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Effects of Pesticides on the Fatty Acid and Phospholipid Composition of *Escherichia coli*

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Cells of *Escherichia coli* contained an altered phospholipid and fatty acid composition when grown in the presence of some pesticides. Whereas parathion increased the concentration of all phospholipid species without changes in their polar head groups, DDT (dichlorodiphenyltrichloroethane) decreased the proportion of neutral serine-derived phosphatides and dieldrin decreased the proportion of negatively charged phospholipids. The saturated/unsaturated plus cyclopropane fatty acid ratio was increased in all cases. The changes suggested that cells adapted their membrane lipids to compensate for the presence of pesticides in the environment.

Persistence of pesticide residues in the environment is particularly marked. Parathion is known to persist in soil surface for long periods of time and to be degraded later (26, 27). Considerable amounts of DDT (dichlorodiphenyltrichloroethane) have been detected in soil even 8 to 10 years after treatment (11). The epoxidation of aldrin to dieldrin was one of the earliest pesticide conversions described (22). Dieldrin is extremely inert to biological and chemical degradation (25). Pesticide residues can interfere with the growth and activity of microorganisms which are largely responsible for continued soil fertility (3, 6, 7, 12, 16, 28, 29). Although the precise mechanisms of action are unknown, the available data are consistent with the idea that most of the compounds interact, presumably with the microbial membrane (2). Bacterial cells adapt their membrane lipids to compensate for the presence of lipophilic compounds in the environment (14, 15). Phospholipids constitute 15 to 30% of the dry weight of bacterial membranes (21).

As an initial step in evaluating the effects of some pesticides on the lipid composition of microorganisms, our study reports the alterations in major phospholipids and fatty acids from *Escherichia coli*.

MATERIALS AND METHODS

Bacteria and growth conditions. *E. coli* 424 CCM-29 WCDF was kindly provided by the Microbial Culture Collection of Buenos Aires University. The cells were grown at 37°C in a defined medium dispensed in conical flasks shaken at a constant rate. The medium contained, in grams per liter of double-distilled water: Na_2HPO_4, 6.0; KH_2PO_4, 3.0; $MgSO_4$, 0.2; NH_4Cl, 1.0; glucose, 4.0; pH adjusted to 6.8. Stock solutions of pesticides were prepared in absolute ethanol and diluted 1,000-fold by volume in the basal medium to give a final concentration of 8.6 μM. The control had the equivalent volume of absolute ethanol. Bacterial growth was monitored by measuring optical density changes at 640 nm on a model DB-GT Beckman spectrophotometer. After 5 h of incubation at 37°C, the cells were harvested at the exponential phase (Fig. 1) and concentrated at low speed in a refrigerated centrifuge. The resulting pellet was washed once with 0.89% NaCl and stored lyophilized.

Assay procedures. Lipids were extracted by the Ames modification of the Bligh-Dyer method (1, 4) and subjected to thin-layer chromatography on Silica Gel H plates in chloroform-methanol-acetic acid-water (80:13:8:0.8). Standard phospholipids were run along on the plates. The spots were visualized with iodine vapor and isolated by scraping off the silica. Total lipid phosphate as well as individual phospholipids were determined according to Dodge and Phillips (9). Statistical analysis was carried out by using Student's *t* test; a probability level of 0.05 or less was accepted as a significant difference.

The methyl esters of the fatty acids were prepared with the Boron trifluoride-methanol reagent and examined in a Varian 2100 gas chromatograph equipped with a dual flame ionization detector. The glass column was packed with 15% diethylene glycol succinate polymer on a 100/120 Gas-Chrom P solid support and was operated at 195°C under 4.5 kg of nitrogen pressure per cm^2. Peaks were identified by comparison with authentic standards and with the known composition of *E. coli* fatty acids (19, 23). The percent composition of the fatty acids was calculated from the areas of each methyl ester by the retention time method (17).

Reagents. All chemicals and solvents were of analytical grade. Standards of phospholipids and fatty acids were purchased from Sigma Chemical Co., St. Louis, Mo. Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8a-hexahydrox-1,4,5,8-endo-exodimethanonaphthalene), dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimetha-

nonaphthalene), DDT [1,1,1-trichloro-2,2-bis(chlorophenyl)ethane], and parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate), were kindly donated by FMC Corp., Middle Port, N.Y.

RESULTS

The effects of various pesticides on *E. coli* were determined. Cells growing in the presence of parathion, DDT, aldrin, or dieldrin achieved slightly increased growth rates. With parathion, there also was a longer lag phase (Fig. 1).

Table 1 shows the changes in phospholipid composition. Parathion and DDT increased the total lipid phosphate concentration. Parathion produced increases in all the phospholipid species, without changes in their distribution patterns. DDT caused larger increases in the contents of phosphatidyl ethanolamine and cardiolipin than parathion but did not significantly alter phosphatidyl serine concentration; therefore, the neutral/acidic phospholipid ratio was decreased. Aldrin and dieldrin produced a marked increase in the content of phosphatidyl serine and a slight increase in the concentration of cardiolipin; they did not modify phosphatidyl ethanolamine, and phosphatidyl glycerol was not detectable. Dieldrin also increased the proportion of neutral phospholipids. It is interesting that phosphatidyl ethanolamine remained the dominant species in all cases.

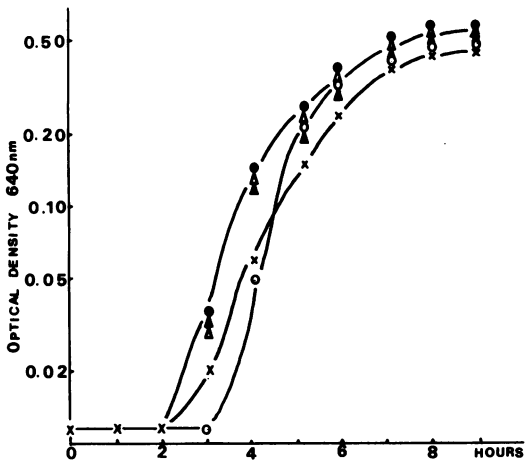

FIG. 1. *Effects of pesticides on the growth of E. coli. Cells were grown as described in the text. Pesticides were added to give an 8.6 μM final concentration. Plotted values are means of duplicate cultures. Symbols: ×, control; ○, parathion; ▲, DDT; △, aldrin; ●, dieldrin.*

The alterations in fatty acid composition are shown in Table 2. The fatty acid profile of the *E. coli* strain studied was in accordance with that found in other strains (19, 23), except for the low *cis*-vaccenic acid data. Parathion, aldrin, and dieldrin all caused increases in the saturated/unsaturated plus cyclopropane fatty acid ratio. With DDT, the increase was very slight.

DISCUSSION

It was apparent from our results that cells of *E. coli* contained an accumulation of phospholipids and an increased saturated fatty acid composition when grown in the presence of some pesticides, with only slight changes in the growth rate. Ingram (15) reported that *E. coli* increased the synthesis of phospholipids containing saturated fatty acids in response to long-chain alcohols in the medium. The alteration in fatty acid composition could be attributed to attempts by the cells to maintain the fluidity of the membrane. A decrease in membrane viscosity is an-

TABLE 1. *Effects of pesticides on the phospholipid composition of E. coli*[a]

Pesticide	μmol of P/g of lyophilized cells[b]					
	Total lipid phosphate	Phosphatidyl serine	Phosphatidyl ethanolamine	Phosphatidyl glycerol	Cardiolipin	Acidic/ neutral
Control	42.50 ± 4.19	0.65 ± 0.06	26.20 ± 1.60	1.70 ± 0.10	4.38 ± 0.18	4.56
Parathion	59.47 ± 1.07[c]	2.25 ± 0.23[d]	50.60 ± 1.50[d]	5.01 ± 0.39[d]	8.08 ± 0.70[d]	4.00
DDT	71.30 ± 0.75[c]	0.82 ± 0.08	65.50 ± 1.70[d]	4.70 ± 0.32[d]	8.70 ± 1.00[d]	2.85
Aldrin	50.47 ± 0.38	5.91 ± 0.20[d]	28.35 ± 0.52	ND[e]	7.89 ± 0.25[d]	4.26
Dieldrin	52.23 ± 0.23	6.60 ± 0.20[d]	27.30 ± 0.70	ND	5.50 ± 0.10[d]	6.14

[a] Pesticides were added to the medium to give a final concentration 8.6 μM. Cells were harvested as described in the text. Phospholipids were isolated by thin-layer chromatography and determined by the procedure of Dodge and Phillips (9).

[b] The results are presented as the means ± standard error of four determinations of lipid phosphate and 5 to 10 determinations of individual phospholipids. Values were compared with the controls by means of the *t* test.

[c] $P < 0.01$.

[d] $P < 0.001$.

[e] ND, Not detectable.

TABLE 2. *Effects of pesticides on the fatty acid composition of E. coli*[a]

Pesticide (*n*)	% of total fatty acids								
	14:0	16:0	16:1	Unknown	17:cyc-9,10	18:0	18:1	19:cyc-9,10	Saturated/unsaturated + cyc
Control (8)	7.01 ± 0.29	37.79 ± 0.42	23.47 ± 0.36	2.00 ± 0.16	23.17 ± 0.93	4.42 ± 0.14	1.56 ± 0.09	0.59 ± 0.07	1.05
Parathion (8)	6.91 ± 0.27	43.17 ± 0.93	19.13 ± 0.55	1.64 ± 0.18	20.07 ± 1.13	6.00 ± 0.31	2.28 ± 0.61	0.76 ± 0.09	1.33
DDT (6)	4.76 ± 0.29	42.14 ± 1.26	20.06 ± 0.55		23.00 ± 0.86	6.58 ± 0.77	2.45 ± 0.82	1.32 ± 0.14	1.14
Aldrin (6)	7.27 ± 0.10	44.88 ± 0.13	17.28 ± 0.22	2.16 ± 0.12	18.77 ± 0.26	6.21 ± 0.20	2.24 ± 0.07	1.19 ± 0.09	1.48
Dieldrin (6)	8.51 ± 0.63	46.12 ± 0.60	17.26 ± 0.34	2.04 ± 0.01	16.66 ± 0.88	6.42 ± 0.24	2.04 ± 0.14	1.13 ± 0.12	1.66

[a] Pesticides were added to the medium to give a final concentration of 8.6 μM. Cells were harvested as described in the text. Fatty acids were analyzed by gas chromatography. Values are reported as the means ± standard errors of *n* determinations. Cyc, Cyclopropane.

tagonized by the synthesis of saturated fatty acids (16).

Our experimental data reveal that changes in phospholipid concentrations were diverse and no single metabolic pathway was involved. Growth of *E. coli* in the presence of parathion resulted in an increase of phospholipids which were the same qualitatively as in the control. Brunson and Shively (5) found the same kind of phospholipid accumulation as a part of mesosome-like intracytoplasmic membranes when an *E. coli* mutant was grown at 42°C in nutritive culture medium. They have suggested a cellular malfunction which prevents phospholipid assimilation.

Growth of *E. coli* in the presence of DDT and dieldrin altered the ratio of polar phospholipid head groups. The less spectacular modification in charge distribution in the bacterial membrane, coupled with changes in fatty acid composition, could affect protein-lipid interactions, transport processes, and membrane-bound enzymatic activities (8, 10, 18, 23, 24). DDT produced a remarkable accumulation of phospholipids with an increased proportion of negatively charged species. When the amount of acidic phospholipids was doubled by genetic means, there was a marked impairment of the growth rate (13). On the other hand, dieldrin increased the proportion of neutral serine-derived phosphatides. Aldrin and dieldrin seemed to inhibit phosphatidyl serine decarboxylase and to activate cardiolipin synthetase, with phosphatidyl glycerol disappearance. Kundig and Roseman (20) demonstrated that phosphatidyl glycerol activates enzyme II of the phosphotransferase system; therefore, the absence of the phospholipid could be conducive to sugar transport alterations in *E. coli*.

We are currently studying lipids and enzymatic activities from isolated membranes of *E. coli* to more fully understand the significance of our results.

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