indicated a significant difference between the means ($p < 0.05$, repeated t-test, right side of the fig.). A substantial improvement in symptoms, as measured by a modified comprehensive psychopathological rating scale[9] (CPRS), was observed after mianserin treatment. Interestingly, no improvement was noted in the patient who did not show a decrease in $\beta$-endorphin immunoreactivity.

Present findings suggest that mianserin may interact with $\beta$-endorphin release from the pituitary and/or its peripheral metabolism. This peptide itself has been tested in depressed patients, but its anti-depressant effectiveness remains questionable[10].

Indirect evidence that mianserin may act as an antagonist of $\beta$-endorphin has recently been provided[11]. In fact, treatment with mianserin prevents the hypotensive effect of peripheral administration of $\beta$-endorphin in rats, possibly by an interaction with serotoninergic transmission.

In view of the possible role of opioids in the etiology of depression, the effect of mianserin on the endorphin system described here could be of relevance for its anti-depressant activity. Interestingly, a reduction in plasma $\beta$-endorphin levels has also been observed in depressed patients after desimipramine treatment[12].

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## Inhibition by carbaryl of DNA, RNA and protein synthesis in cultured rat lung cells[1,2]

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*Summary.* The carbamate pesticide carbaryl rapidly inhibited DNA, RNA and protein synthesis in L-2 cells from rat lung. The inhibiton was partly reversible and was not accompanied by inhibiton of transport of tritiated precursors into intracellular pools or destruction of the integrity of the cell membrane.

The pesticide carbaryl (1-naphthyl-N-methylcarbamate) (C.A.S. 000063252) is used on nearly 100 food, forage and other crops[3]. Because of extensive use of carbaryl in agriculture and for domestic insect control, large numbers of people may be exposed to the pesticide by inhalation. In additon, smokers may inhale small quantities of carbaryl residues in cigarette smoke (less than 1 µg/cigarette[4]). We tested the effects of carbaryl on macromolecule synthesis in lung cells as part of a project aimed at evaluating the biological effects of carbaryl by short-term in vivo and in vitro bioassays.

*Materials and methods.* Carbaryl was extracted with acetone from the commercial formulation Sevin, recrystallized from hexane-acetone solution, and dissolved in dimethyl sulfoxide (DMSO). L-2 cells, a cell line of Type II pneumonocytes from rat lung[5], were used in these studies. L-2 cells retain differentiated functions in vitro, continuing to synthesize lecithin, produce multi-lamellar osmiophilic bodies[6], and make high levels of certain inducible enzymes[7]. L-2 cells were grown in F12K medium[8] (Grand Island Biological Co.) supplemented with 10% fetal bovine serum, penicillin, and streptomycin and were incubated at 37 °C in 5% $CO_2$ in air.

In toxicity studies, cells were harvested by trypsinization 24 h after addition of carbaryl or DMSO (solvent control) and numbers of viable cells were determined by the trypan blue dye exclusion method[9]. The effects of carbaryl on DNA, RNA and protein synthesis were examined by comparing the incorporation of tritiated precursors, $^3$H-methyl-thymidine, 5,6,-$^3$H-uridine and L-(3,4,5-$^3$H(N))-leucine (New England Nuclear, NEN), into trichloroacetic acid (TCA)-insoluble material in control and treated cultures at various intervals after addition of treatments. At the end of the labeling period, the cells were rinsed with cold phosphate buffered saline (PBS) containing the corresponding unlabeled precursor, scraped off the plates, and

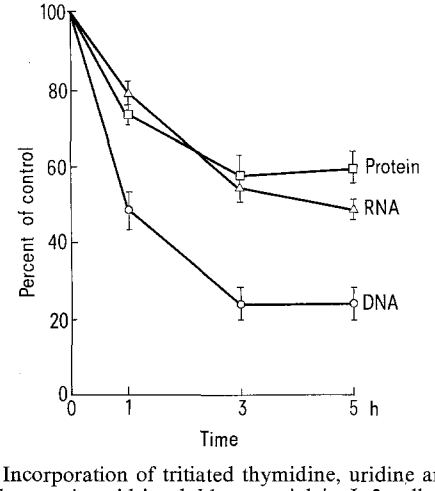


Figure 1. Incorporation of tritiated thymidine, uridine and leucine into trichloroacetic acid-insoluble material in L-2 cells after 1, 3 and 5 h of exposure to $8 \times 10^{-5}$ M (16 ppm) carbaryl. Cultures in triplicate were pulse-labeled with 1 µCi of $^3$H-thymidine (56.4 Ci/mM), 1 µCi of $^3$H-uridine (41.3 Ci/mM) or 2 µCi of $^3$H-leucine (147.0 Ci/mM) per ml of medium for 20 min at each time point.

collected on GF/A glass fiber filters (Whatman) in cold 10% TCA. Filters were rinsed with TCA and methanol and radioactivity was counted in Omnifluor (NEN) by liquid scintillation spectrometry.

To study the effects of treatments on transport of thymidine, uridine and leucine into the cells, cultures were treated with $8\times10^{-5}$ M carbaryl and pulse-labeled for 30 sec at 1, 3 and 5 h after treatment by addition of warm PBS containing tritiated precursors. The cells were rinsed, scraped off, collected by centrifugation, and lysed in 0.5 ml of ice water. An equal volume of cold 20% TCA was added and the samples were held on ice for 30–40 min with occasional mixing. Samples were centrifuged and the radioactivity in aliquots of the supernatant solution was counted in Aquasol (NEN).

*Results and discussion.* Treatment with carbaryl for 24 h caused a dose-related decrease in cell viability. Carbaryl at $5\times10^{-5}$ M (10 ppm) reduced the number of viable cells to 50% of the control number. $2\times10^{-5}$ M (4 ppm) carbaryl was only slightly toxic; $2.5\times10^{-4}$ M (50 ppm) carbaryl reduced the number of cells by 80%.

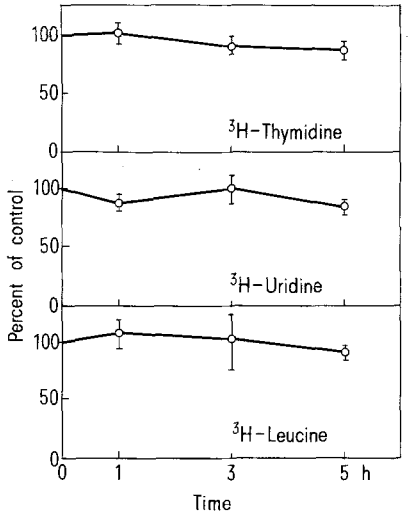


Figure 2. Uptake of tritiated thymidine, uridine and leucine into the acidin-soluble fraction of L-2 cells during 30-sec pulses after 1, 3 of 5 h of exposure to $8\times10^{-5}$ M (16 ppm) carbaryl. Quadruplicate cultures were supplemented with 5 µCi of $^3$H-thymidine ($9.1\times10^{-6}$ M), 5 µCi of $^3$H-uridine ($1.2\times10^{-5}$ M) or 10 µCi of $^3$H-leucine ($6.4\times10^{-6}$ M) per ml of medium. Uptake into control cultures was 1000–1600 cpm.

Carbaryl inhibited macromolecule synthesis in L-2 lung cells, as reported for HeLa[10] and Ehrlich ascites tumor cells[11]. Exposure to $8\times10^{-5}$ M carbaryl for 1 h reduced DNA synthesis by about 50%. This concentration was used to compare the effects of carbaryl on synthesis of DNA, RNA and protein after 1, 3 and 5 h of exposure (fig. 1). Rates of RNA and protein synthesis decreased in parallel with DNA synthesis, but DNA synthesis was more severely inhibited. Additional experiments showed that the rates of synthesis of all 3 macromolecules were reduced within 20 min of application of carbaryl. Cultures treated for 3 h, washed and refed with fresh medium had regained 50–80% of the control rate of macromolecule synthesis when tested 3 h after refeeding.

In tests to determine whether inhibition of macromolecule synthesis was caused by a decrease in transport into intracellular pools, low concentrations of tritiated precursors in the medium and labeling for only 30 sec were used to minimize the effects of diffusion. Our results (fig. 2) indicate that there was no inhibition of transport of thymidine, uridine or leucine into the cells even after 5 h of exposure to $8\times10^{-5}$ M carbaryl, a concentration that severely inhibited incorporation into DNA, RNA and protein. Furthermore, these cells did not show increased staining with trypan blue, indicating that the integrity of the cell membrane was not destroyed. The mechanism for inhibition of macromolecule synthesis remains to be elucidated.

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## Effect of 3-methylcholanthrene on RNA polymerase and protein kinase activities of transcriptionally active chromatin subfraction in rat liver

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*Summary.* The activities of RNA polymerases and nuclear protein kinases were determined in subfractions of rat liver nuclei after a single dose of the polycyclic aromatic hydrocarbon 3-methylcholanthrene (3-MC). Both enzyme activities as well as the in vivo phosphorylation of chromatin proteins were enhanced by 3-MC in the transcriptionally active subfraction of chromatin.

The induction of microsomal aryl hydrocarbon hydroxylase activity by 3-MC is thought to be the most important step in the metabolic activation of polycyclic aromatic hydrocarbons to their ultimate carcinogenic forms[3–5]. The inducing action of 3-MC seems to be regulated mainly at the transcriptional level: RNA polymerase activity, chromatin template activity, the incorporation of orotic acid into nuclear and cytoplasmic RNAs in rat liver are significantly