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THE TOXICOLOGY AND METABOLISM OF NICKEL COMPOUNDS

PROGRESS REPORT

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ABSTRACT OF PROGRESS REPORT

The toxicology and metabolism of nickel compounds [e.g., NiCl_2 , $\alpha\text{Ni}_3\text{S}_2$, and $\text{Ni}(\text{CO})_4$] were investigated in rats and hamsters. The new knowledge that was acquired includes the following:

1. Documentation that nickel carbonyl [$\text{Ni}(\text{CO})_4$] is teratogenic and embryotoxic in hamsters.
2. Observation that measurements of urine beta-2 microglobulin concentrations may serve as a diagnostic test for nephrotoxicity in nickel-exposed workers.
3. Documentation that exposure of rats to $\text{Ni}(\text{CO})_4$ inhibits thymidine- ^3H uptake into DNA in regenerating liver; that sucrose gradient ultracentrifugation of hepatic DNA from $\text{Ni}(\text{CO})_4$ -treated rats does not reveal DNA fragmentation and that parenteral administration of NiCl_2 to rats inhibits thymidine- ^3H uptake into kidney DNA.
4. Development of a new experimental model for chemical induction of ocular neoplasms in rats by intraocular injection of $\alpha\text{Ni}_3\text{S}_2$.
5. Demonstration that admixture of Mn dust suppresses induction of local sarcomas following im injection of benzo(a)pyrene, as well as following im injection of $\alpha\text{Ni}_3\text{S}_2$.
6. Refinement of a sensitive gas chromatographic assay for heme oxygenase activity in renal microsomes, to be used for studies of renal effects of nickel compounds.
7. Identification of several factors that influence the induction of erythrocytosis following intrarenal injection of $\alpha\text{Ni}_3\text{S}_2$ in rats. For example, $\alpha\text{Ni}_3\text{S}_2$ -induced erythrocytosis is suppressed by concurrent ip infusion of sodium diethyldithiocarbamate, but not by d-penicillamine.
8. Development of high-resolution methods for separation and identification of ^{63}Ni -binding proteins by agarose gel electrophoresis and isoelectric focusing, with detection of ^{63}Ni by autoradiography.
9. Development of a reference method for analysis of nickel in serum and urine by electrothermal atomic absorption spectrophotometry, in order to achieve international harmonization of nickel determinations that are used to monitor occupational and environmental exposures to nickel compounds.

THE TOXICOLOGY AND METABOLISM OF NICKEL COMPOUNDS

PRINCIPAL INVESTIGATOR

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SCIENTIFIC ACCOMPLISHMENTS DURING THE PAST YEAR

A. Teratogenicity of Nickel Carbonyl in Hamsters. Last year's annual report included preliminary findings that exposure of pregnant hamsters to inhalation of $\text{Ni}(\text{CO})_4$ causes a variety of congenital anomalies in the progeny. This investigation has now been completed. Nickel carbonyl was administered to groups of pregnant hamsters by inhalation (0.06 mg $\text{Ni}(\text{CO})_4$ /litre/15 min) on days 4, 5, 6, 7, or 8 of gestation. The dams were killed on day 15 of gestation and the fetuses were examined for malformations. Exposure to $\text{Ni}(\text{CO})_4$ on days 4 or 5 of gestation resulted in malformations in 5.5% (8/146) and 5.8% (10/171) of the progeny, respectively, ($P < 0.05$, versus 0/95 in controls). The proportions of litters with malformed fetuses were 33% (4/12) and 24% (4/17) in dams exposed to $\text{Ni}(\text{CO})_4$ on days 4 and 5 of gestation ($P < 0.05$, versus 0/9 in litters of control dams). Progeny of dams exposed to $\text{Ni}(\text{CO})_4$ on days 4 and 5 included 9 fetuses with cystic lungs, 7 fetuses with exencephaly, 1 fetus with exencephaly plus fused rib, and 1 fetus with anophthalmia plus cleft palate. Hemorrhages into serous cavities were found in 18% (26/146) and 25% (42/171) of fetuses of dams exposed to $\text{Ni}(\text{CO})_4$ on days 4 or 5 of gestation. Such hemorrhages were not observed in controls. In progeny of dams exposed to $\text{Ni}(\text{CO})_4$ on days 6 or 7 of gestation, there was 1 fetus with fused ribs and there were 2 fetuses with hydronephrosis. In another experiment, pregnant hamsters were exposed to inhalation of $\text{Ni}(\text{CO})_4$ (0.06 mg/litre/15 min) on day 5 of gestation; these dams were permitted to deliver their litters and to nurse their pups. On the day of delivery, there was no significant difference in the average number of live pups in the $\text{Ni}(\text{CO})_4$ -exposed litters compared to control litters. Neonatal mortality was increased in $\text{Ni}(\text{CO})_4$ -exposed litters; by day 4 postpartum, the number of live pups averaged 7.6 ± 1.5 in $\text{Ni}(\text{CO})_4$ -exposed litters ($P < 0.01$ versus 9.6 ± 1.8 pups in control litters). The observation that $\text{Ni}(\text{CO})_4$ is teratogenic and embryotoxic in Syrian hamsters, and our previous observation that prenatal exposure of rats to $\text{Ni}(\text{CO})_4$ causes anophthalmia and microphthalmia, establishes that $\text{Ni}(\text{CO})_4$ is teratogenic and embryotoxic in two animal species. These findings were reported at the International Symposium on Arsenic and Nickel which was held in Jena, DDR, in July 1980 (abstract (1) submitted herewith), and a paper (2) (submitted herewith) that describes this research has been submitted for publication in "Teratogenesis, Mutagenesis and Carcinogenesis". As a continuation of this avenue of research, the mutagenicity of $\text{Ni}(\text{CO})_4$ in Fischer rats is now being tested in Fischer rats by the dominant lethal mutation test. Experiments to date have indicated that exposure of male rats to inhalation of $\text{Ni}(\text{CO})_4$ causes diminished fertility.

B. Kinetics of Ni[II] Metabolism. Compartmental analyses of Ni[II] metabolism and elimination have been performed in several studies in this research program during the past 4 years. The analyses of Ni[II] kinetics in terms of mathematical models have been compiled in an invited review article (3) (submitted herewith) that will be included in a book on "Nickel in the Environment" (J.O. Nriagu, Editor), to be published by Wiley Interscience Press.

C. Nephrotoxicity of Nickel Compounds in Animals and Man. Preliminary data that demonstrated nephrotoxicity of $\text{Ni}(\text{CO})_4$ in rats were included in last year's annual report. This investigation was completed during the present grant year. Urinary excretions of protein and amino acids were measured before and after exposure of rats to inhalation of nickel carbonyl in LD_{50} dosage (0.06 mg/litre of air/15 min). Urinary excretion of total proteins was increased by an average of 2.4-fold on the first day after exposure and remained elevated (2.0-fold and 1.9-fold) on the second and third days. Urinary excretions of 16 neutral and basic amino acids were all increased on the third day. In comparison to pre-exposure values, the increased excretion of neutral and basic amino acids averaged 3.0-fold, and ranged from 1.2-fold (ornithine) to 7.3-fold (valine). Among the acidic amino acids and amides, only the excretion of glutamic acid was increased (4.6-fold), and the excretions of glutamine, aspartic acid, and asparagine were reduced respectively to 3.8%, 64%, and 52% of the pre-exposure values. Urinary excretion of ammonia was greatly increased after exposure to nickel carbonyl. The alterations of urinary excretions of amino acids were apparently mediated by nephrotoxicity rather than by mobilization of amino acids from tissues, since plasma concentrations of amino acids were not significantly affected by exposure to nickel carbonyl. The pronounced diminution of glutamine excretion and the marked increase of ammonia excretion were consistent with enhanced renal production of ammonia from glutamine by action of glutaminase. A paper (4) (submitted herewith) has been accepted for publication in "Annals of Clinical and Laboratory Science".

To investigate the possible nephrotoxicity of nickel exposures in man, measurements of beta-2 microglobulin concentrations were performed in urine from 38 healthy adults without occupational exposure to nickel; in urine from 30 men who work in an electrolytic nickel refinery, and in the urine from 18 men who perform nickel electroplating. In the healthy adults, urine beta-2 microglobulin concentrations averaged 0.074 mg/litre (range = 0.01 to 0.19 mg/litre). Beta-2 microglobulin concentrations exceeded 0.19 mg/litre in 7 of 30 nickel refinery workers, and in 0 of 18 nickel plating workers (Table 1). Urine beta-2 microglobulin concentrations correlated with urine nickel concentrations in the nickel refinery workers ($r = 0.65$). These findings indicate that measurements of urine beta-2 microglobulin may be useful to monitor nickel-induced nephropathy in refinery workers. An abstract (5) (submitted herewith) that describes these observations was presented in May 1980 at the Boston Meeting of the Association of Clinical Scientists. These data will be included in a manuscript that will be submitted for publication within the next 6 months.

TABLE 1. URINE BETA-2 MICROGLOBULIN (B₂M) CONCENTRATIONS IN NICKEL-EXPOSED WORKERS

Category	No. of Subjects	Urine Ni µg/litre Mean (range)	Urine B ₂ M mg/litre Mean (range)	Subjects with Urine B ₂ M > 0.19 mg/litre
Nickel Electro- plating workers	18	6.1 (0.9-25)	0.068 (0.01-0.18)	0/18
Nickel refinery workers	30	654 (1.2-14,000)	0.112 (0.01-0.45)*	7/30**
Refinery workers (with urine Ni < 100 µg/litre)	19		0.089 (0.01-0.20)	2/19
Refinery workers (with urine Ni > 100 µg/litre)	11		0.152 (0.01-0.45)*	5/11**

* $p < 0.05$ by Mann-Whitney test; ** $p < 0.05$ by Fisher's exact test.

D. Effects of Nickel Compounds on DNA Synthesis. Experiments on $\text{Ni}(\text{CO})_4$ inhibition of ^3H -thymidine incorporation into hepatic DNA were begun at the end of the previous grant year. The experiments on $\text{Ni}(\text{CO})_4$ have been completed, as well as similar studies of the effects of $\alpha\text{Ni}_3\text{S}_2$ and NiCl_2 . *In vivo* incorporation of thymidine- ^3H into DNA was determined in rats at 28 hr after partial hepatectomy. Administration of nickel carbonyl ($\text{Ni}(\text{CO})_4$) at 2 or 4 hr before sacrifice inhibited thymidine- ^3H uptake into liver and kidney DNA. For example, in rats killed 4 hr after iv injection of $\text{Ni}(\text{CO})_4$ (2 mg Ni/100 g), ^3H -labelling of liver DNA averaged 54 (SE $\pm$ 10)% of controls ($p < 0.05$), and ^3H -labelling of kidney DNA averaged 53 (SE $\pm$ 6)% of controls ($p < 0.01$). Injection of NiCl_2 (2 mg Ni/100 g, im) 4 hr before death did not significantly affect thymidine- ^3H uptake into liver DNA, but did inhibit thymidine- ^3H uptake into kidney DNA ($65 \pm 6\%$, $p < 0.02$). Intrarenal injection of $\alpha\text{Ni}_3\text{S}_2$ (6 mg Ni/100 g) on the 14th day before death was attended by a statistically insignificant reduction (21%) of mean thymidine- ^3H uptake into kidney DNA. Binding of ^{63}Ni to DNA in liver and kidney of rats killed 4 hr after injection of $^{63}\text{Ni}(\text{CO})_4$ or $^{63}\text{NiCl}_2$ ranged from 0.3 to 2.2 mol ^{63}Ni /mol of DNA nucleotides. Ultracentrifugation of DNA on alkaline sucrose gradients did not reveal any differences between sedimentation profiles of hepatic DNA from $\text{Ni}(\text{CO})_4$ -treated rats versus paired control rats. A manuscript (6) (submitted herewith) that describes these observations has been published in "Carcinogenesis".

E. Carcinogenesis Experiments with Nickel Compounds. In collaboration with Dr. Daniel Albert of the Ocular Pathology Department at Massachusetts Eye and Ear Infirmary, a technique has been developed for carcinogenesis testing of nickel compounds by intraocular injection. Nickel subsulfide, $\alpha\text{Ni}_3\text{S}_2$, was administered to Fischer rats by a single injection into the vitreous body of the right eye (0.5 mg $\alpha\text{Ni}_3\text{S}_2$ /rat, suspended in 20 μl of NaCl vehicle). Control rats received a similar injection of the vehicle. Malignant tumors developed in the injected eyes of 14/15 $\alpha\text{Ni}_3\text{S}_2$ -treated rats by 8 months (versus 0/11 controls, $P < 0.001$). Five of the injected eyes of $\alpha\text{Ni}_3\text{S}_2$ -treated rats contained multiple tumors. The 22 eye tumors that were induced by $\alpha\text{Ni}_3\text{S}_2$ included 11 melanomas, 4 retinoblastomas, 3 gliomas, 1 phakocarcinoma, and 3 unclassified malignant neoplasms. Three of the melanomas developed extraocular extensions; one of the melanomas metastasized to lungs and brain. This study provides a new experimental model for chemical induction of ocular neoplasms. As a procedure to test the carcinogenicity of nickel compounds, intraocular injection has the advantages of short latent period, high tumor incidence, and ease of tumor detection. A paper (7) (abstract submitted herewith) that describes these findings was presented at the American Association of Pathologists meeting during the FASEB Convention in Anaheim, California in April, 1980, and a manuscript (8) (submitted herewith) has been submitted for publication in "Investigative Ophthalmology and Visual Research".

As a continuation of investigations of relative carcinogenic activities of nickel compounds, 6 crystalline nickel compounds were administered in equal dosages (14 mg Ni/rat) to male Fischer rats by a single im injection, as previously described. The experiment was initiated in December 1979; the test compounds are NiTi_2 , NiTe , NiSb , NiAs , NiFe . Two control groups received im injections of penicillin or glycerol vehicles. At 8 months, 2/20 rats that received nickel telluride (NiTe) have developed sarcomas at the injection site; no tumors have yet occurred in the other experimental groups. These animals will be observed for 2 years for development of neoplasms. The carcinogenic activities of these 6 nickel compounds and of 6 nickel compounds that were previously tested ($\alpha\text{Ni}_3\text{S}_2$, βNiS , $\alpha\text{Ni}_3\text{Se}_2$, Ni dust, NiSe , $[\text{Ni}(\text{CO})_2\text{C}_5\text{H}_5]_2$, and amorphous NiS) will be correlated with their respective solubilities in rat serum and muscle cytosol.

An anticarcinogenic effect of Mn dust upon cancer induction following intramuscular and intrarenal injection of $\alpha\text{Ni}_3\text{S}_2$ was observed during previous grant years. An experiment has been performed to ascertain whether the anticarcinogenicity of Mn dust is specific for nickel compounds or whether it also affects tumor induction by polycyclic aromatic hydrocarbons. Cr dust was also tested, as a control. In Experiment #1, male Fischer rats were given im injections of benzo(a)pyrene (BP, 1.2 mg), Mn dust (4.4 mg), and Cr dust (4.4 mg), alone, and in various combinations. By 100 weeks, sarcomas occurred at the injection site in 17/20 rats that received BP alone versus 10/19 rats that received BP plus Mn dust ($P < 0.05$). The sarcoma incidences were 0/20 in 3 control groups that received the vehicle, Mn dust alone, or Cr dust alone, and 20/20 in rats that received BP plus Cr dust. In Experiment #2, male Fischer rats were given im injections of BP (0.6 mg), 7, 12-dimethylbenz(a)anthracene (DMBA, 0.6 mg), Mn dust (4.4 mg), and Cr dust (4.4 mg), alone, and in various combinations. Local sarcomas occurred in

17/20 rats that received BP alone, versus 5/17 rats that received BP plus Mn dust ($P < 0.001$). Sarcoma induction was not inhibited when BP was injected in the right thigh and Mn dust in the left thigh (sarcoma incidence = 16/20). The sarcoma incidences were 0/19 in a vehicle control group; 0/18 in 2 control groups that received Mn dust or Cr dust alone; 18/20 in rats that received BP plus Cr dust; 14/20 in rats that received DMBA alone; 17/19 in rats that received DMBA plus Mn dust; and 13/18 in rats that received DMBA plus Cr dust. These experiments show that Mn dust inhibits the carcinogenicity of BP when the Mn dust and BP are administered to rats by a single im injection. Under identical conditions, Mn dust does not affect the carcinogenicity of DMBA; Cr dust does not affect the carcinogenicities of BP or DMBA. A paper (9) (abstract submitted herewith) that describes these findings was presented at the American Association for Cancer Research in San Diego, California in May 1980, and a manuscript (10) (submitted herewith) has been accepted for publication in "Carcinogenesis".

The principal investigator participated in a Workshop on Metal Carcinogenesis which was held in Atlanta, Georgia in March 1980, jointly sponsored by several agencies of the U.S. Government. A plenary lecture (10) (submitted herewith) on Recent Research in Nickel Carcinogenesis was presented at the workshop and will be published in "Environmental Health Perspectives".

F. Analyses of Nickel in Biological Materials to Monitor Occupational and Environmental Exposures to Nickel Compounds. A reference method for analysis of nickel in serum and urine by electrothermal atomic absorption spectrophotometry was developed by the principal investigator and has been subjected to critical evaluation by a panel of scientists from seven nations, under the auspices of the International Union of Pure and Applied Chemistry (IUPAC). The method (11) (submitted herewith) has been prepared as a report of the IUPAC Commission on Toxicology for publication in "Pure and Applied Chemistry". The method has been tested by seven laboratories in an International proficiency survey program organized by the principal investigator in collaboration with Dr. Stanley Brown of the National Research Council of Great Britain. The harmonization of nickel analyses that was achieved by this "round-robin" exercise will be reported (12) (abstract submitted herewith) at the Second International Conference on Nickel Toxicology in Swansea, Wales, UK in September 1980.

In collaboration with Drs. Philippe Grandjean and Irving J. Selikoff of Mount Sinai Medical School in New York, an investigation of plasma nickel concentrations in shipyard workers in Baltimore and Boston is underway. A paper (13) (abstract submitted herewith) that describes the results of this study will be presented at the Swansea Conference, and a manuscript will be drafted for publication during the next 6 months. The principal investigator has prepared a chapter (14) (submitted herewith) on nickel analysis in human toxicology for publication in a textbook entitled "Methods of Analysis for Heavy Metals and Human Toxicology" (A. Vercruysse, editor) which will be published by Elsevier Press.

G. Assay of Microsomal Heme Oxygenase Activity. A new Varian gas chromatograph has been installed, and substantial effort has been devoted to determining the optimal instrumental parameters and reaction conditions for assay of microsomal heme oxygenase (HO) activity by gas chromatographic analysis of the enzymatic release of CO from heme. The ingredients of the 2 ml enzyme reaction mixture are: Fe-heme (30 nmol) as substrate; bovine albumin (0.5 mg) to bind heme; desferoxamine (10 μ mol) to bind Fe; oxyhemoglobin (0.1 mg) to bind CO; glucose-6-phosphate (1.7 μ mol), NADP (1.6 μ mol), glucose-6-phosphate-dehydrogenase (1 IU), and Mg [II] (4 μ mol) to generate NADPH; phosphate buffer (200 μ mol) to maintain pH 7.4; and washed kidney microsomes (0.5 mg of protein) as source of HO and NADPH-cytochrome P-450 reductase. After 10 min incubation at 37°C, the HO reaction is stopped by addition of p-hydroxymercuribenzoate (PHMP) (2 μ mol/0.1 ml). A blank contains the same ingredients, but PHMP is added prior to incubation. Aliquots (0.5 ml) of reaction and blank tubes are added to septum vials which contain 0.2 ml of $K_3Fe(CN)_6$ -sterox-citrate reagent. The vials are stirred for 4 min to release CO into the head-space. The head-space gas is swept onto a gas-chromatographic train of 3 columns: (a) drierite (25°C) to remove H_2O ; (b) molecular sieve (25°C) to separate CO from CO_2 ; and (c) Ni catalyst (325°C) to reduce CO to CH_4 . The carrier gas is H_2/N_2 (2:3, v/v). CH_4 is detected with a H_2 -flame ionization detector, and the area under the CH_4 peak is measured. HO activity is expressed as pmol of CO formed/sec/mg of microsomal protein (pkat/mg). The coefficient of variation of HO assays in rat kidney microsomes is $\pm 8\%$ (within-run), and the detection limit is 0.05 pkat/mg. In fasting male Fischer rats, kidney HO activity averages 0.87 pkat/mg (range 0.25 to 2.3 pkat/mg, N = 12). This procedure provides a sensitive, specific, and practical method to measure induction of kidney HO activity by nickel compounds. Preliminary results indicate that sc administration of $NiCl_2$ to rats (0.25 mmol/kg, 16 hr before death) causes 12-fold induction of kidney HO activity. A paper (15) (abstract submitted herewith) that describes these findings was presented at the American Association for Clinical Chemistry in Boston, Massachusetts in July 1980, and an article (16) (submitted herewith) on the effects of xenobiotics on heme oxygenase activity has been prepared for publication by the Commission of the European Communities.

H. Nickel-Induced Erythrocytosis. The erythropoietic effects of sustained intraperitoneal (i.p.) administration of $CoCl_2$ and $NiCl_2$ were compared with the effects of a single intrarenal (i.r.) injection of αNi_3S_2 in female Fischer-344 rats. Infusion of $NiCl_2$ by osmotic minipumps (0.85 mg Ni/day, i.p., for 24 days; total dose = 20 mg Ni/rat) did not alter the blood hematocrit or reticulocyte count during 6 weeks of observation. Under identical conditions, i.p. infusion of $CoCl_2$ (0.85 mg Co/day, i.p., for 24 days; total dose = 20 mg Co/rat) caused significant erythrocytosis (hematocrit at 6 weeks = $62 \pm 4\%$, $P < 0.001$ versus $50 \pm 3\%$ in controls) and reticulocytosis (reticulocyte count at 3 weeks = $2.5 \pm 1.5\%$ versus $0.8 \pm 0.7\%$ in controls). Administration of αNi_3S_2 to rats as a single i.r. injection (7 mg Ni/rat) caused pronounced erythrocytosis (hematocrit at 6 weeks = $79 \pm 2\%$, $P < 0.001$ versus controls) and reticulocytosis (reticulocyte count at 3 weeks = $2.8 \pm 1.2\%$, $P < 0.005$ versus controls). This study demonstrates that i.p. infusion of $Ni[II]$ is ineffective in stimulating erythropoiesis in rats, and that salient differences exist between the enhanced erythropoiesis that occurs in rats following i.p. infusion of $Co[II]$ and that produced by i.r. injection of αNi_3S_2 . A paper (17) (abstract submitted herewith) that

describes these findings will be presented at the Swansea Conference on Nickel Toxicology in September 1980, and a manuscript (18) (submitted herewith) has been submitted for publication in "Annals of Clinical and Laboratory Science".

In a continuation of investigations of the erythrocytosis that occurs following intrarenal (i.r.) injection of nickel subsulfide, four experiments were performed. In Experiment #1, 5 groups of 10-12 male Fischer rats were given a single i.r. injection of $\alpha\text{Ni}_3\text{S}_2$ (2.5 mg), alone, or in combination with 8 mg of Mn, Cr, Cu, or Al dusts. Five control groups received the vehicle (0.4 ml of NaCl solution, 0.15 mol/l) or vehicle with 8 mg of Mn, Cr, Cu, or Al dusts. In rats that received only $\alpha\text{Ni}_3\text{S}_2$, the hematocrit (mean $\pm$ S.D.) at 4 weeks was $64 \pm 4\%$ ($P < 0.001$ versus $47 \pm 5\%$ in controls). In rats that received $\alpha\text{Ni}_3\text{S}_2$ plus Mn dust, the hematocrit at 4 weeks was $50 \pm 2\%$ ($P < 0.001$ versus $64 \pm 4\%$ in rats that received only $\alpha\text{Ni}_3\text{S}_2$). The other metal dusts (Cr, Cu, and Al) did not inhibit $\alpha\text{Ni}_3\text{S}_2$ -induced erythrocytosis; none of the metal dusts, alone, caused significant changes in blood hematocrit. This experiment supports the specificity of Mn-antagonism of $\alpha\text{Ni}_3\text{S}_2$ -induced erythrocytosis. In Experiment #2, paired groups of 10-12 male gerbils, Syrian hamsters, and guinea pigs were given a single i.r. injection of $\alpha\text{Ni}_3\text{S}_2$ (5 mg) or NaCl vehicle. In the guinea pigs, the hematocrit at 4 weeks was $55 \pm 6\%$ ($P < 0.01$ versus $49 \pm 2\%$ in controls). No significant changes of hematocrits occurred in $\alpha\text{Ni}_3\text{S}_2$ -treated gerbils or hamsters. This experiment shows that guinea pigs are susceptible to $\alpha\text{Ni}_3\text{S}_2$ -induced erythrocytosis. In Experiment #3, 8 groups of 10-12 male Fischer rats were treated by splenectomy or sham splenectomy at 5 days before or 5 days after a single i.r. injection of $\alpha\text{Ni}_3\text{S}_2$ (5 mg) or NaCl vehicle. The splenectomy did not significantly influence $\alpha\text{Ni}_3\text{S}_2$ -induced erythrocytosis. In Experiment #4, 3 groups of 8 male Fischer rats were given a single i.r. injection of $\alpha\text{Ni}_3\text{S}_2$ (1.2 mg), followed by i.p. infusion of NaCl solution (controls), d-penicillamine (d-PEN, 10 $\mu\text{mol/day}$), or sodium diethyldithiocarbamate (DDC, 20 $\mu\text{mol/day}$). The i.p. infusions were administered by osmotically driven minipumps and were continued for 4 weeks. Three additional groups of rats received i.r. injections of NaCl vehicle (0.2 ml) and i.p. infusions of vehicle, d-PEN, or DDC. The $\alpha\text{Ni}_3\text{S}_2$ -induced erythrocytosis was inhibited by DDC; d-PEN had no significant effect. A paper (19) (abstract submitted herewith) that described these findings was presented at the Association of Clinical Scientists in Boston, Massachusetts in May 1980. Upon the completion in 6-8 months of additional experiments, a manuscript will be drafted for publication of these results. An invited paper (20) (abstract submitted herewith) on nickel-induced erythrocytosis will be presented at the Erythropoietin Congress in Knoxville, Tennessee in October 1980.

1. Separation and Identification of ^{63}Ni -Binding Proteins. A high-resolution method has been developed for identification of ^{63}Ni -binding proteins in rat kidney cytosol by high-voltage electrophoresis and isoelectric focusing in agarose gels, and detection of ^{63}Ni by autoradiography. This procedure reveals three distinct ^{63}Ni -binding proteins in renal cytosol at 1 to 6 months after im or iv injection of tracer doses of $^{63}\text{NiCl}_2$. In addition to albumin, the ^{63}Ni is bound to a high-molecular weight protein ($>300,000$ daltons) and to a low molecular weight protein ($<30,000$ daltons) based upon partitions in gel chromatographic and ultrafiltration experiments. These experiments are in progress; a manuscript should be drafted during the next year.

COMPLIANCE WITH CONTRACT REQUIREMENTS

As specified in last year's renewal proposal, the specific goals of this year's research were as follows:

1. Embryotoxicity, Teratogenicity, and Mutagenicity of Nickel Compounds. The studies of embryotoxicity and teratogenicity of $\text{Ni}(\text{CO})_4$ in hamsters have been completed, and the dominant lethal mutation test in rats is well underway (see Section A). Our collaborator, Dr. Daniel Albert (Professor of Ophthalmic Pathology at Harvard Medical School), has not yet had time to complete a detailed ultrastructural study of the ocular malformations induced by $\text{Ni}(\text{CO})_4$ in rats.

2. Metabolism, Detoxification, Excretion, and Chelation of Nickel Compounds. Substantial progress has been made towards the specified goals. A high-resolution method has been developed for fractionation of ^{63}Ni -binding proteins in renal cytosol, and characterizations of the proteins are in progress (see Section I). The study of efficacy of chelating drugs administered by implanted minipumps in preventing $^{63}\text{NiS}_2$ -induced erythrocytosis in rats has been completed (see Experiment #4, discussed in Section H, paragraph 2).

3. Studies of Nickel Carcinogenesis. The first and second studies that were outlined in the renewal proposal have been completed (see Section E), and the third study is in progress. The sucrose gradient ultracentrifugation experiment to detect DNA strand-breaks or fragmentation induced by $\text{Ni}(\text{CO})_4$ has been completed (see Section D), and DNA damage was not observed.

4. Nickel Analysis in Body Fluids and $\text{Ni}(\text{CO})_4$ Analyses in Air. Development of the provisional reference method for electrothermal atomic absorption spectrometry of nickel in serum and urine has been completed (see Section F). The measurements of nickel concentrations in plasma and urine of shipyard workers have been completed and the data are being analyzed (see Section F). Pressure of other tasks has precluded study of the indomethacin effect on nickel excretion in urine. The chemiluminescent $\text{Ni}(\text{CO})_4$ detector and new gas chromatograph have been installed and are in operation.

5. Hematological Effects of Nickel Compounds, Including Nickel Stimulation of Microsomal Heme Oxygenase Activity. The specific experiments that were outlined to study induction of erythrocytosis by nickel compounds have been completed, and several additional experiments have been performed (see Section H). The gas chromatographic technique for heme oxygenase assay has been refined and improved, (see Section G). The specific experiments that were proposed in last year's renewal proposal will be initiated during the next 3 months.

In addition to the work towards these specified goals, contributions have been made to knowledge of the nephrotoxicity of nickel (see Section C). The scientific literature on nickel analysis in biological materials and on recent research on nickel carcinogenesis has been comprehensively reviewed.

EFFORT OF THE PRINCIPAL INVESTIGATOR

The principal investigator is devoting 25% of his time and effort to this project during the period from December 1, 1979 to November 30, 1980.

SIGNIFICANCE OF THE RESEARCH: ITS RELEVANCE TO NATIONAL PRIORITIES

Developments within the past year indicate that national and international concern regarding environmental pollution and industrial toxicity from nickel compounds is reaching a crescendo. At the International Symposium on Nickel held at Jena, DDR, in July 1980, Cumbrowski et al (21) reported analyses of nickel concentrations in tissues obtained at autopsy of persons who resided in the vicinity of East German nickel smelters and in comparable tissues from control subjects who resided in rural areas. Significantly increased nickel concentrations were found in kidney, liver, pancreas, and lungs of the persons with environmental exposure to nickel. Similarly, Balazova et al (22) reported analyses of nickel concentrations in tissues obtained at necropsy of mongrel dogs that resided in the vicinity of Czechoslovakian nickel smelters and in control dogs from other areas. Exposure to environmental emissions of nickel was associated with significant increases in nickel concentrations in canine lung, liver, kidney, and bone. These data (21,22) are the first reported evidence of tissue accumulation of nickel from environmental exposures. Lessard et al. (23) observed 3-fold increase in lung cancer risk in persons who lived in a zone less than 1 km from a nickel refinery in New Caledonia, in comparison to persons who lived more than 3 km from the refinery. This excess risk was independent of employment in the refinery (23). In papers to be presented at the International Conference on Nickel Toxicity to be held in Swansea, Wales, in September 1980, Cuckle et al. (24) will report a significant increase in lung cancer mortality in workers in a Welsh chemical plant which manufactures soluble nickel salts, and Burges et al. (25) will report a significant increase in gastric cancer in nickel electroplating workers. These are the first epidemiological studies that indicate nickel-associated cancer risks in workers who were not employed in nickel refineries. In the light of these observations, the risks of carcinogenesis from exposure to nickel compounds may not be restricted to nickel refinery workers, as was previously believed.

Schneider et al. (26) reported analyses of nickel in bone and kidney of a malformed human baby that died 13 days after delivery. The nickel concentrations in bone (27 mg/kg, dry wt) and in kidney (4.7 mg/kg, dry wt) were markedly increased, in comparison to reference values in bone and kidney of human babies that died of various causes before 1 year of age (bone nickel concentration = 5.0 ± 2.9 mg/kg, N = 18; kidney nickel concentration = 1.5 ± 1.6 mg/kg, N = 20). Schneider et al (26) speculated that prenatal nickel exposure might have been a teratogenic factor in the malformed infant. This case report, in conjunction with several recent reports of teratogenic effects of nickel compounds in animals (see reference 2), suggests that nickel compounds might be teratogenic in man.

An outbreak in the USA of acute nickel intoxication induced by extracorporeal hemodialysis has been reported by Webster et al. (27). Stein et al. (28) have observed substantial increases in cerebral nickel concentrations in patients with chronic renal insufficiency. These reports raise the possibility that nickel intoxication may contribute to the development of dementia

and other CNS manifestations in patients with renal insufficiency, especially during dialysis therapy. With the recent expansion of chronic dialysis treatment centers in the USA, and with the increasing age distribution of the US population, neurological complications of dialysis therapy are steadily increasing in clinical importance.

Insofar as the principal investigator can ascertain, this research program at the University of Connecticut is the only one in the United States which specifically focuses upon the toxicology and metabolism of nickel compounds. The relevance of this research to national priorities has been enhanced by the introduction of nickel catalysts in several industrial processes for gasification of coal. Formation and release of volatile nickel compounds (e.g. $\text{Ni}(\text{CO})_4$) and of nickel sulfides (e.g., $\alpha\text{Ni}_3\text{S}_2$) may represent serious hazards to industrial workers and even to the general population. The data on toxicity, carcinogenicity, teratogenicity, and mutagenicity of nickel compounds and the biochemical indices and analytical methods for detection of nickel exposures that are being derived from this research program will become especially important to national priorities if nickel-catalyzed gasification technologies for coal achieve widespread adoption. Moreover, there is increasing interest in the use of Ni-Zn and Ni-H₂ storage batteries for propulsion of motor vehicles. If such batteries become widely adopted, there will inevitably be toxicological problems associated with the fabrication and disposal of nickel-containing components.

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