Hexachlorophene-Induced Alterations in the Metabolism of Cultured Human Lung Cells (37067)

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(Introduced by James C. Fritz)

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Hexachlorophene (2,2′-methylenebis[3,4,6-trichlorophenol]) is a germicidal agent which has been used in bacteriostatic amounts in soaps, deodorant products, medicinals, and cosmetic preparations (1). This agent has also been used internally in man for treatment of blood flukes (2). This compound is thought to exert its bacteriostatic effects by inhibiting several bacterial enzyme systems, including glucose, lactate, and succinic dehydrogenases and cytochrome oxidase (3). It has also been reported to affect cell membrane permeability (4).

Because of the widespread use of hexachlorophene, this study was undertaken to investigate its effect on mammalian cell biosynthetic processes.

Materials and Methods. Growth conditions. Approximately 2.5×10^6 heteroploid L-132 human embryonic lung cells (Flow Laboratories, Rockville, Md) were inoculated per petri dish and incubated in Eagle's minimum essential medium supplemented with 10% glutamine, 10% calf serum, 5% nonessential amino acids, and 100 units of penicillin-streptomycin per ml. Cultures were incubated for 24 hr at 37° in a humidified atmosphere of 4% CO_2. Hexachlorophene (Sindar Corp., New York) was added at various concentrations to the cultures at the time of inoculation. The purity of the hexachlorophene used in these studies was not determined directly; however, statistically similar results were obtained with another hexachlorophene sample (G-11), which was shown to be dioxin-free up to 50 ppb and exhibited one peak in the gas–liquid chromatographic analysis (A. Ulsamer, Food and Drug Administration, personal communication).

DNA, RNA, and protein synthesis. L-132 cell cultures were pulsed in the last 2 hr of incubation with [^{3}H-]thymidine (1 μCi/ml), [^{3}H-]uridine (1 μCi/ml), or [^{3}H-]lysine (1 μCi/ml) to measure the rates of DNA, RNA, and protein synthesis, respectively. After the 2-hr pulse, the cell sheets were rinsed in cold physiological saline, trypsinized, and resuspended in saline. Aliquots of the suspensions were taken for cell count determinations with a Coulter counter. The DNA and RNA samples were prepared from aliquots of the cells by the method of Regan and Chu (5), and protein samples were prepared by the method of Mans and Novelli (6). A Mark I Nuclear Chicago scintillation counter was used to measure radioactivity, and results were calculated as counts per minute of isotope incorporated per cell.

Results. The effect of hexachlorophene on the growth of L-132 cells was determined after one generation. As seen in Fig. 1, the data seem to indicate that hexachlorophene at 1 μg/ml had a slight inhibitory effect on cell growth. In one of two experiments performed at this concentration, the decrease was statistically significant at the $p < 0.05$ level. Hexachlorophene concentrations of 5 and 10 μg/ml markedly inhibited cell division.

The data in Table I indicate the effect of hexachlorophene on macromolecular biosynthesis in L-132 cells. At 1 μg/ml hexachlorophene had no inhibitory effect on RNA, protein, or DNA synthesis, but concentrations of 5, 7.5, and 10 μg/ml inhibited the synthesis of these macromolecules.

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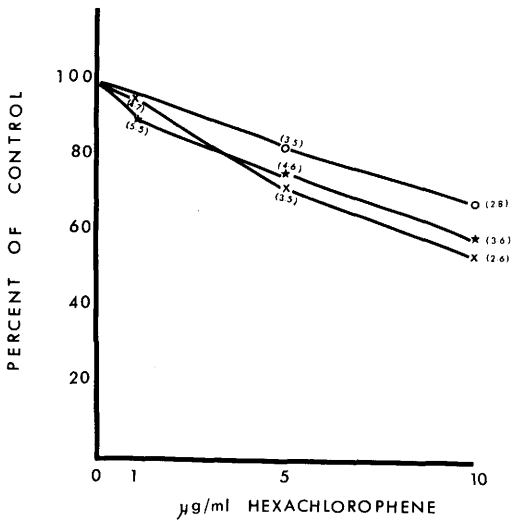

FIG. 1. Effect of hexachlorophene on L-132 cell growth. Each culture contained approximately 2.5 × 10^6 cells at 0 time. X - - - - Experiment 1 (Average of 2 cultures for control and each dose level). ★ - - - - Experiment 2 (Average of 7 cultures for control and each dose level). O - - - - Experiment 3 (Average of 6 cultures for control and each dose level). The numbers in parentheses represent the number of cells × 10^6 contained in hexachlorophene-treated cultures at 24 hr.

In order to show that the biochemical effects measured were not in dead or dying cells, cell cultures were treated for 24 hr with hexachlorophene, rinsed once with Hank's balanced salt solution, and then allowed to grow an additional 24 hr in untreated medium. Figure 2 shows that the remaining cells in the cultures treated with hexachlorophene at 1 or 5 μg/ml divided as efficiently as did cells in the control cultures. In the cultures treated with 10 μg/ml of hexachlorophene, there was a 48% increase in cell number (2.5 × 10^6 to 3.7 × 10^6) as compared with a 66% increase in the controls (3.5 × 10^6 to 5.8 × 10^6). The decreased rate of division probably occurred because of cells that were dead or dying after 24 hr of incubation with hexachlorophene (the time of the macromolecular determinations), however, the amount of the decrease could not account entirely for the drastic reductions seen in macromolecular biosynthesis in these cells.

Discussion. The present results show that hexachlorophene inhibits cell division and the synthesis of RNA, protein, and DNA in L-132 cultures. Cells that were rinsed after treatment with hexachlorophene at 1 or 5 μg/ml divided as efficiently as untreated cultures. In cultures treated with 10 μg/ml, a portion of the cells were irreversibly damaged and did not divide after rinsing.

Not much is known about the metabolic effects of hexachlorophene. It is thought to exert its bacteriostatic effect by inhibiting several bacterial enzyme systems associated with oxidative phosphorylation (7). Hexachlorophene has been shown to uncouple oxidative phosphorylation *in vivo* at a concentration as low as 10^{-7} *M* and is considered to be a more potent uncoupler than is 2,4-dinitrophenol (8). The bacteriostatic action of hexachlorophene has been shown to be reversible by tissue fluids and other materials

TABLE I. Effect of 24-Hr Hexachlorophene Treatment on DNA, RNA, and Protein Synthesis in L-132 Cells.

Compound	Hexachlorophene (μg/ml)	Experiment no. [a] 1 and 2 (3)	3 (3)	4 (2)	5 (5)
DNA (cpm/cell × 10^{-3})	Control	9.3	6.6	8.7	6.1
	1	10.9[b]			
	5	11.0[c]	5.8[b]	7.3[b]	
	7.5			3.9[c]	1.9[c]
	10	5.3[c]	1.9[c]	2.9[c]	1.4[c]
RNA (cpm/cell × 10^{-3})	Control	7.3	6.7	5.2	6.5
	1	7.2			
	5	5.5[c]	4.8[c]	3.0[c]	
	7.5			1.7[c]	1.8[c]
	10	2.6[c]	2.4[c]	1.4[c]	1.6[c]
Protein (cpm/cell × 10^{-3})	Control	5.0		4.7	4.8
	1	5.6			
	5	3.6[c]		2.8[c]	
	7.5			2.1[c]	2.3[c]
	10	2.4[c]		1.6[c]	1.5[c]

[a] The number of cultures at each dose level is given in parentheses. The mean over replicate cultures at each dose level was compared to the control mean by a *t* test.

[b] Significantly different from control ($p < 0.05$).

[c] Significantly different from control ($p < 0.01$).

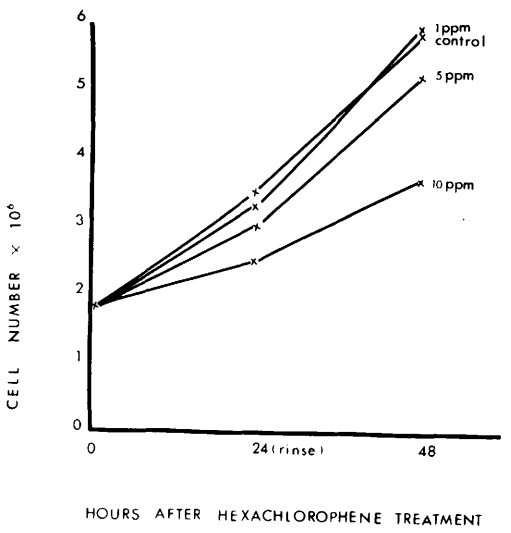

FIG. 2. Cell division after 24 hr of hexachlorophene treatment followed by rinsing and 24 hr of incubation in control medium. Each point is the average of 5 cultures.

that are present on the skin (9). This bacteriostatic action is not demonstrable after a single application of hexachlorophene, especially if followed by rinsing (1) but builds up to an effective level after repeated use.

The possibility that 1 μg/ml hexachlorophene inhibits L-132 cell growth but not macromolecular biosynthesis indicates that the inhibition of macromolecular biosynthesis may not be the primary cause of cell death. Since the energy of oxidative phosphorylation (ATP) is required for the synthesis of RNA, DNA, and protein, their inhibition by hexachlorophene in the L-132 cells is consistent with the finding that hexachlorophene uncouples oxidative phosphorylation.

Summary. Treatment of cultured human lung cells for 24 hr with hexachlorophene resulted in an inhibition of cell division and the synthesis of DNA, RNA, and protein. Normal cell division could be restored by rinsing cultures that had been treated with hexachlorophene at 1 or 5 μg/ml.

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