

Incorporation of [^3H]Uridine into RNA under the Influence of Dieldrin and Polychlorinated Biphenyls

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The present study was conducted to determine the influence of two organochlorines, i.e., polychlorinated biphenyl (Aroclor 1242) and dieldrin, on RNA synthesis in elicited peritoneal macrophages. Tritiated uridine (^3H]uridine) incorporation into RNA was used as an index of RNA synthesis. RNA synthesis was depressed in macrophages incubated in the presence of dieldrin in both a time- and dose-related manner. The dieldrin-induced inhibition of RNA synthesis was further exacerbated when the macrophages were actively phagocytosing latex particles. Aroclor 1242 similarly inhibited RNA synthesis in resting macrophages; however, inhibition was not seen until a concentration exceeding 7 ppm of Aroclor 1242 was used in the incubation medium. Dieldrin-induced inhibition of RNA synthesis was observed at levels of 1 ppm. The inhibition of RNA synthesis provides a corroborative index to support the hypothesis previously advanced that the observed *in vivo* organochlorine-induced immune suppression may be macrophage mediated. Since the observed *in vivo* immune suppression was manifested in the absence of any morphological changes in the target lymphoid-macrophage tissues, a biochemical lesion, perhaps at the level of RNA synthesis, may be a site for the organochlorine-induced immunosuppression.

In the framework of a research program studying immunological aspects of environmental chemicals, one of the objectives was to study direct effects of chemicals on the biochemical function of macrophages, cells to which a major role in the humoral and cellular immune system has been attributed. Peritoneal exudate (PE) cells from Balb/c mice were chosen as a test system for the biochemical studies; the same protocol was used for the immunological investigation component of this program (Loose *et al.*, 1977, 1978). After intraperitoneal stimulation of the donor animals with sodium caseinate, the cell population collected 4 days later consisted of about 90% macrophages.

The synthesis and metabolism of RNA is a molecular function which—unlike that of DNA—is permanent in nonproliferating cells. RNA synthesis not only is a good indicator of cell viability, but has also been suggested to be important in the acquisition and transfer of immunity (Gottlieb and Waldman, 1972). After the discovery that immune macrophages, i.e., macrophages isolated from immunized animals, can transfer immunity, RNA-antigen complexes were isolated from these cells, which apparently act as carriers in the transfer of immunity. Using purified RNA fractions from macrophages containing these complexes, it could be

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shown that they can induce, specifically, the formation of antibodies *in vitro* and *in vivo* (Adler *et al.*, 1966). The RNA was isolated from immune macrophages or from lymphocytes and was purified by sucrose gradient centrifugation or column chromatography. These RNA fractions were therefore called "transfer agents of immunity" (Mikami *et al.*, 1971).

The incorporation of uridine into RNA has been measured in macrophages from immunized and nonimmunized animals. It was shown that macrophages from mice which had been immunized against sheep erythrocytes incorporated significantly more uridine than nonimmunized macrophages when sheep erythrocytes were present in the culture medium (Hartmann, 1969). In studies with pulse-labeled RNA from mouse PE cells using gel electrophoresis for fractionation, Soderberg *et al.* (1976) showed that the RNA species of normal and immune macrophages exhibited the same pattern as those of other mammalian cells. In the presence of inactivated *Salmonella enteritidis* the synthesis of ribosomal RNA in phagocytosing macrophages was found to be enhanced over that of resting cells, while the RNA profile was unchanged.

Using dieldrin and PCB (Aroclor 1242) as model compounds, we studied the influence of these environmental chemicals on RNA synthesis in mouse PE cells. Chemical concentration and time of incubation were experimental variables. Furthermore, the influence of the chemicals on RNA synthesis in resting and in phagocytosing macrophages was compared. Incorporation of tritiated uridine was used to measure RNA synthesis.

MATERIALS AND METHODS

Experimental design. Four days after injection of sodium caseinate (1 cc of 6% Na⁺-caseinate) into the peritoneal cavity of Balb/c mice, the exudate cells were harvested by washing the peritoneal cavity with saline. After the saline wash, the cells were suspended in culture medium (TC-199, Difco) and the percentage of living cells was determined using trypan blue dye exclusion. The medium was enriched with 10% fetal calf serum (FCS). Incubations were carried out at 37°C under an atmosphere of 95% O₂:5% CO₂. Blank samples containing FCS-enriched medium and cells only were included in the incubations as well as cell-free blanks with enriched medium and chemicals.

After incubating for 15 min with the test chemicals, the experiment was started by adding [³H]uridine. After the incubation period, the test was stopped by immersing the test tubes in an ice bath. The cells (5×10^6 per tube) were separated by centrifugation. Samples of the supernatant were counted in a scintillation counter and served as controls for the applied concentrations of [¹⁴C]dieldrin and [³H]uridine. After a single saline wash, the cell pellet was dissolved in 0.5% sodium dodecyl sulfate (SDS) solution. Samples of this solution were used to measure the uptake of tritium by scintillation counting as well as to determine the protein content by a modified Lowry method (Massaro *et al.*, 1970). From the measured values the specific ³H activity per milligram of protein was calculated for test samples and blanks. Background counts from cell-free samples were subtracted from test and blank samples to eliminate the error caused by wall adsorption of the hydrophobic chemicals.

TABLE 1
 $[^3\text{H}]$ URIDINE INCORPORATION OF RESTING MACROPHAGES IN THE PRESENCE
 OF $[^{14}\text{C}]$ DIELDRIN^a

Incubation time (hr)	Dieldrin concentration (ppm)	RNA incorporation (%)			Total incorporation (%)		
		Mean	$\pm$ SD	No.	Mean	$\pm$ SD	No.
2	0.1	94.1	—	1	117.7	—	1
	1.0	71.4	± 26.69	3	75.14	± 28.95	3
	10.0	69.6	± 22.9	3	75.00	± 21.96	3
4	0.1	87.8	± 8.44	4	82.1	± 3.30	3
	1.0	77.2	± 12.02	3	75.16	± 20.32	3
	10.0	65.3	± 15.8	3	53.9	± 8.52	2
8	0.1	21.7	—	1	16.0	—	1

^a Specific incorporation, bound ^3H cpm/ μg RNA or bound ^3H cpm/mg protein, respectively, is expressed as a percentage of the respective blank samples. The mean, its standard deviation, and the number of duplicate or triplicate determinations are given.

To determine the degree of uridine incorporation into RNA, proteins and nucleotides were precipitated with perchloric acid in an ice bath and centrifuged. The centrifugate was treated with 0.3 N KOH at 37°C for 1 hr. After neutralization and duplicate washes of the precipitate with 0.2 N perchloroacetic acid (PCA), the concentration of ribonucleotides in the supernatant was determined by measuring the uv absorption between 310 and 230 nm. From the ^3H count in the supernatant and the RNA concentration the specific radioactivity of RNA was calculated. The amount of uridine incorporation in test samples was expressed as a percentage of blank incorporation.

RESULTS

The results of uridine incorporation of resting macrophages in the presence of 0.1, 1.0, and 10.0 ppm dieldrin are listed in Table 1 for incubation periods of 2 to 8 hr. The results are expressed as a percentage of the respective blanks. The mean, the standard deviation, and the number of tests for each concentration and incubation time are given; duplicate or triplicate samples were measured in each test. For clarity, the results were plotted as graphs shown in Figs. 1 and 2. Total ^3H uptake and uridine incorporation in RNA, in as a percentage of the blank are shown for 2 and 4 hr, respectively, as a function of the dieldrin concentration.

As can be seen from Fig. 1, the incorporation of $[^3\text{H}]$ uridine decreased at a concentration of 1 ppm dieldrin after an incubation period for 2 hr. At this duration of incubation, an increase of the dieldrin concentration resulted in no further significant reduction of uridine incorporation.

With an incubation period of 4 hr, an inhibition of RNA synthesis was found at a concentration of 0.1 ppm dieldrin. The inhibition increased with rising concentrations, and at 10 ppm dieldrin in the medium, the uridine incorporation in RNA was only 65% of the blank.

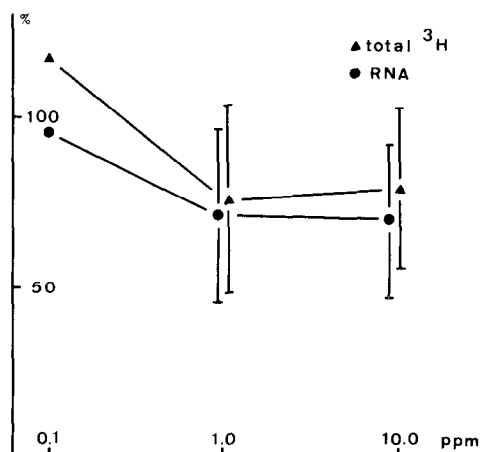


FIG. 1. Total ^3H uptake and [^3H]uridine incorporation in RNA, expressed as a percentage of control, by resting macrophages after 2 hr of incubation with dieldrin.

To stimulate phagocytosis, latex beads with an average diameter of $8\ \mu\text{m}$ (Difco) were used at a ratio of 25 particles per cell. The beads were added together with the [^3H]uridine. Generally, the total tritium uptake as well as the uridine incorporation into RNA was markedly lower in phagocytosing than in resting macrophages. RNA incorporation was only $28 \pm 17\%$ of resting cells; for this reason the specific activity of [^3H]uridine had to be increased.

The results of ^3H uptake and uridine incorporation of phagocytosing macrophages in the presence of dieldrin are listed in Table 2 and shown graphically in Figs. 3 and 4.

As can be seen from Fig. 3, the inhibition of RNA synthesis in phagocytosing macrophages at 2 hr and 1 ppm dieldrin did not differ much from that of resting cells. At 2 hr and 10 ppm, however, the inhibition was increased by about 20% in phagocytosing macrophages.

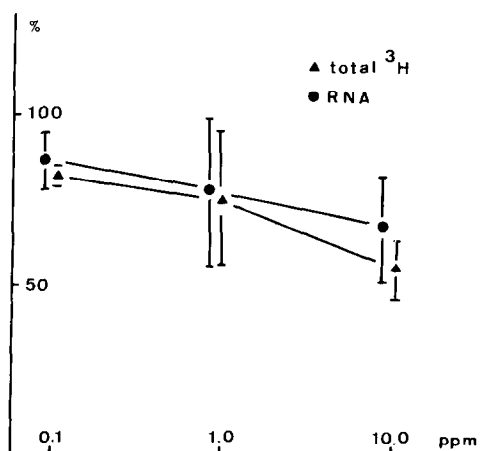


FIG. 2. Total ^3H uptake and [^3H]uridine incorporation in RNA, expressed as a percentage of control, by resting macrophages after 4 hr of incubation with dieldrin.

TABLE 2
 $[^3\text{H}]$ URIDINE INCORPORATION OF PHAGOCYTOSING MACROPHAGES
 IN THE PRESENCE OF $[^{14}\text{C}]$ DIELDRIN^a

Incubation time (hr)	Dieldrin concentration (ppm)	RNA incorporation (%)			Total incorporation (%)		
		Mean	$\pm$ SD	No.	Mean	$\pm$ SD	No.
2	1.0	90.99	± 1.01	2	84.6	± 11.78	2
	10.0	55.5	± 3.30	2	44.2	± 4.18	3
4	0.1	97.1	± 13.79	2	100.3	± 12.80	2
	1.0	63.53	± 0.31	2	57.0	—	1
	10.0	45.6	± 7.99	4	40.6	± 13.0	4
8	0.1	89.7	—	1	84.9	± 11.22	3

^a Specific incorporation, bound ^3H cpm/ μg RNA or bound ^3H cpm/mg protein, respectively, is expressed as a percentage of the corresponding blank samples. The mean, its standard deviation, and the number of duplicate or triplicate determinations are given.

A similar trend can be seen after an incubation period of 4 hr: With both 1 and 10 ppm dieldrin in the medium, the inhibition was about 15% stronger in phagocytosing than in resting macrophages. The RNA synthesis at 10 ppm and 4 hr decreased to 45% of the blank.

At the 0.1 ppm level a decrease in uridine incorporation to 85% of the blank was only seen after 8 hr; the 4-hr samples showed no effect of this dieldrin concentration in phagocytosing macrophages.

In addition to the tests with dieldrin, the effects of Aroclor 1242 on the same system were examined. The results with resting macrophages are listed in Table 3. In these experiments the PCB concentrations in the incubation medium were deter-

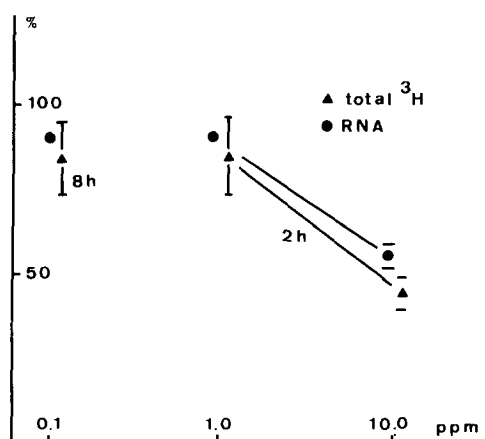


FIG. 3. Total ^3H uptake and $[^3\text{H}]$ uridine incorporation in RNA, expressed as a percentage of control, in phagocytosing macrophages after incubation with 1.0 and 10.0 ppm of dieldrin for 2 hr and with 0.1 ppm for 8 hr.

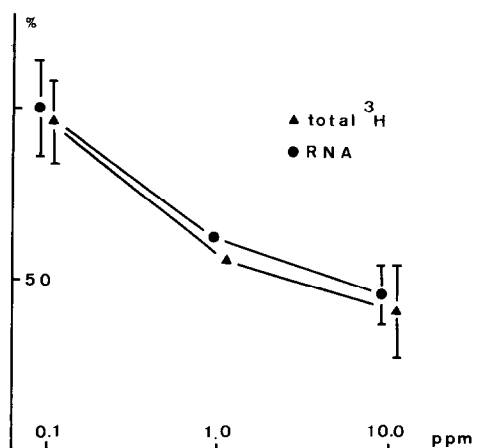


FIG. 4. Total ^3H uptake and [^3H]uridine incorporation in RNA, expressed as a percentage of control, in phagocytosing macrophages after 4 hr of incubation with dieldrin.

mined by gas-liquid chromatography. At incubation periods of 2 and 4 hr, no effect on RNA synthesis was seen at concentrations of 1 and 7 ppm. Tests with concentrations above 20 ppm showed very strong inhibition of RNA synthesis (up to 90%) corresponding to 10% of the blank incorporation.

DISCUSSION

The reported results show that both dieldrin and PCB (Aroclor 1242) can significantly inhibit the RNA synthesis of PE cells. The degree of inhibition depends both on chemical concentration and on duration of incubation. The threshold values for a significant inhibition are 0.1 ppm and 8 hr for dieldrin. Apparently the effect of dieldrin is enhanced when macrophages are actively phagocytosing, which might be explained by increased uptake of the chemical by pinocytosis as well as by

TABLE 3

[^3H]URIDINE INCORPORATION OF RESTING MACROPHAGES IN THE PRESENCE OF Aroclor 1242^a

Incubation time (hr)	Aroclor 1242 concentration (ppm)	RNA incorporation (%)	Total incorporation (%)
2	1.0	99.1	87.6
	28.3	13.0	28.3
4	1.0	93.0	—
	7.25	99.0	87.6
	22.6	7.9	26.3
	25.0	14.9	27.0
8	0.1	116.9	122.6

^a Specific incorporation, bound ^3H cpm/ μg or bound ^3H cpm/mg protein, respectively, is expressed as a percentage of the corresponding blank samples.

adsorption to the phagocytosed particles. Inhibition of RNA synthesis by PCB (Aroclor 1242) was not seen until a concentration of approximately 7 ppm was exceeded in the culture medium containing the PE cells. The inhibition of RNA synthesis in the PE cells did not appear to be as time dependent as that seen when dieldrin was used. In addition, at a concentration of 0.1 ppm PCB a slight stimulation of RNA synthesis occurred (Table 3).

Previous studies have demonstrated that PCB and hexachlorobenzene (HCB) influence humoral immunity (Loose *et al.*, 1977), host resistance to endotoxin and malaria (Loose *et al.*, 1978), and cell-mediated immunity (Silkworth and Loose, 1978). Immunological alterations occurred in the absence of any light microscopic changes in lymphoid-macrophage tissues. However, a high concentration of both compounds was found in the same tissues examined histologically. For this research, it was suggested that the presence of the chemical induced a functional alteration without any concomitant morphological change in the lymphoid tissues. Since the tissues of interest, i.e., liver, lung, and spleen, also contain the greatest population of fixed tissue macrophages and since macrophage participation is an integral component in the production of antibody and host defense to endotoxin and malaria, it was suggested that macrophage function may be compromised by the chemicals (PCB and HCB). Therefore, the present study was conducted to ascertain the influence of PCB (Aroclor 1242) and dieldrin on RNA synthesis, an essential component of macrophage biochemical and functional integrity.

As was demonstrated, the inhibition of RNA synthesis by PCB and dieldrin appeared to be dose and time related. Further studies will be conducted to determine the relationship between the intracellular concentration of the xenobiotics relative to the rate of RNA synthesis in the macrophages in an attempt to describe a possible mechanism of action. These studies will also determine the reversibility of the inhibition and the cell specificity.

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