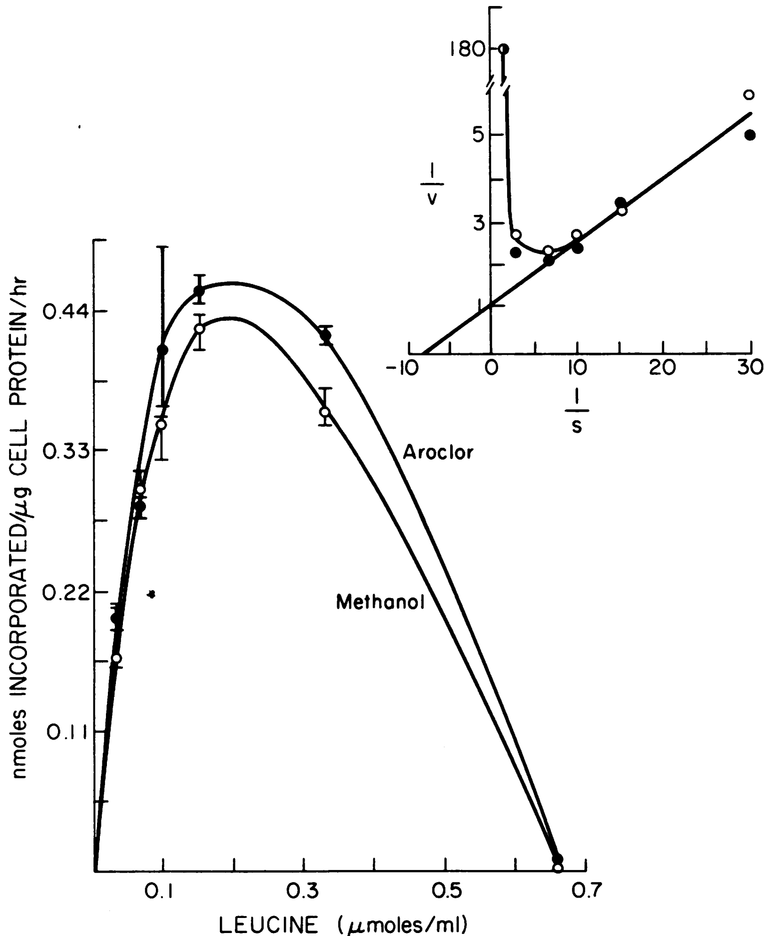

FIG. 2. *Effects of PCBs on the rate of protein synthesis by growing cells in DNS medium.* (*I*) *and* (●) *indicate the ranges and means, respectively, of measured rates by cells grown in the presence of 100 µg of Aroclor per liter.* (*I*) *and* (○) *are for rates by cells in the absence of PCBs. Inset shows a Lineweaver-Burke transformation of the mean data points.*

dium (1). Despite the fact that cells were not washed, merely concentrated, for studies of macromolecular synthesis, the possibility remained that effects of PCBs may have been ameliorated by harvesting cells from growth medium containing the chlorinated hydrocarbons. To avoid this possible problem, direct measurements were made of rates of protein (and nucleic acid) synthesis by growing populations over the 5-h interval they were in exponential growth.

Fractions of an exponentially growing culture in SWCAA medium (Fig. 1A) were diluted 10-fold into each of three flasks containing 500 ml of fresh medium. One resulting subculture was treated with 100 µg of Aroclor per liter (Fig. 1D) and another (Fig. 1B) with methanol. The third served as an untreated control (Fig. 1C). At intervals, rates of protein and nucleic acid synthesis (see below) were determined, using small fractions from each culture. The results shown in Table 2 indicated no significant differences in the rate of amino acid incorporation per cell in samples from control or treated populations. The measured rates of protein synthesis by cells in the PCB-treated population were approximately the same per cell after adding the chlorinated hydrocarbons as they had been before it was added (Table 2). Cells from each population incorporated radioactivity in amino acids into protein at constant rates of approximately 3.0 $\times 10^{-7}$ cpm/cell per min for over 3 h after the PCBs or methanol were added, indicating that they were in balanced growth.

Thus, although cells grown with growth-inhibiting concentrations of Aroclor appeared to have somewhat higher amounts of protein than

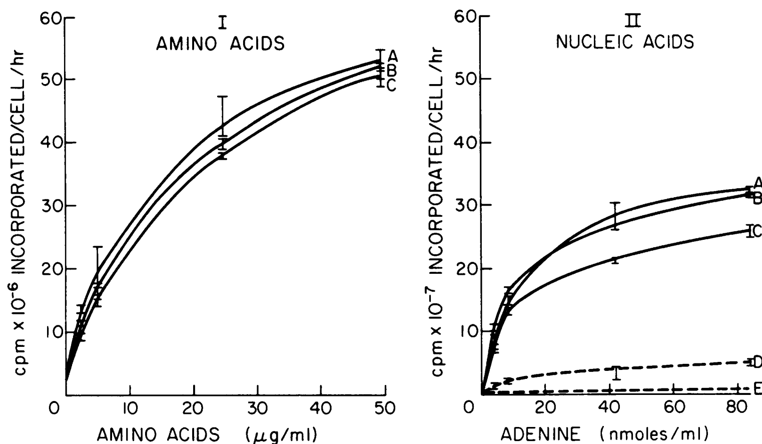

FIG. 3. *Effects of PCBs on rates of amino acid (I) and nucleic acid (II) synthesis by cell suspensions. Cells were treated 2 h prior to assay with PCBs or methanol as described by Fig. 1. Assay intervals (minutes): I, 5.5; II, 7. In I, A = PCB treated; B = untreated control; C = methanol control. In II, A and D = untreated control; B = methanol control; C and E = PCB treated. In II, A through C are for total nucleic acids, and D and E are for DNA.*

TABLE 2. *Effects of PCBs on rates of macromolecular synthesis by growing cells*[a]

Substance	Culture treatment	Rate (cpm incorporated/cell per min) assayed at interval:[b]			
		I	II	III	IV
Protein	None	$3.6 \pm 0.0 \times 10^{-7}$	$2.7 \pm 0.2 \times 10^{-7}$	$2.9 \pm 0.3 \times 10^{-7}$	$2.7 \pm 0.0 \times 10^{-7}$
	Methanol	$3.1 \pm 0.0 \times 10^{-7}$	$3.2 \pm 0.2 \times 10^{-7}$	$2.7 \pm 0.0 \times 10^{-7}$	$2.6 \pm 0.1 \times 10^{-7}$
	PCBs (100 μg/liter)	$2.7 \pm 1.0 \times 10^{-7}$	$2.6 \pm 0.2 \times 10^{-7}$	$2.5 \pm 0.1 \times 10^{-7}$	$2.4 \pm 0.3 \times 10^{-7}$
Total nucleic acids	None	$2.5 \pm 0.2 \times 10^{-6}$	$2.9 \pm 0.2 \times 10^{-6}$	$3.6 \pm 0.4 \times 10^{-6}$	$2.8 \pm 0.5 \times 10^{-6}$
	Methanol	$2.5 \pm 0.1 \times 10^{-6}$	$3.0 \pm 0.2 \times 10^{-6}$	$3.9 \pm 0.5 \times 10^{-6}$	$3.6 \pm 0.1 \times 10^{-6}$
	PCBs (100 μg/liter)	$2.5 \pm 0.0 \times 10^{-6}$	$8.8 \pm 0.5 \times 10^{-7}$	$8.5 \pm 0.4 \times 10^{-7}$	$6.0 \pm 0.0 \times 10^{-7}$
DNA	None	$7.6 \pm 0.4 \times 10^{-8}$	$7.2 \pm 0.9 \times 10^{-8}$	$8.0 \pm 0.8 \times 10^{-8}$	
	Methanol	$2.6 \pm 0.7 \times 10^{-8}$	$7.7 \pm 0.1 \times 10^{-8}$	$9.4 \pm 0.4 \times 10^{-8}$	
	PCBs (100 μg/liter)	$1.7 \pm 0.5 \times 10^{-8}$	$1.7 \pm 1.4 \times 10^{-9}$	$1.9 \pm 0.0 \times 10^{-9}$	

[a] Values are means plus or minus the ranges obtained in duplicate assays with 2-ml fractions of cultures in SWCAA medium. Growth of cultures is indicated in Fig. 1. Assay intervals (minutes): proteins, 9; nucleic acids, 10.

[b] Interval numerals correspond to those indicated in Fig. 1.

controls, no evidence was obtained for stimulated rates of protein synthesis by such cells under similar conditions of growth. More importantly, cells whose growth was partially inhibited by PCBs continued to synthesize proteins at undiminished rates for at least 3 h posttreatment.

Rates of nucleic acid synthesis. Figure 3 also shows the measured rates of synthesis of total nucleic acids (DNA + RNA) and DNA by concentrated-cell suspensions. In contrast to the results of amino acid incorporation, the rate of total nucleic acid synthesis measured at nonlimiting adenine concentrations was 20% lower in PCB-treated cells (Fig. 3II, curve C) compared with controls (Fig. 3II, curves A and B), and the rate of DNA synthesis by PCB-treated cells (Fig. 3II, curve E) was reduced by more than 90% (Fig. 3II, curve D), to an undetectable level. Furthermore, by 2.5 h after adding sufficient PCBs to inhibit growth of this organism to 20% of the control value, adenine incorporation rates had dropped to 20 to 30% of the control values and of the rate measured before adding the chlorinated hydrocarbons (Table 2). Rates of protein synthesis remained within 14% of the control value and the initial value over the same interval (Table 2). The inhibition of nucleic acid synthesis in growing cells persisted for over 5 h, at which time cells in control populations began to enter stationary growth as evidenced by changes in optical density (Fig. 1B and C), and the experiment was terminated.

Table 2 also shows the measured rates of DNA synthesis by portions of exponentially growing cultures with and without PCBs. The

values are undoubtedly maximal since several of the measurements of PCB-treated cultures were based upon recovery of counts which were low (5 to 10 cpm) after correction for nonspecific binding of adenine to filters (generally about 100 cpm). Nevertheless, the results indicated that DNA synthesis was inhibited to about 2% of that of the control rates by 2.5 h after PCBs were added (Table 2, interval II). Inhibition persisted for at least 3.8 h after the PCBs were added, after which rate measurements of DNA synthesis were not made.

The results indicated that PCBs inhibited cell nucleic acid synthesis very shortly after they were added to exponentially growing cells. To determine whether this inhibition was on transport of adenine into cells or was specifically involved with nucleic acid biosynthesis, the rate of [^{14}C]adenine uptake was compared with its rate of incorporation into total nucleic acids. Measurements were made using exponentially growing cells in a manner similar to that described in Fig. 1. The Aroclor added inhibited the growth rate to 20% of that of the control (methanol) culture within 1 h. Cells were harvested in exponential growth, 3 h after adding PCBs or methanol. They were concentrated but not washed and were used immediately. Measured rates of [^{14}C]adenine incorporation were constant and identical to [^{14}C]adenine uptake rates after 1 min in PCB-treated cells and after 5 min in control cells. Differences in establishing constant rates may reflect differences in the rate at which cells equilibrated intracellular pools of adenine. The rates observed with PCB-treated cells (6.5×10^{-11} nmol/cell per min) were only 25% of those of control cells (2.6×10^{-10} nmol/cell per min) (Fig. 4). The initial rate at which adenine accumulated in intracellular pools was the same, or faster, in PCB-treated cells compared with controls. This fast initial rate rapidly slowed after 1 min in PCB-treated cells, resulting in approximately 30% lower pool sizes in these as compared with controls after 3 min (Fig. 4).

Intracellular adenine pools in cells exposed to PCBs had apparently not equilibrated until after 10 min, the longest interval studied. The rate of adenine accumulation in intracellular pools by PCB-treated cells appeared to increase at a slow, constant rate of about 56 pmol/min after 1 min. It is not known if intracellular pool levels in cells from each population achieved similar steady-state values after 10 min. The data suggest, however, that inhibition by PCBs of nucleic acid synthesis may have resulted, at least in part, from inhibition of adenine transport into the cell and/or its accumulation in intracellular pools.

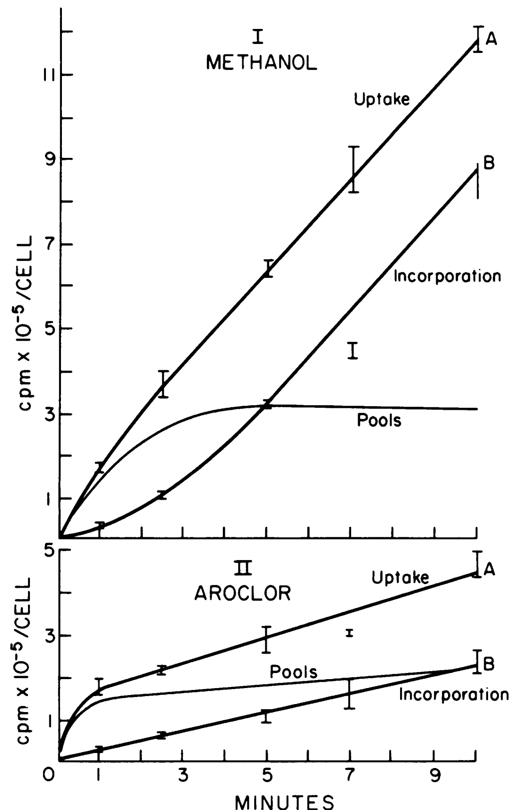

FIG. 4. *Rates of adenine uptake (A) and adenine incorporation (B) by exponentially growing cells pretreated as in Fig. 1 with methanol (I) or Aroclor (II). Data bars indicate the range of values obtained in duplicate assays. Curves lacking data points depict calculated intracellular pools of adenine. Cells per assay (cells per ml × 10^8): I, 3.32; II, 1.28.*

DISCUSSION

With 10 to 100 μg of Aroclor per liter added to their culture medium during the period of exponential growth, cells of the pseudomonad studied grew at lower rates and to lower final cell yields than those in control populations. Although unable to divide at normal rates with PCBs present, cells continued to respire (1) and to synthesize proteins at undiminished rates for at least 5 h (two doubling times) from the time PCBs were added. Moreover, the oxidizable substrates or amino acid precursors used were supplied exogenously to intact cells. Thus, any effect of the chlorinated hydrocarbons on transport of these into the cell was not sufficient to cause rate limitations on respiration or protein synthesis.

In contrast, both the synthetic rate and total amount of nucleic acids per cell were signifi-

cantly reduced in cells inhibited in their growth by PCBs. By 3 to 4 h posttreatment, DNA synthesis proceeded at only 2% of the control rate in PCB-treated cells whose growth was inhibited to 50% of the control rate.

Similar intracellular adenine pools and a longer interval required to equilibrate them in cells exposed to PCBs suggest impaired adenine transport and/or its leakage back to the external environment. The observed decrease in pool size was 30% of that of control cells. It seems unlikely that this would result in the 98% decrease in the rate of DNA synthesis measured under similar conditions. Thus, the effect of PCBs on nucleic acid synthesis may involve complex regulatory processes associated with transport and accumulation of adenine and perhaps other required precursors. Sensitivity of various bacteria to PCBs might reflect differences in rates at which they accumulate precursors of nucleic acids. Less sensitive bacteria may be more efficient at compensating for impaired uptake of these performed exogenous nutrients, e.g., via de novo synthesis. Alternatively, PCBs may interfere specifically with enzymic synthesis of nucleic acids from purine, pyrimidine, or nucleotide precursors. It is also possible that these chlorinated hydrocarbons cause alterations in the cell membrane that could then result in secondary effects, including those involved with DNA synthesis at membrane attachment sites. Since DNA synthesis is closely coupled to cell division in other gram-negative bacteria (3, 6), the observed inhibition could explain the inhibitory effects of PCBs on cell growth.

Known inhibitors of nucleic acid synthesis in other bacteria, including hydroxyurea, mitomycin C, and nalidixic acid (3, 6), are normally applied in concentrations many times higher (microgram to milligram per milliliter) than those of the Aroclor used in this study. The hydrophobic nature of the chlorinated hydrocarbons presumably enhances their toxic properties by promoting efficient binding to cells.

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