Studies of DNA, RNA, and Protein Synthesis in HeLa S Cells Exposed to DDT and Dieldrin

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HeLa S cells were cultured in the presence of different levels of DDT and dieldrin. The total cell count decreased as DDT concentration in the culture medium was increased from 0 to 0.5 p.p.m., and this decrease became progressively less to 50 p.p.m. despite the lack of change in total protein content. When DDT and dieldrin concentrations were increased to 0.5 p.p.m., C^{14} leucine incorporation into cellular protein increased but decreased as the DDT and dieldrin concentrations were increased to 10 and 50 p.p.m. The degree of change with dieldrin was less than with DDT. The changes in RNA synthesis at different DDT levels were similar to those at different dieldrin levels but dieldrin had a greater effect. At 10- and 50-p.p.m. DDT and dieldrin levels, the DNA synthesis changed very little.

The toxicity of the chlorinated hydrocarbon group of pesticides for mammals is exhibited primarily by central nervous system excitation, and, in some instances, growth inhibition and weight loss occur (*1–4, 9*). The cytotoxicity of pesticides in cultured mammalian cells is evidenced by progressive inhibition of cell growth, and this is associated with or followed by cytopathic changes in cell morphology which lead to the destruction of the culture (*5, 6*). The mechanism of action of these pesticides including DDT and dieldrin still is not well understood. This paper reports studies of some of the molecular events following exposure of HeLa S cell culture to DDT and dieldrin. Such a system is ideal for the evaluation of population development in terms of cellular RNA, DNA, and protein synthesis, since the concentration of the pesticide may be adjusted easily.

Materials and Methods

Cell responses to DDT and dieldrin were studied in HeLa S cell cultures derived from a human epidermoid carcinoma of the cervix (*11*). The HeLa S cell line is a laboratory line developed in the Carver Research Foundation laboratory from the original strain HeLa and maintained as a separate line by serial transfer over a period of about 8 years before storage under liquid nitrogen.

DDT (*p,p'*-isomer, 100% purity) and dieldrin (technical grade, ≥85%) were purchased from City Chemical Corp. L-Leucine-C^{14} (uniformly labeled, 230 mc. per mmole) was purchased from Volk Radiochemical Co. and thymine-2-C^{14} (6.3 mc. per mmole) and uridine 2-C^{14} (30.0 mc. per mmole) from New England Nuclear Corp. All isotopic tracers were diluted to the concentration of 1 μc. per ml. with Dulbecco phosphate-buffered saline solution and kept at 5° C. as the stock solution.

Stock solutions of DDT and dieldrin were prepared by dissolving 0, 5, 100, and 500 mg. of the pesticide in 5 ml. of Tween 80. After sterilization by filtration, 0.1 mg. of each of these solutions was added to 100 ml. of culture medium (80% medium 199 plus 20% human serum) to produce solutions of 0, 1, 20, and 100 p.p.m. concentrations, respectively.

An accurately determined number of HeLa S cells (1.3×10^6 to 2.3×10^6) were suspended in 5 ml. of growth medium (80% medium 199 plus 20% human serum) in 240-ml. culture bottles. Five milliliters of the 0, 1, 20, and 100 p.p.m. stock pesticide solutions were added to give final concentrations of 0, 0.5, 10, and 50 p.p.m., respectively. In the first set of experiments using DDT the cultures were incubated at 36° C. for 24 hours following the addition of 0.1 μc. of L-leucine-C^{14}, 0.5 μc. of thymine-C^{14}, or 0.5 μc. of uridine-C^{14}. In the second set of experiments using dieldrin the cultures were incubated at 36° C. for 48 hours following the addition of 0.1 μc. of L-leucine-C^{14}, 0.25 μc. of thymine-C^{14}, or 0.25 μc. of uridine-C^{14}. Each experiment was run in duplicate and repeated three times.

At the end of the incubation period cytological changes were observed under the light and phase microscopes. The culture medium was decanted and then the cells were trypsinized with 8 ml. of 0.5% trypsin 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 Hank's balanced salt solution.

A 1.0-ml. aliquot of the evenly dispersed cellular suspension was filtered through Millipore filter (3 micron pore size) and washed with 10 ml. of Hank's balanced salt solution, 10 ml. of 5% trichloroacetic acid solution, and finally with 5 ml. of 5% trichloroacetic acid containing 1 mg. of unlabeled leucine per ml. Radioactivity was determined in a Packard Tri-Carb liquid scintillation counter by the method described by Wang and Jones (*12*). The filter papers were dried at room temperature overnight, and then placed in 20-ml. radiation counting glass vials containing 18 ml. of the following liquid scintillation counting mixture: 5 grams of PPO (2,5-diphenyloxazole) plus 0.3 grams of POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] in 1 liter of toluene. The remaining 9.0-ml. portion was centrifuged to obtain a cell pellet which was extracted twice with 5 ml. of warm 95%

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ethanol for 15 minutes in a water bath at 90° C. to extract the free amino acids, and finally with 10 ml. of 5% trichloroacetic acid solution. The protein content was then determined by Nesslerization (*8*).

The DNA and RNA were separated by a modification of the procedure of Hutchison and Munro (*7*). The cell pellet was resuspended in 0.5*M* perchloric acid (PCA) at 4° C. for 10 to 20 minutes and then centrifuged. The resulting precipitate was washed once with absolute ethanol containing 0.2*N* potassium acetate; twice with 95% ethanol–ether, 3 to 1; and then digested for 18 hours with 0.1*N* KOH at 37° C. The digest was carefully neutralized at 4° C. to pH 7 to 7.5, and the DNA and protein were precipitated with cold 0.5*M* PCA. The supernatant (RNA fraction) was diluted to 8 ml. and 0.2 ml. spotted on Carl Schleicher and Schuell Co. No. 589 Black Ribbon filter paper, 1.5 inches in diameter. Radioactivity was determined by the method described by Wang and Jones (*12*). Ultraviolet absorption was determined at 257 mμ. The precipitate was washed with cold 0.5*M* PCA and the DNA hydrolyzed by treatment with 0.5*M* PCA at 90° C. for 30 minutes. The hydrolyzate (DNA fraction) was diluted to 4 ml. and 0.2 ml. spotted on Carl Schleicher and Schuell Co. No. 589 Black Ribbon filter paper, 1.5 inches in diameter. Radioactivity was determined by the method described by Wang and Jones (*12*). Ultraviolet absorption was determined at 260 mμ. The concentration of RNA and DNA was determined from standard curves of different concentrations of yeast-RNA and sperm-DNA (Nutritional Biochemicals Corp., Cleveland, Ohio) *vs.* ultraviolet absorption at 257 and 260 mμ, respectively.

Results and Discussion

Effect of DDT and Dieldrin on Protein Synthesis. Figure 1 shows the effect of different levels of DDT on cell development expressed as total cell count. The total cell count decreased markedly as DDT concentration in the medium was increased from 0 to 0.5 p.p.m. As the concentration of DDT was increased to 50 p.p.m., the decrease in total cell count became progressively less. The changes in specific activity (c.p.m. per 10^6 cells) paralleled the changes in total cell count at the different DDT concentration levels. Microscopic observations showed increased granulation, elongation, and vacuolation, all of which are manifestations of irregular cell growth and cell destruction. The decrease in the incorporation of labeled leucine per cell indicated a decrease in protein synthesis or turnover rate in each cell. When the changes in cell development were expressed in terms of total protein content per culture, there was very slight or no change due to the presence of DDT (Figure 2). The rate of protein synthesis (c.p.m. per mg. of protein) increased as DDT concentration in the medium was increased from 0 to 0.5 p.p.m. No effect was observed at 10 p.p.m., but at 50 p.p.m. protein synthesis decreased. The decrease in total cell count and the lack of change in total protein content would indicate that cell division was decreased with a concurrent increase in protein content per cell due to an increase in cell size as the DDT concentrations were increased.

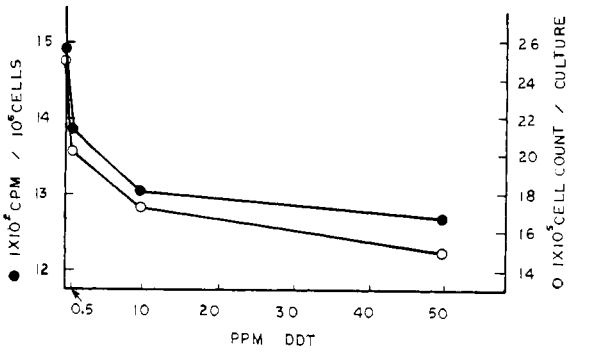

Figure 1. Incorporation of C^{14} leucine per 10^6 cells as DDT concentration is increased

Cell cultures containing no DDT and Tween 80:25.2 × 10^2 c.p.m. per 10^6 cells and 2.5 × 10^6 cells per culture

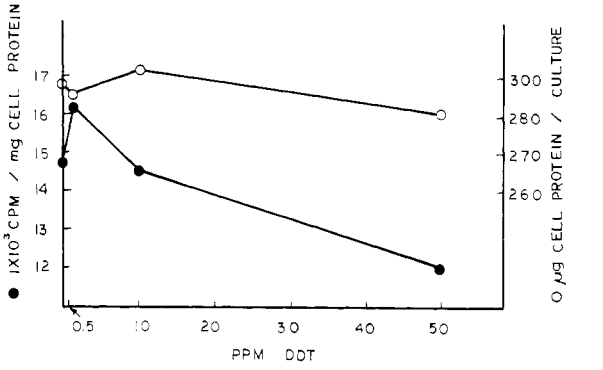

Figure 2. Incorporation of C^{14} leucine per mg. of cell protein as DDT concentration is increased

Cell cultures containing no DDT and Tween 80:18 × 10^3 c.p.m. per mg. of cell protein and 307 μg. protein per culture

The increased incorporation of labeled leucine in cell protein as DDT concentration in the medium was increased from 0 to 0.5 p.p.m. (Figure 2), despite the decrease in total cell count (Figure 1) and the lack of change in total protein content (Figure 2), would indicate that, at this concentration level, proteins were catabolized and synthesized at faster rates than at the other DDT levels, but this proceeded at a rate to keep the total protein content constant. Furthermore, the increased protein synthesis, when the DDT concentration was increased from 0 to 0.5 p.p.m., may indicate an initial stimulation of the enzymes responsible for protein synthesis. Gabliks and Friedman (*6*) reported that decrease in total protein content resulted from the presence of several pesticides, including DDT. They used this criterion as a measurement of growth inhibition, because it was reported previously that an increase in total cellular protein generally was proportional to the increase in total cell number (*10*). In the present study, changes in total cell protein content per culture did not show a correlation with the changes in total cell number per culture. This apparently is due to the increase in cell size such that the total protein content per cell increased while the total number of cells per culture decreased.

Figure 3 shows a slight but linear decrease in total protein content with the increase in dieldrin concentration greater than 0.5 p.p.m. There was a slight increase in total protein content as dieldrin concentration in the medium was increased to 0.5 p.p.m. The trend in the changes in protein synthesis (c.p.m. per mg. of protein) was similar to that resulting from different levels of DDT concentrations. However, the degree of change was less with dieldrin than with DDT. Since the cells were exposed to dieldrin for 48 hours, it is not known whether these differences are due to the difference in time of exposure or to the difference in pesticide or to both. The results of Gabliks and Friedman (*6*) indicate that the difference in time of exposure would contribute in part to these differences in protein synthesis. Furthermore, in the present study the presence of the carrier Tween 80 (0 p.p.m.) increased the protein synthesis at 48 hours, whereas the protein synthesis was decreased at 24 hours over the blank (contains no Tween 80) culture.

The fact that the total protein content in the presence of DDT remained constant but changed with varying concentrations of dieldrin would indicate differences in the mechanism of action of these two pesticides.

Effect of DDT and Dieldrin on RNA Synthesis. Figure 4 shows the effect of DDT on RNA synthesis. As DDT concentration was increased from 0 to 0.5 p.p.m. a very slight decrease in total RNA content occurred, but at higher concentrations of DDT there was no further change. RNA synthesis (c.p.m. per mg. of RNA) decreased markedly as DDT concentration in the medium was increased from 0 to 0.5 p.p.m. and decreased less markedly from 0.5 to 10 p.p.m. The specific activity at 50 p.p.m. was slightly greater than that at 0 p.p.m. The lack of change in total RNA content correlated with the constant values of total protein content over the different concentration levels of DDT. This would strongly indicate the importance of RNA in protein synthesis.

The total RNA content increased as dieldrin concentration in the medium was increased from 0 to 0.5 p.p.m. and from 0.5 to 10 p.p.m. and decreased from 10 to 50 p.p.m. (Figure 5). The trend in RNA synthesis (c.p.m. per mg. of RNA) at different dieldrin levels was similar to that at the different DDT levels (Figure 5). However, the effect due to dieldrin was greater than that of DDT.

The carrier Tween 80 (0 p.p.m. culture) decreased RNA synthesis after 24 hours' incubation, whereas after 48 hours' incubation RNA synthesis increased.

Effect of DDT and Dieldrin on DNA Synthesis. There were slight increases in total DNA content as the DDT concentration increased from 0 to 0.5 p.p.m. and from 0.5 to 10 p.p.m. but a slight decrease occurred from 10 to 50 p.p.m. DDT (Figure 6). DNA synthesis (c.p.m. per mg. of DNA) decreased at 0.5 and 50 p.p.m., but no change occurred at 10 p.p.m. as compared with that at 0 p.p.m. DDT level.

The trend in total DNA content was opposite to that in RNA synthesis at different levels of DDT. Perhaps 0.5 and 10 p.p.m. DDT stimulated the enzymes involved in DNA synthesis while 50 p.p.m. DDT inhibited these enzymes.

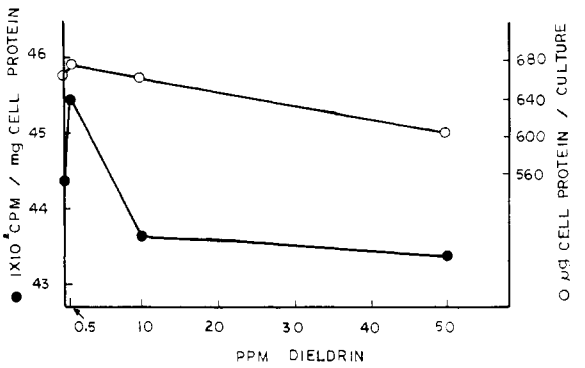

Figure 3. Incorporation of C^{14} leucine per mg. of cell protein as dieldrin concentration is increased

Cell cultures containing no dieldrin and Tween 80: 40.5 × 10^2 c.p.m. per mg. of cell protein and 606 μg. of protein per culture

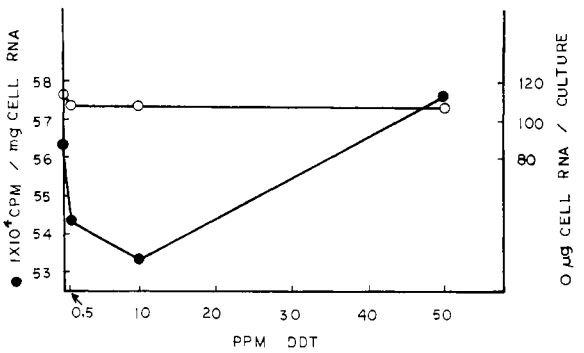

Figure 4. Incorporation of C^{14} uridine per mg. of cell RNA as DDT concentration is increased

Cell cultures containing no DDT and Tween 80: 65 × 10^4 c.p.m. per mg. of cell RNA and 116 μg. of RNA per culture

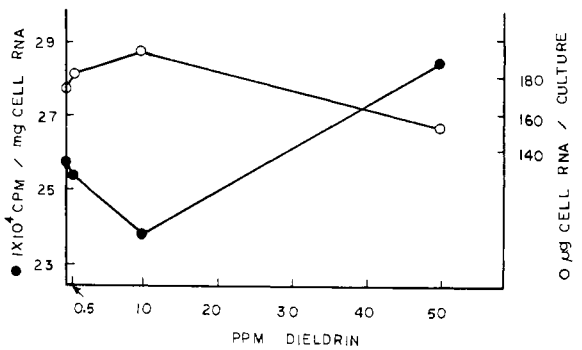

Figure 5. Incorporation of C^{14} uridine per mg. of cell RNA as dieldrin concentration is increased

Cell cultures containing no dieldrin and Tween 80: 23 × 10^4 c.p.m. per mg. of cell RNA and 199 μg. of RNA per culture

As dieldrin concentration was increased from 0 to 0.5 p.p.m., the total DNA content was decreased (Figure 7). Dieldrin at 10 and 50 p.p.m. levels did not affect the total DNA content as compared to 0 p.p.m. dieldrin level.

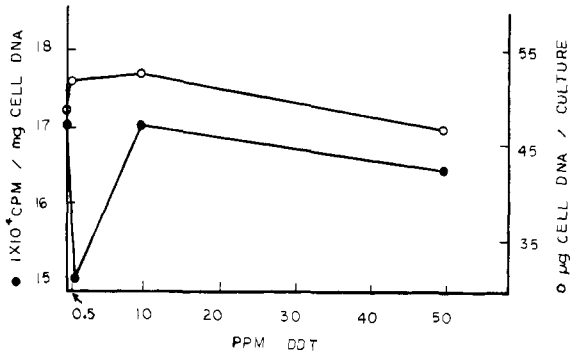

Figure 6. Incorporation of C^{14} thymine per mg. of cell DNA as DDT concentration is increased

Cell cultures containing no DDT and Tween 80: 18.5×10^4 c.p.m. per mg. of cell DNA and 52 μg. of DNA per culture

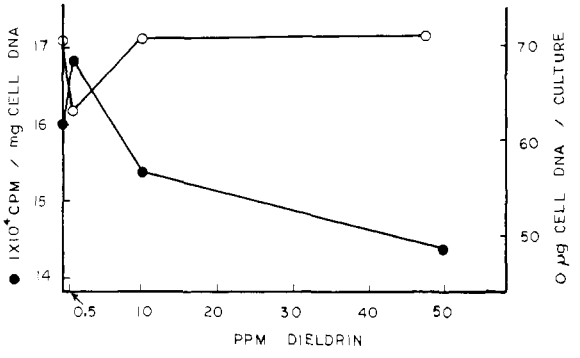

Figure 7. Incorporation of C^{14} thymine per mg. of cell DNA as dieldrin concentration is increased

Cell cultures containing no dieldrin and Tween 80: 18.3×10^4 c.p.m. per mg. of cell DNA and 71 μg. of DNA per culture

The changes in DNA synthesis (c.p.m. per mg. of DNA) paralleled the changes in protein synthesis (c.p.m. per mg. of protein) at the different levels of dieldrin. In contrast, the changes at 0.5 and 50 p.p.m. were opposite to those in RNA synthesis at these dieldrin levels.

The presence of Tween 80 in the culture medium decreased DNA synthesis after 24-hour and 48 hour incubation periods.

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