Effect of DDT and Dieldrin on RNA and Protein Synthesis in Subcellular Fractions of HeLa S Cells

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HeLa S cells were cultured in the presence of 0.5, 10, and 50 p.p.m. DDT or dieldrin and then fractionated by differential ultracentrifugation into four subcellular fractions. Carbon-14–leucine incorporation into each fraction increased when DDT and dieldrin concentrations were increased to 0.5 p.p.m. The changes in the total RNA specific activity reported previously paralleled those in the nucleus and supernatant fractions at 0.5- and 10-p.p.m. DDT evels and in the microsome and supernatant fractions at 0.5- and 10-p.p.m. dieldrin levels.

Several reports (Conney *et al.*, 1967; Johnson, 1951; Kinoshita *et al.*, 1966; Torda and Wolf, 1948) have indicated that DDT has an inhibitory effect on several enzyme systems. Parker (1960) has suggested that the high lipide solubility of DDT might determine the distribution of the pesticide in the mitochondria and other sites of metabolic activity. In this laboratory, Chung *et al.* (1967) have observed that C^{14}-leucine incorporation into cellular protein increased when DDT and dieldrin concentrations were increased to 0.5 p.p.m., but decreased when the levels were increased to 10 and 50 p.p.m. Since these changes may be a reflection of a number of alterations in the subcellular activity, this work was conducted to study the specific activity of RNA and protein in the subcellular fractions of HeLa S cells exposed to different levels of DDT and dieldrin.

MATERIALS AND METHODS

HeLa S cells were obtained from the Carver Research Foundation laboratory. An accurately determined number of cells (2×10^6 to 5×10^6) were cultured in 10 ml. of culture medium (80% medium 199 plus 20% calf serum) in the presence of 0, 0.5, 10, and 50 p.p.m. DDT or dieldrin for 48 hours following the addition of 0.1 to 0.3 μc. of L-leucine-C^{14} or uridine-C^{14} as described by Chung *et al.* (1967). Each experiment was run in duplicate and repeated three times.

At the end of the incubation period, the culture medium was decanted and the cells were removed from the walls of the culture bottle with 10 ml. of 0.02% versene (EDTA) solution for 5 to 10 minutes at 36° C. The cells were isolated by centrifugation, then washed with and finally resuspended in 10 ml. of 0.25*M* sucrose solution.

The cells in 0.25*M* sucrose solution were homogenized in a loose-fitting Potter-Elvehjem-type homogenizer (Schneider, 1948). The homogenate was separated into the major subcellular fractions (nuclei, mitochondria, microsomes, and supernatant) by differential ultracentrifugation with the Spinco Model-L preparative ultracentrifuge by the method of Uyeki (1963).

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The RNA and protein in the subcellular fractions were separated by a modification of the Schmidt-Thannhauser procedure (1945) as described by Chung *et al.* (1967).

Protein content was determined by nesslerization (Koch and McMeekin, 1924) and RNA content by the Mejbaum reaction (Mejbaum, 1939). Radioactivity in each subcellular fraction was determined in a Packard Tri-Carb liquid scintillation counter by the method described by Bruno and Christian (1961). Aliquots of 0.5-ml. samples were digested with 0.5 ml. of hydroxide of hyamine (10X) overnight in 20-ml. radiation counting glass vials, and then 18 ml. of the following scintillation counting mixture were added: 4 grams of BBOT [2,5 bis-(5-*tert*-butylbenzoxazolyl)thiopene] plus 80 grams of naphthalene in 400 ml. of methyl cellusolve and 600 ml. of toluene.

RESULTS AND DISCUSSION

Chung *et al.* (1967) found that C^{14}-leucine incorporation into total cellular protein of HeLa S cells increased when DDT and dieldrin concentrations were increased from 0 to 0.5 p.p.m. but decreased as the DDT and dieldrin concentrations were increased to 10 and 50 p.p.m. The radioactivity at the 50-p.p.m. DDT level was less than that at 10 p.p.m., whereas at both dieldrin levels the radioactivity was the same. The results of the present study (Table I) can explain these changes in protein synthesis by the increase in protein specific activity at 0.5-p.p.m. DDT and dieldrin concentrations in all four cellular fractions. Similarly, from 0.5- to 10-p.p.m. and from 10- to 50-p.p.m. levels, protein specific activity decreased in the mitochondrion, microsome, and supernatant fractions. From 0.5- to 10-p.p.m. dieldrin levels protein specific activity decreased, and from 10- to 50-p.p.m. dieldrin levels there was little or no change in protein specific activity in the nucleus, mitochondrion, and microsome fractions.

At the 10-p.p.m. DDT level the protein specific activity was less than that at the 0.5-p.p.m. level but was the same as that at the 0-p.p.m. level, and at the 50-p.p.m. level the protein specific activity was greater than that at the 0- and 10-p.p.m. levels but the same as that at the 0.5-p.p.m. level in the nucleus fraction. From 0.5- to 10-p.p.m. and from 10- to 50-p.p.m. dieldrin levels, there was little or no change in the supernatant fractions.

Table I. Effects of Pesticides on Specific Activity of Proteins in Different Cellular Fractions

Treatment, P.P.M.[a]	Specific Activity[b]			
	Nucleus	Mitochondrion	Microsome	Supernatant
		DDT		
0	23.4 ± 0.9	90.7 ± 2.0	83.5 ± 4.4	95.0 ± 4.8
0.5	30.8 ± 2.1	124.1 ± 8.3	108.4 ± 12.1	115.3 ± 4.6
10	23.9 ± 2.2	83.8 ± 1.8	61.4 ± 4.4	83.3 ± 5.5
50	30.5 ± 1.3	58.9 ± 7.5	40.6 ± 3.4	44.1 ± 2.9
		DIELDRIN		
0	14.4 ± 0.7	20.4 ± 1.2	17.7 ± 0.8	17.6 ± 0.9
0.5	16.6 ± 0.6	27.2 ± 1.4	28.8 ± 1.1	20.2 ± 0.3
10	12.4 ± 1.3	16.0 ± 1.3	15.9 ± 1.3	19.1 ± 1.6
50	13.5 ± 1.1	16.2 ± 1.0	17.9 ± 1.5	18.7 ± 0.8

[a] 0.1 to 0.3 μc. of L-leucine-C^{14}/10 ml. culture medium was used in each culture. Initial cell count was 2×10^6 to 5×10^6/10 ml. culture medium.
[b] Value expressed as c.p.m./mg. protein $\times 10^{-3}$; average ± S.E. of mean for 6 samples.

Table II. Effect of Pesticides on Specific Activity of RNA in Different Cellular Fractions

Treatment, P.P.M.[a]	Specific Activity[b]			
	Nucleus	Mitochondrion	Microsome	Supernatant
		DDT		
0	77.5 ± 2.3	89.9 ± 2.9	168.6 ± 13.6	329.0 ± 12.4
0.5	65.6 ± 1.2	82.8 ± 5.5	211.7 ± 14.2	240.3 ± 24.9
10	56.2 ± 5.5	91.8 ± 7.1	222.7 ± 24.8	204.9 ± 3.7
50	53.9 ± 0.1	91.0 ± 7.1	172.1 ± 19.4	303.9 ± 32.1
		DIELDRIN		
0	72.9 ± 4.4	73.8 ± 2.8	134.5 ± 8.7	252.4 ± 29.8
0.5	74.7 ± 3.4	91.0 ± 9.2	101.5 ± 15.3	184.5 ± 16.7
10	65.7 ± 3.3	70.3 ± 5.0	100.5 ± 2.4	207.4 ± 12.0
50	77.6 ± 3.4	81.6 ± 7.0	113.4 ± 16.2	271.1 ± 2.5

[a] 0.1 to 0.2 μc. of uridine-2-C^{14}/10 ml. culture medium was used in each culture.
[b] Value expressed as c.p.m./mg. RNA $\times 10^{-3}$; average ± S.E. of mean for 6 samples.

Chung *et al.* (1967) also reported that the specific activity of total RNA decreased at 0.5- and 10-p.p.m. DDT and dieldrin levels as compared with the 0-p.p.m. level. Table II shows that a similar trend in RNA specific activity was exhibited in the nucleus and supernatant fractions at 0.5- and 10-p.p.m. DDT levels, in the microsome and supernatant fractions at 0.5- and 10-p.p.m. dieldrin levels, and in the nucleus fraction at the 10-p.p.m. dieldrin level. Similarly, RNA specific activity increased from 10 to 50 p.p.m. in the four cellular fractions of dieldrin-treated cells and in the supernatant fraction of DDT-treated cells.

RNA specific activity increased at 0.5- and 10-p.p.m. DDT levels in the microsomal fraction as compared with that at 0 p.p.m. (Table II). There was a decrease in RNA specific activity as the DDT concentration increased from 10 to 50 p.p.m. in the microsome fraction, but there was no change in the nucleus and mitochondrion fractions.

Changes in the specific activity of DNA at different dieldrin levels observed by Chung *et al.* (1967) paralleled changes in the specific activity of protein in all four cellular fractions (Table I) and of RNA in the mitochondrion fraction (Table II).

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