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 8×10^{-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 5×10^{-5} M (10 ppm) reduced the number of viable cells to 50% of the control number. 2×10^{-5} M (4 ppm) carbaryl was only slightly toxic; 2.5×10^{-4} M (50 ppm) carbaryl reduced the number of cells by 80%.

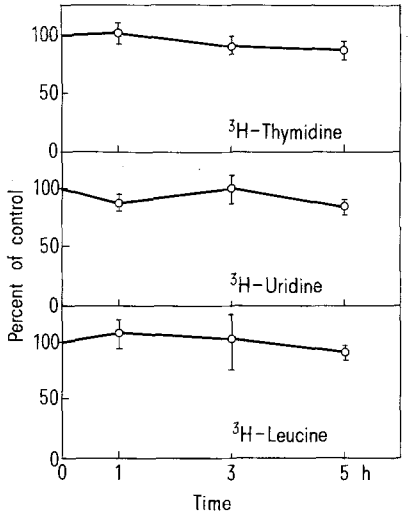

Figure 2. Uptake of tritiated thymidine, uridine and leucine into the acidin-soluble fraction of L-2 cells during 30-sec pulses after 1, 3 of 5 h of exposure to 8×10^{-5} M (16 ppm) carbaryl. Quadruplicate cultures were supplemented with 5 µCi of ^{3}H-thymidine (9.1×10^{-6} M), 5 µCi of ^{3}H-uridine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

1 We thank W.H.J. Douglas for his gift of L-2 cells.
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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

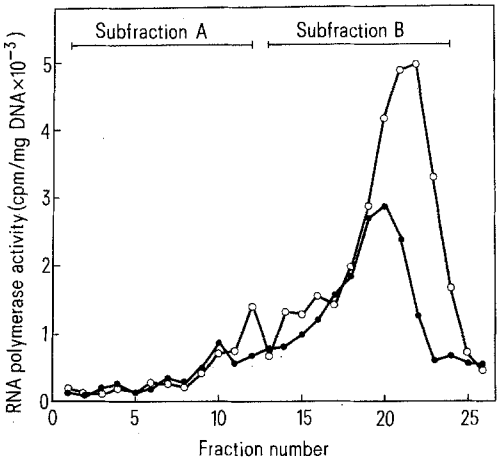

Figure 1. RNA polymerase activity of hepatic nuclear subfractions of control (●——●) and 3-MC treated (○——○) young rats.

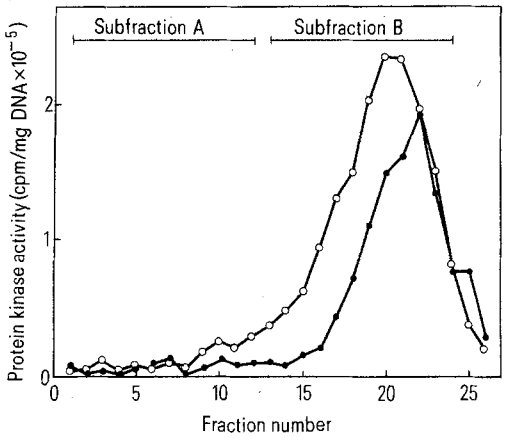

Figure 2. Protein kinase activity of hepatic nuclear subfractions of control (●——●) and 3-MC treated (○——○) young rats.

elevated after a single i.p. dose of 3-MC[6]. It was shown in our laboratory that the activation of the free and template-engaged forms of RNA polymerases showed different patterns in the nucleolar and extranucleolar fractions[7] and that the activities of nuclear protein kinases, which may play a key role in the regulation of gene expression, were also enhanced by 3-MC treatment[8]. These biochemical events are accompanied by a pronounced hypertrophy of the nucleoli[9].

The present experiments were designed to investigate the effect of 3-MC on the extent of in vivo protein phosphorylation, RNA polymerase and protein kinase activities in chromatin subfractions of different transcriptional activities.

Materials and methods. 10-day-old CFY rats weighing 20–30 g were given a single i.p. dose of 3-MC (100 mg/kg b.wt) 24 h prior to being sacrificed. The control animals received only the solvent (10 ml of corn oil/kg b.wt).

Liver nuclei were isolated by the method of Chauveau et al.[10]. Fractionation of nuclei into transcriptionally active and inactive subfractions was carried out according to Webster et al.[11]. Briefly, pure nuclear fractions were washed with a buffer containing 0.5 M sucrose, 0.2% Triton X-100, 0.05 M Tris, 0.025 M KCl, 0.002 M $MgCl_2$, pH 7.5, and then with a solution containing 0.08 M NaCl and 0.02 M EDTA. Washed nuclear pellets were suspended in 5.0 ml of 0.01 M Tris, pH 8.0 and sonicated. Sheared

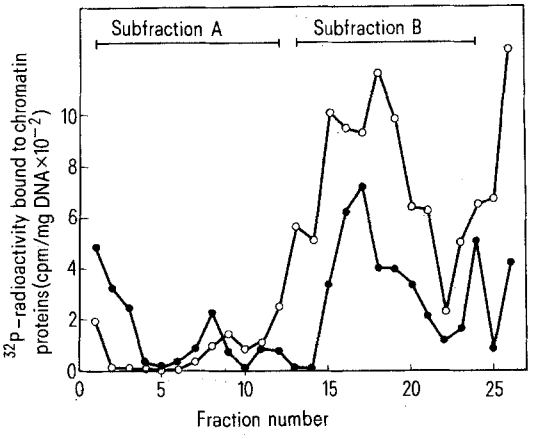

Figure 3. In vivo phosphorylation of chromatin proteins in hepatic nuclear subfractions of control (●——●) and 3-MC treated (○——○) young rats.

nuclear suspensions from control and 3-MC treated animals corresponding to 5 mg chromatin protein were layered over 0.17–1.7 M linear sucrose gradients (in 0.01 M Tris, pH 8.0). Gradients were centrifuged for 5 h at 24,000 rpm in the SW 27 Beckman rotor. Gradient fractions were collected from the bottom of the tubes and tested for endogenous RNA polymerase activity[12] and protein kinase activity using caseine as phosphate acceptor[13]. In vivo phosphorylation of the proteins of the chromatin subfractions was studied after i.p. injection of the animals with 13 MBq [^{32}P] orthophosphate 2 h before killing. Protein bound phosphate in gradient fractions was determined by measuring the trichloroacetic acid precipitable radioactivity after excessive DNase and RNase digestion. Protein and DNA concentrations were determined by the method of Lowry[14] and that of Burton[15], respectively.

Results and discussion. Selective binding of strong polycyclic aromatic carcinogens to the transcriptionally active chromatin subfractions of AKR mouse embryo cells was demonstrated by Spelsberg and coworkers[16–18]. About 84% of ^{3}H-3-MC binds to the transcriptionally active subfractions of isolated rat liver nuclei (unpublished observation of the authors). Thus it seems to be highly probable that the specific macromolecular target(s) which is/are responsible for mediation of the inducing effect of polycyclic aromatic hydrocarbons is/are localized in the active chromatin regions.

It was demonstrated in the present experiments that using sucrose gradient centrifugation for the fractionation of sheared nuclei most of the activities of RNA polymerases (fig. 1) and nuclear protein kinases (fig. 2) were present in the slow-sedimenting nuclear subfraction containing actively transcribed chromatin (designated subfraction B on the sedimentograms). The majority of phosphoproteins phosphorylated in vivo were also localized in this chromatin subfraction (fig. 3).

Treatment with 3-MC caused stimulation of the RNA polymerases (fig. 1) and protein kinases (fig. 2) mainly in the transcriptionally active chromatin subfraction, whereas the enzyme activities of the fast-sedimenting nuclear subfraction containing mostly inactive chromatin (subfraction A on the figures) remained near to the low level characteristic of the control nuclei. The enhancement of in vivo protein phosphorylation is also restricted to the active subfraction (fig. 3). Thus it is concluded that RNA polymerases, protein kinases and nuclear phosphoproteins may be among the candidates for nuclear target macromolecules mediating the inducing action of 3-MC at the level of gene expression.

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Subcellular distribution of mercury in liver of lake trout *(Salvelinus namaycush)*[1]

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Summary. Mercury was found primarily (80%) in the submicroscopic material (magnification $\times < 7700$) of environmentally exposed adult lake trout from Lake Michigan, USA.

Determination of subcellular distribution of contaminants in fish holds promise as a means of predicting metabolic pathways most likely affected by toxicants and of predicting possible modes of detoxification. Heavy metals have been reported to be sequestered in the cytosol of fish liver as protein-bound metallothioneins. Studies thus far have involved short-term laboratory exposure[3,4]. The objective of our work was to determine the subcellular distribution of mercury in liver of environmentally exposed lake trout, *Salvelinus namaycush* (Walbaum), from Lake Michigan (USA). Lake trout contain high levels of common Great Lakes contaminants[5].

Two adult lake trouts were captured by gill net in Lake Michigan near Saugatuck, Michigan (42°41′N, 86°18′W). A male (2640 g, 643 mm, 5 years old) collected in October 1975 was killed and the liver was immediately removed, frozen, and stored at −20 °C. A female (4400 g, 732 mm, 7 years old) collected in September 1977 was transported alive to the laboratory, where it was killed by a blow to the head and the fresh liver sampled. Subcellular fractions of liver were prepared by differential centrifugation in sucrose as described by Hogeboom[6] except that frozen liver was homogenized in room-temperature sucrose to quick-thaw the tissue[7]. Subcellular fractions for electron microscopy were preserved in 2% phosphate buffered glutaraldehyde and fractions for contaminant analysis were frozen and stored at −20 °C. Electron microscopy preparation[8] and examination of fractions were performed by Clinical Laboratories, Veterans Administration Medical Center, Ann Arbor. They determined the average percent volume of each cell organelle in each fraction using 100 points in 10–15 electron micrographs of each fraction[9]. We measured total mercury (inorganic and organic) of subcellular fractions and whole fresh liver by a combustion-amalgamation technique[10].

Of the total mercury in fresh liver, 69% was present in the soluble fraction (table 1). The value of 1.2 µg total mercury/g (table 1) was confirmed by analysis of samples of whole liver. In frozen liver, 58% of the mercury was in the soluble fraction, 19% in the nuclear fraction, and 14% in the microsomal fraction; however, data from frozen liver were not used for further calculations because electron microscopy revealed that the mitochondria were ruptured. Of the polychlorinated biphenyls and *p,p′*DDE (saponified *p,p′*DDT plus *p,p′*DDE), 96 to 97% were in the soluble fraction of this frozen lake trout liver, but these data should be considered preliminary since we have no data for organochlorine distribution in fresh liver.

We calculated the proportion of mercury in each cell organelle in fresh liver by multiplying the proportion of mercury in each fraction (table 1) by the proportion of each organelle in the same fraction (table 2) and summing across all fractions for a particular organelle (table 3). The SE of the proportion was calculated according to Cochran[11]. These calculations assume that the distribution of mercury is proportional to the volume representation of the different organelles. The highest proportion of mercury (0.799) was in the submicroscopic material, i.e., material not visible at magnification × 7700.

Our determination of subcellular distribution of mercury in liver is the only study of fish exposed environmentally for several years – assuredly long enough for the distribution of the metal in tissues and subcellular organelles to reach equilibrium. After exposure of rainbow trout *(Salmo gairdneri)* for 24 h to methyl mercury chloride in water, Olson et al.[4] reported that mercury was highest in the cytosol

Table 1. Total mercury (wet wt) present in subcellular fractions of fresh liver of Lake Michigan lake trout

Subcellular fraction	Total mercury µg/g[a]	Proportion
Nuclear fraction	0.110	0.0917
Heavy mitochondria	0.064	0.0534
Light mitochondria	0.052	0.0434
Microsomal fraction	0.149	0.1243
Soluble fraction	0.824	0.6872
Total	1.199	1.0000

[a]Calculated as µg/g of whole liver (wet wt).