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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., $NiCl_2$, αNi_3S_2, βNiS, Ni_3Se_2, Ni powder and $Ni(CO)_4$] were investigated in rats and hamsters. The new knowledge includes:

1. Demonstration that nickel carbonyl [$Ni(CO)_4$] is teratogenic for hamsters, causing exencephaly, anophthalmia, pulmonary cysts, and other anomalies.

2. Elucidation of physiological factors which influence αNi_3S_2-induced erythrocytosis in rats, and demonstration that the erythrocytosis is mediated by increased plasma erythropoietin activity.

3. Development of a sensitive assay for heme oxygenase activity in renal microsomes for use in studies of renal effects of nickel compounds.

4. Demonstration that administration of $Ni(CO)_4$ to rats inhibits incorporation of ^3H-thymidine into DNA during hepatic regeneration after partial hepatectomy.

5. Demonstration that clones of Syrian hamster fetal cells which have been transformed by in vitro exposure to αNi_3S_2 consistently cause sarcomas following sc injection into nude mice.

6. Demonstration that nickel carbonyl-cyclopentadiene dimer induces rhabdomyosarcomas following im injection in rats and observation of differences in carcinogenic activities of several insoluble nickel compounds.

7. Discovery that intraocular injection of αNi_3S_2 induces amelanotic melanomas in rats.

8. Refinement of analytical methods for nickel in biological materials and application of urine nickel analyses for monitoring nickel-exposures in electroplating workers.

PRINCIPAL INVESTIGATOR

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SCIENTIFIC ACCOMPLISHMENTS DURING THE PAST YEAR

A. Analyses of Nickel in Biological Materials. The Perkin-Elmer model 5000 electrothermal atomic absorption spectrometer that was purchased by the Department of Energy under last year's contract has been installed. This new instrument has vastly enhanced the sensitivity, precision, and convenience of nickel analyses in body fluids. The analytical sensitivity for nickel that has been achieved with the new instrument is 20 times that of our previous instrument. By use of the new atomic absorption spectrometer, the proposed IUPAC reference method for analysis of nickel in urine and serum has been greatly improved and comprehensively revised. The new draft procedure will be reviewed by the IUPAC Commission on Toxicology in September 1979 and thereafter will be published as a provisional reference method in Pure and Applied Chemistry, the official IUPAC journal. Independent trials of the draft procedure are in progress in governmental, industrial, and university laboratories in Canada, England, West Germany, Netherlands, Norway, Sweden, Finland, U.S.S.R., Poland, Yugoslavia, Guatamala, New Caledonia, and Japan. During the past year progress has been made towards international harmonization of nickel analyses in biological fluids through the principal investigator's efforts as Chairman of the IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel.

Prior to installation of the new atomic absorption spectrometer, an investigation was performed of fluctuations of nickel concentrations in urine of nickel plating workers. Nickel analyses were performed by our previous atomic absorption spectrometer upon urine specimens obtained from electroplating workers at the beginning, middle, and end of the work-shift. The means (±S.D.) for nickel concentrations in urine specimens from 7 electroplating workers on 3 regular workdays were: 34±32 μg/L (pre-shift); 64±63 μg/L (mid-shift), and 46±32 μg/L (end-shift), compared to 2.7±1.6 μg/L (pre-shift) in 19 controls (hospital workers). Nickel concentrations in urine specimens from 6 electroplating workers on the first workday after a two-week vacation averaged: 5±3 μg/L (pre-shift); 9±6 μg/L (mid-shift), and 12±6 μg/L (end-shift). Nickel concentrations in personal air samples (7-hr) collected from the breathing zones of 5 electroplating workers on 3 regular workdays averaged 9.3±4.4 $\mu g/m^3$. Nickel concentrations in the air samples were correlated with nickel concentrations in end-shift urine specimens (corr. coef. = 0.70; $P < 0.05$) but were not correlated with nickel concentrations in pre-shift or mid-shift urine specimens. In view of the fluctuations of urine nickel concentrations that occur during the work-shift, nickel analyses of 8-hr urine specimens are recommended to monitor occupational exposures to nickel. In situations where timed urine collections are impractical, analyses of end-shift urine specimens are the best alternative. A paper that describes these findings was presented at the meeting of the Association of Clinical Scientists in May 1979 (abstract[1] submitted herewith),

and the manuscript[2] (submitted herewith) has been accepted for publication in Annals of Clinical and Laboratory Science.

By use of the new electrothermal atomic absorption spectrometer, it is now possible to perform reliable nickel analyses upon 0.5 ml specimens of plasma. In collaboration with Drs. Philip Grandjean and Irving J. Selikoff of Mount Sinai Medical School in New York, an investigation has been initiated of plasma nickel concentrations in shipyard workers. To date 110 subjects in several trades (welders, pipe-fitters, mechanics, etc.) have been studied, and increased concentrations of plasma nickel have been found in 39 subjects (35%). A few of the shipyard workers have remarkably high concentrations of plasma nickel, indicative of potentially hazardous exposures. These studies will be completed during the next 6 months, and a manuscript will be submitted for publication within the next year.

B. Effects of Chelating Agents on Nickel Metabolism. To compare the effectiveness of chelating drugs in enhancing renal excretion of Ni(II), studies of $^{63}NiCl_2$ metabolism were performed in rats. Continuous administration of $^{63}NiCl_2$ solution (2 μg Ni/hr) was performed by ip implantation of osmotically-driven minipumps. Renal ^{63}Ni clearance averaged 6.4 (S.D.±0.9) ml/hr (N=8) during the interval from 24 to 96 hr after initiation of the infusion. Three chelating drugs were administered to groups of 6 rats by sc implantation of minipumps during simultaneous ip infusion of $^{63}NiCl_2$. d-Penicillamine (1 μmole/hr) increased renal ^{63}Ni clearance by 53% (mean=9.4±1.3 ml/hr, $P < 0.001$), and triethylenetetramine (1 μmole/hr) increased renal ^{63}Ni clearance by 26% (mean=7.8±1.1 ml/hr, $P < 0.025$) during the interval from 24 to 96 hr. Under the same conditions, sodium diethyldithiocarbamate (2 μmole/hr) did not affect renal ^{63}Ni clearance (mean=6.1±0.8 ml/hr, vs 6.2±1.1 ml/hr in NaCl-treated control rats). A manuscript[3] (submitted herewith) that describes these findings has been accepted for publication in Toxicology and Applied Pharmacology. As the next phase of these studies, efficacies of several chelating drugs in prevention of αNi_3S_2-induced erythrocytosis are currently being evaluated.

C. Nickel Subsulfide-Induced Erythrocytosis. Investigations of αNi_3S_2-induced erythrocytosis have been continued during the past year. Following ir administration of 5 mg of αNi_3S_2 to Fischer rats, blood hematocrit became increased within 1 wk and reached 76% (S.D.±1%) at 8 wk ($P < 0.001$ vs mean hematocrit of 46 ± 1% in control rats). The αNi_3S_2-induced erythrocytosis was attended by intense stimulation of erythropoiesis with reticulocytosis, increased ^{59}Fe-uptake into erythrocytes, and erythroid hyperplasia in bone marrow and spleen. The half-life of ^{51}Cr-labelled erythrocytes from αNi_3S_2-treated rats was prolonged, consistent with increased proportion of circulating juvenile erythrocytes. Histological examination of rat kidneys after ir injection of αNi_3S_2 showed mild inflammation at 1 and 2 wk. Dense black particles of αNi_3S_2 were visible in histological sections of rat kidneys for 8 wk. Erythrocytosis did not occur in rats after (a) ir implantation of semi-permeable cellulose tubules containing αNi_3S_2, (b) repeated im injections of αNi_3S_2, or (c) intrahepatic injection of αNi_3S_2 after partial hepatectomy. Erythrocytosis did not occur in male BALB/c mice or ground squirrels after ir injection of αNi_3S_2. A paper that describes these findings was presented

at the meeting of the Association of Clinical Scientists in May 1979 (abstract[4] submitted herewith), and the manuscript[5] (submitted herewith) has been accepted for publication in Annals of Clinical and Laboratory Science.

Studies in our laboratory have shown that αNi_3S_2-induced erythrocytosis is mediated by increased serum erythropoietin activity. Measurements of erythropoietin activities were performed upon (a) pooled serum from rats at 2 wk after ir injection of αNi_3S_2 (5 mg/rat) and (b) pooled serum from control rats at 2 wk after ir injection of sterile NaCl vehicle (0.4 ml/rat). A sensitive erythropoietin bioassay was employed, which entailed repetitive administration of test serums to post-hypoxic polycythemic mice in divided doses (12 sc injections of 0.5 ml of serum at 6 hr intervals for 3 da; total dose = 6 ml of serum/mouse). The erythropoietin detection limit was ≃ 20 I.U./L of serum. In mice which received pooled serum from αNi_3S_2-treated rats, erythrocyte ^{59}Fe-uptake averaged 28% (S.D.±5) (vs 3.7±1.1% in control rats; $P < 0.001$). Based upon a 7-point calibration plot, the erythropoietin activity in pooled serum from αNi_3S_2-treated rats averaged 130 I.U./L (S.D.±18) (vs 27±6 I.U./L in control rats; $P < 0.001$). In vitro addition of Ni(II) to rat serum (100 μg/L) had no effect upon serum erythropoietin activity. This investigation has substantially advanced knowledge regarding the physiological mechanism of αNi_3S_2-induced erythrocytosis. A manuscript[6] (submitted herewith) which describes this research has been published in Research Communications in Chemical Pathology and Pharmacology.

D. Microsomal Heme Oxygenase Activity. As discussed in last year's research proposal, the principal investigator has proposed the following hypothetical sequence of physiological events whereby ir injection of αNi_3S_2 might induce erythrocytosis in rats: (a) ir injection of αNi_3S_2 causes sustained induction of renal heme oxygenase activity, (b) induction of heme oxygenase diminishes intracellular pO_2 in specific renal cells which are responsible for erythropoietin production, (c) diminution of intracellular pO_2 in these cells stimulates erythropoietin release into plasma, and (d) increased plasma erythropoietin activity stimulates erythrocyte production in the bone marrow. In order to test this hypothetical mechanism, sensitive and specific measurements of microsomal heme oxygenase activity are essential. Several published assays for heme oxygenase proved inadequate for the investigation, so that it was necessary to develop an improved procedure. Optimum reaction conditions for oxidative degradation of heme to yield biliverdin-IXa, Fe, and CO were established, and the most sensitive conditions for gas chromatographic determination of liberated CO were determined. A paper that describes our protocol for assay of heme oxygenase activity in rat kidney microsomes will be presented at an Applied Seminar on Biochemical Hematology, which will be held in Philadelphia in October 1979. The heme oxygenase assay will be demonstrated at this seminar, and preliminary findings in regard to αNi_3S_2-stimulation of renal heme oxygenase activity will be presented. The paper[7] (submitted herewith) will be published in the Seminar Manual.

E. Aminoaciduria and Proteinuria Induced by Nickel Carbonyl Inhalation. Previous investigations in our laboratory showed that parenteral administration of Ni(II) to rats promptly induced proteinuria and aminoacid-

uria. As a continuation of this avenue of research, the effects of acute exposure to inhalation of $Ni(CO)_4$ were investigated in Fischer rats. Rats were kept in metabolism cages so that urine could be collected quantitatively for 24 hr before and for 72 hr after inhalation of $Ni(CO)_4$ for 15 min. Specimens of plasma were obtained at 72 hr after the $Ni(CO)_4$ exposure. Control rats were treated similarly but were given sham exposures. Amino acids in urine and plasma were measured by high-pressure ion-exchange chromatography, and urine protein was determined by the biuret reaction. The results of this investigation (Table 1) indicate that the acute exposure to $Ni(CO)_4$ induced proteinuria and aminoaciduria. Unlike our previous findings that proteinuria and generalized aminoaciduria returned almost to normal by the third day after parenteral administration of Ni(II), the effects of $Ni(CO)_4$ inhalation were more protracted, and the aminoaciduria was more selective. The aminoaciduria was mediated by renal toxicity of $Ni(CO)_4$ rather than by mobilization of amino acids from tissues, since plasma concentrations of amino acids were generally not significantly affected by exposure to $Ni(CO)_4$ (Table 2). This investigation has just been concluded, and a manuscript that describes these findings will be drafted within the next 6 months.

F. Effect of $Ni(CO)_4$ upon ^{3}H-Thymidine Incorporation into Hepatic DNA. Previous studies in our laboratory demonstrated that administration of $Ni(CO)_4$ to rats by iv injection caused acute inhibition of hepatic RNA synthesis, as demonstrated by (a) diminished incorporation of ^{14}C-orotic acid into RNA, in vivo, (b) decreased RNA polymerase activity in hepatic nuclei, in vitro, and (c) decreased synthesis of RNA in vitro by a chromatin-RNA polymerase complex that was isolated from hepatic nuclei. On the other hand, administration of $Ni(CO)_4$ had little effect upon hepatic protein synthesis, as measured by uptake of ^{14}C-leucine into microsomal proteins. As a continuation of this study, the effect of $Ni(CO)_4$ injection (at 4 hr before sacrifice) upon hepatic DNA synthesis has been measured in male Fischer rats which were subjected to partial hepatectomy at 28 hr before sacrifice. ^{3}H-thymidine was injected ip at 1 hr before sacrifice, and incorporation of ^{3}H-thymidine into DNA was determined by the method of Mirvish et al.[8] DNA was measured by the method of Setaro and Morley.[9] As shown in Table 3, injection of $Ni(CO)_4$ significantly inhibited DNA synthesis in regenerating liver, as measured by ^{3}H-thymidine uptake into DNA. $Ni(CO)_4$ injection also significantly suppressed ^{3}H-thymidine incorporation into kidney DNA, even though the rate of DNA synthesis in the kidneys of control rats was low. These studies are being continued, and a manuscript that describes these investigations should be completed during the next year.

G. In Vitro Studies of Nickel Carcinogenesis. A major accomplishment during the past year has been the unequivocal demonstration of in vitro carcinogenicity of nickel subsulfide for Syrian hamster fetal cells. In vitro exposure of Syrian hamster fetal cells to αNi_3S_2 yielded positive colony assays for morphological transformation. A dose-response relationship was found between the concentration of αNi_3S_2 and the incidence of morphological transformation. Morphological transformation occurred at concentrations of αNi_3S_2 (0.1 or 1.0 µg/ml of culture medium) which did not impair cell plating efficiency. Nickel monosulfide (NiS) did not induce morphological transformation of Syrian hamster fetal cells under the same conditions. Clones of αNi_3S_2-transformed cells were able to grow in

soft-agar medium and demonstrated increased basal and induced activities of ornithine decarboxylase. Undifferentiated sarcomas developed in 26 of 27 nude mice at the site of sc injection of clones of αNi_3S_2-transformed cells. No tumors developed in 19 control nude mice which received sc injections of nontransformed Syrian hamster fetal cells which had not been exposed to αNi_3S_2. This study demonstrated that fetal cells which undergo transformation following exposure to αNi_3S_2 are capable of producing malignant tumors in nude mice. A manuscript[10] (submitted herewith) that describes this research has been accepted for publication in Cancer Research (Sept. or Oct. 1979).

H. In Vivo Carcinogenesis with Nickel Compounds. To compare their relative carcinogenic activities, 6 nickel compounds were administered in equal dosages (14 mg Ni/rat) to male Fischer rats by a single im injection as previously described. At 100 wk after injection, the incidences of sarcomas at the injection sites were: αNi_3S_2, 100% (9/9); NiS, 100% (14/14); Ni_3Se_2, 91% (21/23); Ni dust, 65% (13/20); NiSe (crystalline), 50% (8/16); nickel carbonyl-cyclopentadiene dimer $(Ni(CO)_2C_5H_5)_2$, 19% (3/16); NiS (amorphous), 0% (0/10); and vehicle controls, 0%, (0/44). The marked difference in sarcoma incidence after im injection of crystalline βNiS and amorphous NiS suggests that the physical form of nickel sulfides has a critical influence upon their carcinogenic activities. Although nickel carbonyl and nickel-bis-cyclopentadiene ("nickelocene") have previously been shown to be carcinogenic in rats, carcinogenicity of nickel carbonyl-cyclopentadiene dimer has not previously been reported. The physical and chemical properties of $(Ni(CO)_2C_5H_5)_2$ make this organometallic compound particularly suited for in vitro studies of nickel carcinogenesis and mutagenesis. A paper (abstract[11] submitted herewith) that summarized these findings was presented in May 1979 at the meeting of the Association of Clinical Scientists. These findings were also presented in a plenary lecture at an International Symposium on Environmental Carcinogenesis (Amsterdam, May 1979), and the results are to be published in an article (submitted herewith) in the Symposium Proceedings.

A notable discovery during the past year has been our observation that an intraocular injection of 2 mg of αNi_3S_2 (into the posterior chamber of the eye of male Fischer rats) regularly induces the development of amelanotic melanomas of the orbit. Such tumors have occurred in less than 12 months in 11/12 rats, and distant metastases were frequent. This experimental model of carcinogenesis is especially attractive since minute tumors can be detected by ophthalmoscopy. Thus, this technique may prove useful for carcinogenicity testing. Moreover, it results in tumors that are interesting from biochemical and immunological viewpoints. This avenue of research is being continued in collaboration with Dr. Daniel Albert, Professor of Ophthalmic Pathology at Harvard Medical School.

I. Teratogenicity of Nickel Carbonyl in Hamsters. Last year's annual report included our findings that exposure of pregnant Fischer rats to inhalation of $Ni(CO)_4$ on the seventh or eighth days of gestation induces ophthalmic anomalies, including anophthalmia and microphthalmia. As a continuation of this avenue of research, the embryotoxicity and teratogenicity of $Ni(CO)_4$ have been studied in Syrian golden hamsters. These investigations have demonstrated (Tables 4 and 5) that exposure of pregnant hamsters to inhalation of $Ni(CO)_4$ on the fifth day of gestation

causes (a) exencephaly, (b) polycystic disease of the lung, (c) skeletal anomalies, (d) anophthalmia, and (e) esophageal dilatation. Illustrative photographs of hamster fetuses with exencephaly, anophthalmia, or polycystic pulmonary disease are shown in Figures 1 to 6. Despite the teratogenicity of $Ni(CO)_4$, there was little evidence of embryotoxicity at the dosage that was used for these studies (Tables 4 and 5). A manuscript that describes the teratogenic effects of $Ni(CO)_4$ in hamsters will be drafted during the next 6 months.

J. Miscellaneous Accomplishments. During the past year there has been remarkable increase in scientific interest in metal carcinogenesis and in the toxic effects of metal compounds. The principal investigator has accepted invitations to write reviews or chapters (which are submitted herewith) on "Mechanisms of Metal Carcinogenesis,"[13] "Radioactive ^{63}Ni in Biological Research,"[14] and "Nickel."[15] These articles present summaries of current knowledge of nickel metabolism, toxicity, carcinogenicity, and teratogenicity. The principal investigator has also been selected by the International Agency for Research on Cancer to serve as a member of the IARC Working Group on Beryllium and Lead (Lyon, France, October 1979). In preparation for this assignment, the principal investigator has comprehensively reviewed animal data on the carcinogenicity of beryllium and lead compounds. This manuscript[16] is submitted herewith, since the literature search was supported by the Department of Energy Contract, and since the carcinogenicity of beryllium and lead compounds are particularly relevant to our nation's energy programs.

COMPLIANCE WITH CONTRACT REQUIREMENTS

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

1. Embryotoxicity, Teratogenicity and Mutagenicity of Nickel Compounds. The study of embryotoxicity and teratogenicity of $Ni(CO)_4$ in hamsters is almost completed (see Section I). Dominant lethal mutation testing in rats is now being initiated.

2. Metabolism, Detoxification, Excretion and Chelation of Nickel Compounds. These studies have been accomplished (see Section B).

3. Studies of Nickel Carcinogenesis. Substantial progress has been made in each aspect of this problem (see Sections F, G, and H).

4. Nickel Analysis in Body Fluids and Nickel Carbonyl Analyses in Air. Considerable progress has been made in nickel analyses in body fluids by electrothermal atomic absorption spectrometry (see Section A). The chemiluminescent nickel carbonyl analyzer that was mentioned in last year's proposal has recently been installed in our laboratory and is currently being tested and calibrated.

5. Effects of Nickel Upon Microsomal Heme Oxygenase Activity. An improved method for heme oxygenase activity has been developed (see Section D). Preliminary tests have shown that this assay has the requisite sensitivity, specificity, and precision for measurements of heme oxygenase activity in renal microsomes from αNi_3S_2-treated and control rats. Studies of

the effects of αNi_3S_2 upon renal heme oxygenase activity and upon the effects of ambient O_2 concentrations upon renal heme oxygenase activity will be undertaken within the next 6 months.

In addition to the work towards these specified goals, valuable contributions have been made to knowledge of the hematological effects of ir injection of αNi_3S_2 in rodents (see Section C). Comprehensive reviews have been prepared of the scientific literature that pertains to nickel metabolism and toxicity and to the carcinogenicity of metal compounds (see Section J).

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, 1978 to November 30, 1979.

SIGNIFICANCE OF THE RESEARCH AND ITS RELEVANCE TO NATIONAL PRIORITIES

There is growing national and international concern regarding environmental pollution and industrial toxicity from nickel and its compounds, as indicated (a) by the report on "Nickel" of the Committee on Medical and Biological Effects of Environmental Pollutants of the National Academy of Sciences,[17] (b) by the monograph on "Carcinogenic Risk to Man from Exposure to Nickel and Inorganic Nickel Compounds," issued by the International Agency for Cancer Research,[18] (c) by the "Criteria Document on Occupational Exposures to Inorganic Nickel," issued by the National Institute of Occupational Safety and Health,[19] (d) by the Health/Risk Assessment Document for Nickel which will soon be issued by the U.S. Environmental Protection Agency,[20] and (e) by the deliberations of the Subcommittee on Environmental and Occupational Toxicology of Nickel of the International Union of Pure and Applied Chemistry.[21] The World Health Organization is completing an "Environmental Criteria Document for Nickel" which will focus additional attention upon the human hazards from exposure to nickel compounds.

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., $Ni(CO)_4$) and of nickel sulfides (e.g., αNi_3S_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 $Ni-H_2$ storage batteries for propulsion of motor vehicles. If such storage batteries become widely adopted for this purpose, there will inevitably be toxicological problems associated with the fabrication and disposal of nickel-containing components.

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TABLE 1

EFFECT OF INHALATION OF $Ni(CO)_4$ UPON URINARY EXCRETION OF PROTEINS AND AMINO ACIDS IN RATS (N = 10; $Ni(CO)_4$ EXPOSURE = 0.9 MG/LITER OF AIR/15 MIN.)

Constituent	Excretion of Protein (mg/kg/day) and Amino Acids (μmole/kg/day) in Urine			
	Day Prior to Exposure	First Day	Second Day	Third Day
Total protein	21±4	51±28[a]	43±29[a]	37±11[a]
Neutral α-amino acids				
Glycine	21±3	21±4	22±3	28±4
Alanine	13±2	9±2	9±2	17±7[b]
Valine	2.2±0.5	2.6±1.0	3.7±0.8[a]	16±28[b]
Leucine	2.1±0.2	3.2±1.1[b]	4.0±0.8[b]	12±3[b]
Isoleucine	1.7±0.3	2.0±0.5	2.0±0.5	5.5±1.6[b]
Serine	8.1±0.5	8.4±0.3	9.1±1.2	13±5[a]
Threonine	8.7±0.9	6.8±0.9	6.6±1.0	14±2[b]
Phenylalanine	2.3±0.2	3.2±0.5[a]	3.7±0.8[b]	8.7±1.1[b]
Tyrosine	2.0±0.3	2.8±0.5[a]	3.2±0.4[b]	7.8±0.9[b]
Methionine	2.7±0.3	2.2±0.2	3.0±0.2	4.1±0.4[a]
Cystine	2.1±0.2	2.4±0.4	3.0±0.4[a]	4.1±0.3[b]
Homocystine	1.0±0.2	0.8±0.2	0.6±0.1	0.6±0.1
Acidic α-amino acids and amides				
Aspatic acid	14±2	10±2	9±2	11±2
Asparagine	7.3±0.4	4.7±0.9[b]	3.8±0.5[b]	4.3±0.8[b]
Glutamic acid	7.1±0.7	12±4[a]	15±3[b]	33±9[b]
Glutamine	13±2	4.5±1.2[b]	0.9±0.4[b]	0.5±0.6[b]
Basic α-amino acids				
Histidine	4.8±0.6	12±1[b]	6.1±0.8[a]	7.1±1.3[a]
Arginine	4.6±1.4	2.9±1.0[a]	2.2±0.9[b]	6.7±3.0
Lysine	5.7±0.9	6.0±0.8	8.1±1.1[a]	25±5[b]
Hydroxylysine	51±10	19±4[b]	12±3[b]	4.4±2.7[b]
Citrulline	0.9±0.2	0.9±0.2	0.9±0.4	2.2±0.7[b]
Ornithine	2.0±0.3	1.9±0.5	1.9±0.4	2.3±0.5
Substituted and Miscellaneous Amino Acids				
α-Aminobutyric acid	2.4±0.5	1.6±0.4	1.7±0.4	3.1±0.5
γ-Aminobutyric acid	1.8±0.2	2.5±0.6	3.6±0.6[b]	3.7±0.7[b]
β-Aminoisobutyric acid	5.4±0.6	3.0±0.7[a]	2.5±0.3[b]	2.2±0.3[b]
1-Methylhistidine	4.4±0.4	3.7±0.6	2.5±0.3[b]	4.8±0.6
3-Methylhistidine	5.0±0.6	7.4±1.5[a]	7.9±2.3[b]	7.4±1.6[b]
β-Alanine	7.3±0.5	8.1±0.6	8.5±1.0	7.7±0.8
Sarcosine	3.0±0.4	1.5±0.3[b]	1.1±0.2[b]	1.4±0.3[b]

[a] $P < 0.01$ vs corresponding pre-exposure value, computed by paired-sample "t" test.

[b] $P < 0.001$ vs corresponding pre-exposure value, computed by paired-sample "t" test.

TABLE 2

EFFECT OF INHALATION OF $Ni(CO)_4$ UPON PLASMA AMINO ACID CONCENTRATIONS (N = 6; $Ni(CO)_4$ EXPOSURE = 0.9 MG/LITER OF AIR/15 MIN.)

Constituent	Plasma Amino Acid Concentrations (μmole/liter) Day Prior to Exposure	72 Hr after Exposure
Neutral α-amino acids		
Glycine	340±41	286±72[a]
Alanine	750±82	637±195
Valine	240±58	211±61[a]
Leucine	296±74	258±113
Isoleucine	148±56	132±29
Serine	362±54	338±88
Threonine	327±41	306±65
Phenylalanine	110±47	129±45
Tyrosine	112±30	114±40
Methionine	67±11	71±18
Acidic α-amino acids and amides		
Aspartic acid	64±31	51±17
Asparagine	62±17	56±13
Glutamic acid	208±43	225±128
Glutamine	622±219	475±249
Basic α-amino acids		
Histidine	108±24	107±37
Arginine	81±38	98±42
Lysine	498±74	529±194
Citrulline	83±24	66±31
Ornithine	242±56	270±165
Other amino acids		
Taurine	348±147	271±128

[a] $P < 0.05$ vs pre-exposure value, computed by paired-sample "t" test.

TABLE 3

EFFECT OF $Ni(CO)_4$ INJECTION UPON ^3H-THYMIDINE INCORPORATION INTO DNA[a]

Parameter	Organ	Control Rats[b] [N = 10]	$Ni(CO)_4$-Treated Rats[b] [N = 10]	P versus Controls[c]
DNA Content (mg/g tissue)	Liver	1.65±0.08	1.72±0.11	N.S.
	Kidney	2.19±0.26	2.22±0.29	N.S.
^3H-Thymidine Uptake (dpm/μg DNA)	Liver	160±83	79±47	< 0.01
	Kidney	2.5±0.5	1.3±0.5	< 0.01

[a] Male Fischer rats were subjected to (a) partial hepatectomy at 28 hr before death; (b) iv injection of $Ni(CO)_4$ (2.2 mg Ni/kg body wt) or H_2O (50 μl/kg body wt in controls) at 4 hr before death; and (c) ip injection of ^3H-thymidine (0.44 mCi/kg body wt) at 1 hr before death. The rats were killed by guillotine, and the liver and kidney were perfused *in situ*. Incorporation of ^3H-thymidine into DNA was measured by the method of Mirvish et al,[8] and DNA was measured by the method of Setaro and Morley.[9]

[b] Each value is the mean±S.D.

[c] *P* values computed by Student's "t" test.

TABLE 4

TERATOGENICITY OF $Ni(CO)_4$ IN SYRIAN GOLDEN HAMSTERS[a]

Observations	Controls	Dams Exposed to $Ni(CO)_4$ Inhalation (0.08 mg/liter for 15 min)				
Day of Exposure	5	4	5	6	7	8
Corporea lutea/dam[b]	12.4±1.9	14.3±1.3	14.1±2.0	12.4±2.1	12.8±0.7	13.3±1.7
Live fetuses/litter[b]	10.8±2.7	13.1±1.5	11.1±3.2	11.1±3.3	11.0±2.0	12.3±2.4
Live fetuses/total products of conception	97/102 (95%)	157/171 (92%)	233/275[c] (85%)	122/129 (95%)	121/131 (92%)	111/118 (94%)
Wt of live fetuses (g)[b]	1.89±0.26	1.80±0.25	1.85±0.26	1.83±0.42	1.85±0.35	1.82±0.23
Litters with malformed fetuses	0/9	9/12[d]	12/21[d]	4/11	4/11	2/9
Malformed fetuses (total)	0(0%)	14(9%)	24(10%)	7(14%)	8(7%)	3(3%)
Exencephaly	0	0	7	0	0	0
Anophthalmia	0	0	1	0	0	0
Polycystic Lungs	0	0	5	0	0	0
Stomach in Thorax	0	0	3	0	0	0
Hydronephrosis	0	0	0	1	1	0
Skeletal Anomalies	0	14	34	6	7	3

[a] Pregnant dams were exposed to inhalation of $Ni(CO)_4$ (0.08 mg/liter for 15 min) on the specified days of gestation. Control dams were given sham exposures (to ambient air). The dams were delivered by Cesarean section on day 15 of gestation.

[b] Mean± S.D.

[c] $P < 0.01$; [d] $P < 0.001$ vs controls, computed by Fisher's exact test or Yates' χ^2 test.

TABLE 5

EFFECTS OF PRENATAL EXPOSURE TO $Ni(CO)_4$ UPON SYRIAN GOLDEN HAMSTERS[a]

Observations	Control Dams [N = 14]	$Ni(CO)_4$-Exposed Dams [N = 9]	P versus Controls
Live Pups/Litter			
At birth	12.1±1.5	11.0±1.8	N.S.
At 3 days	9.7±1.9	7.6±1.5	< 0.01
At 2 months	8.3±2.0	7.2±1.4	N.S.
Male/Female Ratio			
At 2 months	60/56	29/36	N.S.
Weight of Pups (g)			
At 2 months			
Males	108±14	112±14	N.S.
Females	95±13	98±15	N.S.

[a] Pregnant dams were exposed to inhalation of $Ni(CO)_4$ (0.08 mg $Ni(CO)_4$/liter for 15 min) on the 5th day of gestation. Control dams were given sham exposures (to ambient air). The dams all delivered on day 16, and they were permitted to nurse their pups. P values were computed by Student's "t" test or Yates' χ^2 test.

Fig. 1. Exencephalic hamster fetus from a dam exposed to $Ni(CO)_4$ on day 5 of gestation. Note that the eye appears normal in size.

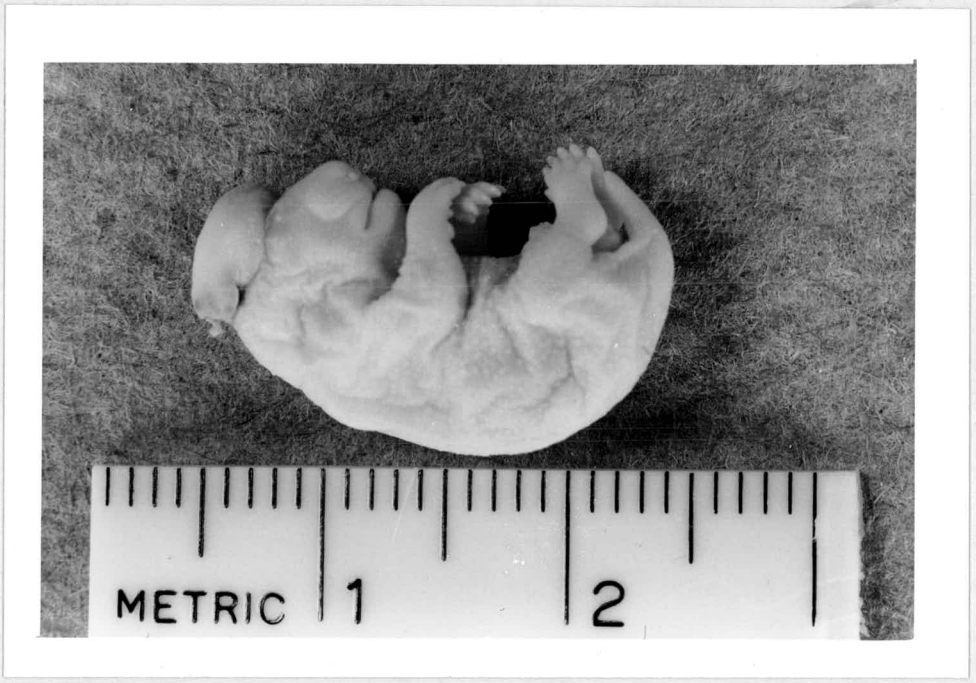

Fig. 2. Exencephalic hamster fetus from a dam exposed to $Ni(CO)_4$ on day 5 of gestation. Note that no eye is visible.

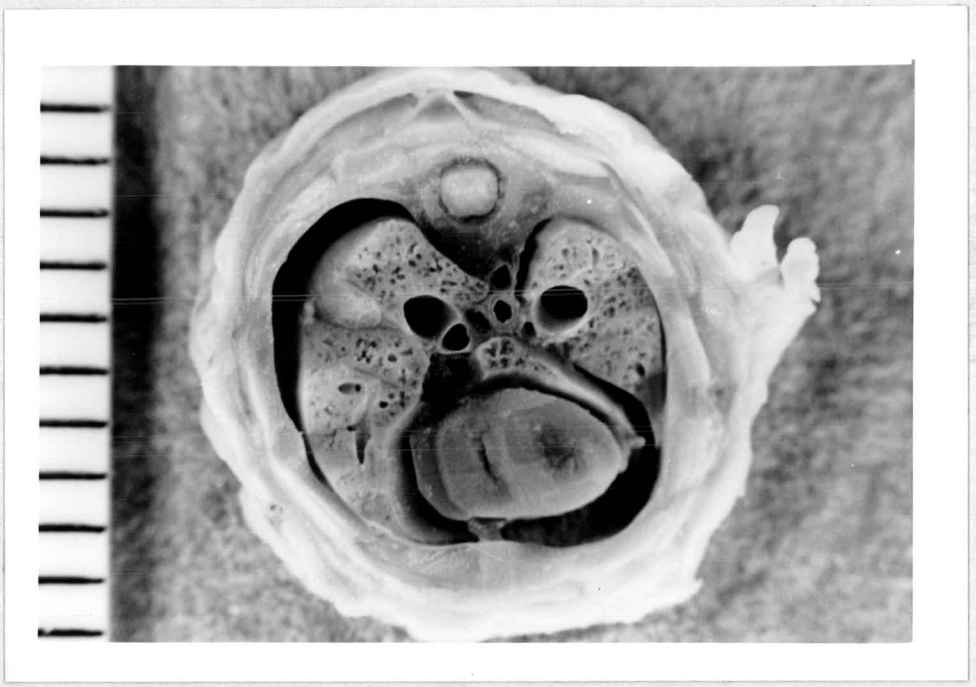

Fig. 3. Transected thorax of hamster fetus from a dam exposed to $Ni(CO)_4$ on day 5 of gestation. The main-stem bronchi are grossly dilated and cystic dilatations of bronchioles are present throughout the pulmonary parenchyma.

Fig. 4. Head of hamster fetus from a dam exposed to $Ni(CO)_4$ on day 5 of gestation. No eye is evident.

Fig. 5. Transected head of the fetus that is illustrated in Fig. 4 to demonstrate the bilateral absence of ophthalmic globes. A small conjunctival space can be seen in its customary position on the right side of the transected head. No conjunctival space is evident on the left side.

Fig. 6. Transected head of a normal hamster fetus from a control dam to illustrate the appearance of normal ophthalmic structures on day 15 of gestation.