Nickel Carbonyl Inhibition of ^{14}C-Orotic Acid Incorporation into Rat Liver RNA* (33867)

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Earlier studies demonstrated that administration of nickel carbonyl, $Ni(CO)_4$, to rats by inhalation or intravenous injection blocks the induction of enzymes in lung and liver (1–4). Nickel carbonyl was shown to inhibit phenobarbital induction of aminopyrine demethylase (1) and cytochrome P-450 (2), and to inhibit phenothiazine induction of benzpyrene hydroxylase (3). Nickel carbonyl prevents the induction of hepatic tryptophan pyrrolase by cortisone but not by trytophan (4). In our most recent investigation, there was 60% inhibition of DNA-dependent RNA-polymerase activity in hepatic nuclei at 24 hr after iv injection of $Ni(CO)_4$ in LD_{50} dosage (5). These observations suggested that $Ni(CO)_4$ inhibits the synthesis of hepatic RNA. To test this hypothesis, we undertook the present measurements of the effect of $Ni(CO)_4$ upon *in vivo* incorporation of ^{14}C-orotic acid into rat liver RNA.

Methods. The experimental animals were 29 male rats of the Sprague-Dawley strain, weighing from 180–200 g. The rats were fasted for 24 hr before sacrifice. Nickel carbonyl was administered iv in LD_{50} dosage of 5 μl of $Ni(CO)_4$/100 g of body weight (equivalent to 2.2 mg of Ni/100 g) 24 hr before sacrifice. The $Ni(CO)_4$ was injected into a tail vein by means of a 10-μl syringe, as previously described (6, 7). In one set of experiments, the rats were also given phenobarbital (10 mg/100 g, ip) 20 hr before sacrifice. This dosage of phenobarbital produced a 30% increase in RNA polymerase activity in hepatic nuclei (5, 8). All of the rats received ^{14}C- orotic acid in dosage of 45 μg (10 μCi)/100 g, ip, 40 min before sacrifice. The rats were killed by guillotine and were exsanguinated. The livers were washed in cold isotonic sodium chloride solution. Determinations of ^{14}C incorporation into liver RNA were performed as described by Roberts and Warwick (9). The radioactivity of the isolated RNA was measured by liquid scintillation counting with internal standardization. The ribose content of the RNA was analyzed by Ceriotti's orcinol procedure (10).

Results. The results (Table I) are expressed as micromoles of ^{14}C-orotic acid incorporated into RNA per mole of RNA ribose, and as micromicromoles of ^{14}C-orotic acid incorporated into RNA per gram of liver. By both methods of computation, $Ni(CO)_4$ significantly inhibited and incorporation of ^{14}C-orotic acid into RNA of rat liver, with or without phenobarbital pretreatment. The phenobarbital administration did not produce any significant effect upon *in vivo* incorporation of ^{14}C-orotic acid into hepatic RNA.

Discussion. Previous reports from our laboratory elucidated the metabolism and excretion of $^{63}Ni(CO)_4$ (6, 7). The epidemiological and experimental evidence which implicated $Ni(CO)_4$ as a carcinogen was summarized in a recent publication (14). In the present work, injection of $Ni(CO)_4$ in rats produced marked inhibition of the incorporation of a pyrimidine precursor into hepatic RNA. This finding is consistent with the previous observation that $Ni(CO)_4$ inhibits hepatic RNA-polymerase activity (5), and supports the hypothesis that $Ni(CO)_4$ inhibits synthesis of hepatic RNA. An attempt is currently being made in our laboratory to ascertain whether $Ni(CO)_4$ inhibition of hepatic

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TABLE I. Effect of Nickel Carbonyl upon Orotic Acid Incorporation into Hepatic RNA.

No. of rats	$Ni(CO)_4$ treatment[a]	Phenobarbital treatment[b]	^{14}C-Orotic acid[c] (mmoles/mole of ribose) Mean ± SD	Range	p vs control	^{14}C-Orotic acid[c] ($\mu\mu$moles/g of liver) Mean ± SD	Range	p vs control
9	−	−	318 ± 133	175–564	—	193 ± 89	86–349	—
8	+	+	81 ± 23	38–109	<0.01	71 ± 71	14–187	<0.01
6	−	+	292 ± 94	153–399	—	159 ± 68	89–280	—
6	+	+	164 ± 62	98–276	<0.02	77 ± 29	41–100	<0.02

[a] Nickel carbonyl, iv, 2.2 mg of Ni/100 g, 24 hr prior to sacrifice.

[b] Phenobarbital, ip, 10 mg/100 g, 20 hr prior to sacrifice.

[c] ^{14}C-Orotic acid, ip, 45 μg (10 μCi)/100 g, 40 min prior to sacrifice.

RNA synthesis is produced by a direct inhibition of RNA-polymerase, or whether it is an indirect result of alterations of liver chromatin or DNA.

Summary. Nickel carbonyl, a metallic carcinogen inhibited ^{14}C-orotic acid incorporation into liver RNA. Forty min after an ip injection of ^{14}C-orotic acid, the mean specific activity of RNA from livers of control rats was 318 (SD, ± 133) μmoles of ^{14}C/mole of ribose. In rats which also received an intravenous injection of nickel carbonyl (2.2 mg of Ni/100 g) 24 hr before sacrifice, the mean specific activity of liver RNA was 81 (SD, ± 23) μmoles of ^{14}C/mole of ribose (p vs controls = <0.01). The incorporation of ^{14}C-orotic acid into liver RNA was not significantly affected by injection of phenobarbital (10 mg/100 g, ip) 20 hr before sacrifice. This work supports the hypothesis that nickel carbonyl inhibits the synthesis of hepatic RNA.

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