MASTER

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The Toxicology and Metabolism of Nickel Compounds

Comprehensive Report of Overall Activities

During the Three-Year Period from

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MAIN RESEARCH ACCOMPLISHMENTS WITH SPECIAL REFERENCE TO THE ORIGINALLY STATED OBJECTIVES

This comprehensive report is submitted upon the completion of 12 years of operation of this contract by the Principal Investigator at the University of Connecticut. The four objectives of this contract, as originally stated in August 1968, are as follows:

A. Elucidation of the molecular mechanisms of nickel toxicity and carcinogenesis;
B. Understanding of the normal physiological role of nickel and its pathological alterations;
C. Development of new methods for prevention, detection, and therapy of poisoning by nickel compounds; and
D. Introduction of new techniques for measurement of nickel in biological materials and environmental samples.

The five long-range questions to be answered by this research program, as stated in August 1977, are as follows:

A. What are the principal mechanisms for detoxification, extracellular transport, and excretion of nickel compounds?
B. What are the physiological functions of nickel?
C. What are the mechanisms of nickel toxicity, carcinogenicity, teratogenicity, and mutagenicity?
D. To what degree do nickel compounds represent toxic, carcinogenic, teratogenic, or mutagenic hazards in the human environment? and
E. What are the optimal methods for prevention, detection, and therapy of nickel poisoning?

The six focuses of this research program, as stated in August 1977, are as follows:

A. Embryotoxicity, teratogenicity, and mutagenicity of nickel carbonyl;
B. Metabolism, detoxification, and excretion of nickel compounds;
C. Studies of nickel carcinogenesis;
D. Nickel analyses in body fluids and tissues to monitor occupational exposures;
E. Nephrotoxicity of nickel compounds; and
F. Hematological effects of nickel compounds.

The main research accomplishments of this project during the past three years are summarized under headings which correspond to the principal areas of investigation. The publications supported by this contract are listed at the beginning of each section.

A. Methods for Nickel Analysis in Biological Materials

(1) Mikac-Dević, D., Sunderman, F.W., Jr., and Nomoto, S.: Furildioxime method for nickel analysis in serum and urine by electrothermal atomic absorption spectrometry. Clin. Chem. 23:948-956, 1977.

(2) Adams, D.B., Brown, S.S., Sunderman, F.W., Jr., and Zachariasen, H.: Interlaboratory comparisons of nickel analyses in urine by atomic absorption spectrometry. Clin. Chem. 24:862-867, 1978.

(2A) Grove, T.H. and Sunderman, F.W., Jr.: Interference by tris-(hydroxymethylamino)methane in nickel analyses by electrothermal atomic absorption spectrometry. (Abstract), Ann. Clin. Lab. Sci. 8:495, 1978.

(3) Brown, S.S., Nomoto, S., Stoeppler, M., and Sunderman, F.W., Jr.: IUPAC reference method for analysis of nickel in serum and urine by electrothermal atomic absorption spectrophotometry. Pure Appl. Chem. (submitted).

(3B) Brown, S.S., DiMichiel, A., and Sunderman, F.W., Jr.: Interlaboratory comparison of nickel analyses in serum and urine by the IUPAC provisional reference method of atomic absorption spectrophotometry. (Abstract), In: Proceedings of the Swansea Conference on Nickel Toxicology, (S.S. Brown and F.W. Sunderman Jr., Eds.), Academic Press, New York (in press).

(4) Sunderman, F.W., Jr.: Analytical biochemistry of nickel. Pure Appl. Chem. 52:529-544, 1980.

(5) Sunderman, F.W., Jr.: Nickel. In: Methods of Analysis for Heavy Metals and Human Toxicology (A. Vercruysse, Ed.), Elsevier Scientific Publishing Co., Amsterdam, (in press).

A major achievement was to secure international consensus regarding analytical methods and reference values for nickel in serum and urine. A new procedure for nickel analysis was developed which significantly improved the sensitivity, accuracy, and precision of electrothermal atomic absorption spectrophotometry of nickel in serum and urine (1). Two international surveys were conducted of nickel analyses in urine by atomic absorption spectrophotometry (2). These surveys showed that widely discordant analytical results were being obtained by participating laboratories in seven countries; the surveys documented the need for improved accuracy of nickel determinations in body fluids. The Principal Investigator, as Chairman of the Commission on Toxicology of the International Union of Pure and Applied Chemistry (IUPAC), organized a Subcommittee on Environmental and Occupational Toxicology of Nickel. After five meetings, extensive evaluations, and wide consultations, the IUPAC Nickel Subcommittee formulated a reference method for nickel analysis in serum and urine, based primarily upon procedures developed at the University of Connecticut (3). A third international survey was conducted in which laboratories in seven countries used the reference method to

analyze serum and urine specimens. This survey demonstrated that the reference method harmonized the results of nickel analyses (3B). The consequence of this activity has been improved standardization of analytical methods that are used to monitor human exposures to nickel compounds. As a correlated effort, comprehensive review articles on the analytical biochemistry of nickel have been prepared in order to delineate (a) precautions in sample collection, (b) procedures to minimize nickel contamination, (c) relative sensitivities of instrumental techniques for nickel analysis, and (d) application of nickel analyses in serum, urine, and other body fluids for surveillance of human exposures to nickel compounds (4,5). Work to achieve international cooperation and agreement upon analytical methods is time-consuming and oftimes frustrating, but the accomplishments during the past three years have been considerable. Formerly, there was ten-fold disparity among the reference values for nickel concentration in serum and urine of healthy persons. Presently, there is no serious disagreement regarding these reference values (5).

B. Applications of Laboratory Methods to Assess Human Exposures to Nickel Compounds

(6) Bernacki, E.J., Parsons, G.E., Roy, B.R., Mikac-Dević, M., Kennedy, C.D., and Sunderman, F.W., Jr.: Urine nickel concentrations in nickel-exposed workers. Ann. Clin. Lab. Sci. 8:184-189, 1978.

(7) Bernacki, E.J., Zygowicz, E., and Sunderman, F.W., Jr.: Fluctuations of nickel concentrations in urine of electroplating workers. Ann. Clin. Lab. Sci. 10:33-39, 1980.

(7A) Grandjean, P., Sunderman, F.W., Jr., Shen, S.K., and Selikoff, I.A.: Measurements of nickel in plasma and urine of shipyard workers. (Abstract), In: Proceedings of the Swansea Conference on Nickel Toxicology, (S.S. Brown and F.W. Sunderman Jr., Eds.), Academic Press, New York (in press).

(7B) Horak, E., Shea, P.H., Maher, J.F., Kennedy, T.L., Randolph, M.T., McLean, R.H., and Sunderman, F.W., Jr.: Radioimmunoassays of beta-2 microglobulin in body fluids of healthy adults, children, patients with renal diseases, and nickel-exposed workers. (Abstract), Ann. Clin. Lab. Sci. (in press).

Study of workers in various occupations exposed to nickel compounds revealed significant increases of nickel concentrations in urine of nickel battery workers, metal sprayers, nickel electroplaters, and particularly, in nickel refinery workers (6,7,7A). Investigation of nickel electroplating workers showed that nickel concentrations in urine samples obtained at the end of the work-shift were correlated with exposures to nickel in air, determined by personal atmospheric monitoring with cassette samplers (7). Measurements of beta-2 microglobulin concentrations in urine samples were found to be useful for detection of renal tubular injury in nickel refinery workers (7A). The principal investigator and the technical personnel supported by this contract provided analytical

assistance to state and local public health authorities for investigations of the possible etiologic role of nickel carbonyl in the pathogenesis of the Legionnaires' Disease epidemic in Philadelphia. Although the etiologic agent was ultimately identified as Legionella pneumophila, the episode demonstrated the importance of nickel analyses for differential diagnosis of idiopathic pneumonia. Three years ago, the University of Connecticut was the only facility in the United States that regularly analyzed nickel concentrations in body fluids. Now, there are at least a dozen laboratories that perform nickel analyses in order to monitor exposures of industrial workers to nickel compounds. This research program has a significant role in fostering such nickel analyses.

C. Embryotoxicity and Teratogenicity of Nickel Compounds

(8) Sunderman, F.W., Jr., Shen, S.K., Mitchell, J.M., Allpass, P.R., and Damjanov, I.: Embryotoxicity and fetal toxicity of nickel in rats. Toxicol. Appl. Pharmacol. 43:381-390, 1978.

(9) Sunderman, F.W., Jr., Allpass, P.R., Mitchell, J.M., Baselt, R.C., and Albert, D.M.: Eye malformations in rats: Induction by prenatal exposure to nickel carbonyl. Science 203:550-553, 1979.

(10) Sunderman, F.W., Jr., Shen, S.K., Reid, M.C., and Allpass, P.R.: Teratogenicity and embryotoxicity of nickel carbonyl in Syrian hamsters. Teratogenesis, Carcinogenesis, and Mutagenesis (in press).

In 1977, the Department of Energy's scientific project officer for this contract suggested that the principal investigator should study the embryotoxicity and teratogenicity of nickel compounds, in view of the paucity of information that was then available on this topic. This avenue of investigation proved to be scientifically fruitful. In our initial study, treatment of pregnant rats with $NiCl_2$ resulted in embryotoxicity but did not induce fetal malformations (8). In contrast, exposure of pregnant rats and hamsters to inhalation of $Ni(CO)_4$ during early gestation resulted in a variety of congenital malformations in the progeny (9,10). Ocular malformations were the principal congenital anomalies in rats (9); cystic lungs and exencephaly were the most common congenital anomalies in hamsters (10). These observations suggest the possibility of teratogenic effects of nickel carbonyl upon human fetuses, as a consequence of accidental exposures of pregnant women to nickel carbonyl in the workplace. Moreover, the finding that $Ni(CO)_4$ exposure results in anophthalmia and microphthalmia in rats has furnished an experimental model to investigate the behavioral effects of congenital blindness.

D. Nickel Carcinogenesis

Explanatory Note: Investigations on nickel carcinogenesis have been primarily supported by NIESH Grant ES-01337 ("Nickel Carcinogenesis"), and have received minor support by DOE Contract EV-03140.

(11) Sunderman, F.W., Jr., Maenza, R.M., Allpass, P.R., Mitchell, J.M., Damjanov, I., and Goldblatt, P.J.: Carcinogenicity of nickel subsulfide in Fischer rats and Syrian hamsters after administration by various routes. In: Inorganic and Nutritional Aspects of Cancer (G.N. Schrauzer, Ed.), Plenum Publishing Corp., New York, 1978, Chapter 4, pp.57-67.

(12) Damjanov, I., Sunderman, F.W., Jr., Mitchell, J.M., and Allpass, P.R.: Induction of testicular sarcomas in Fischer rats by intratesticular injection of nickel subsulfide. Cancer Res., 38:268-276, 1978.

(13) Sunderman, F.W., Jr., Trudeau, E.A., Jr., Horak, E., Mitchell, J.M., and Allpass, P.R.: Serum ceruloplasmin concentrations in rats with primary and transplanted sarcomas induced by nickel subsulfide. Ann. Clin. Lab. Sci. 9:60-67, 1979.

(14) Costa, M., Nye, J.S., Sunderman, F.W., Jr., Allpass, P.R., and Gondos, B.: Induction of sarcomas in nude mice by inplantation of Syrian hamster fetal cells exposed in vitro to nickel subsulfide. Cancer Res. 39:3591-3597, 1979.

(15) Sunderman, F.W., Jr.: Carcinogenicity and anticarcinogenicity of metal compounds. In: Environmental Carcinogenesis (P. Emmelot and E. Kriek, Eds.), Elsevier/North Holland Press, New York, 1979, pp. 165-192.

(16) Sunderman, F.W., Jr., Maenza, R.M., Hopfer, S.M., Mitchell, J.M., Allpass, P.R., and Damjanov, I.: Induction of renal cancers in rats by intrarenal injection of nickel subsulfide. J. Environ. Pathol. Toxicol. 2:1511-1527, 1979.

(17) Albert, D.M., Gonder, J.R., Papale, J., Craft, J.L., Dohlman, H.G., Reid, M.C., and Sunderman, F.W., Jr.: Induction of ocular neoplasms in Fischer rats by intraocular injection of nickel subsulfide. Invest. Ophthalmol. Visual Res. (submitted).

(18) Hui, G. and Sunderman, F.W., Jr.: Effects of nickel compounds on incorporation of thymidine-^{3}H into DNA in rat liver and kidney. Carcinogenesis 1:297-304, 1980.

(19) Sunderman, F.W., Jr., McCully, K.S., Taubman, S.B., Allpass, P.R., Reid, M.C., and Rinehimer, L.A.: Manganese inhibition of sarcoma induction by benzo(a)pyrene in rats. Carcinogenesis (in press).

(20) Sunderman, F.W., Jr.: Mechanisms of metal carcinogenesis. Biol. Trace Element Res. 1:63-86, 1979.

(21) Sunderman, F.W., Jr.: Recent research on nickel carcinogenesis. Environ. Health Perspect. (in press).

An important achievement of our research on nickel carcinogenesis has been demonstration that in vitro exposure of Syrian hamster embryo cells to nickel subsulfide (αNi_3S_2) results in morphological transformation, and that the transformed cells regularly induce sarcomas following sc inplantation in nude mice (14). Other accomplishments have included (a) establishment of the dose-response relationship for induction of local sarcomas following im injection of αNi_3S_2 in rats (15), (b) development of a new model for induction of ocular neoplasms (e.g., melanoma) by intraocular injection of αNi_3S_2 in rats (17), (c) demonstration that manganese inhibits the induction of sarcomas in rats after im injection of benzo(a)pyrene as well as after im injection of αNi_3S_2 (19), and (d) observation that $Ni(CO)_4$ inhibits the incorporation of thymidine-^3H into DNA in rat liver and kidney (18). Crystalline nickel monosulfide (βNiS), nickel monoselenide (NiSe), nickel subselenide (Ni_3Se_2), and nickel carbonyl-cyclopentadiene dimer ($(Ni(CO)_2 \cdot C_5H_5)_2$) have been found to be carcinogenic in rats following im injection; under the same conditions, amorphous nickel monosulfide (NiS) did not induce any tumors (15). Our studies indicate that the physical form of particulate nickel compounds has a critical effect upon their carcinogenic activities. The principal investigator has prepared review articles on mechanisms of metal carcinogenesis (20) and on recent research on nickel carcinogenesis (21).

E. Hematologic Effects of Nickel Compounds

(22) Morse, E.E., Lee, T.Y., Reiss, R.F., and Sunderman, F.W., Jr.: Dose-response and time-response study of erythrocytosis in rats after intrarenal injection of nickel subsulfide. Ann. Clin. Lab. Sci., 7:17-24 1977.

(23) Hopfer, S.M. and Sunderman, F.W., Jr.: Manganese inhibition of nickel subsulfide induction of erythrocytosis in rats. Res. Commun. Chem. Pathol. Pharmacol. 19:337-345, 1978.

(24) Hopfer, S.M., Sunderman, F.W., Jr., Fredrickson, T.N., and Morse, E.E.: Nickel-induced erythrocytosis: Efficacies of nickel compounds and susceptibilities of rat strains. Ann. Clin. Lab. Sci. 8:396-402, 1978.

(25) Hopfer, S.M., Sunderman, F.W., Jr., Fredrickson, T.N., and Morse, E.E.: Increased serum erythropoietin activity in rats following intrarenal injection of nickel subsulfide. Res. Commun. Chem. Pathol. Pharmacol. 23:155-170, 1979.

(26) Hopfer, S.M., Sunderman, F.W., Jr., Morse, E.E., and Fredrickson, T.N.: Effects of intrarenal injection of nickel subsulfide in rodents. Ann. Clin. Lab. Sci. 10:54-64, 1980.

(27) Oskarsson, A., Reid, M.C., and Sunderman, F.W., Jr.: Comparison of the effects of cobalt chloride, nickel chloride, and nickel subsulfide upon erythropoiesis in rats. Ann. Clin. Lab. Sci. (submitted).

(27A) Sunderman, F.W., Jr., Reid, M.C., Delaney, L., Shen, S.K., and Hopfer, S.M.: Factors which influence the induction of erythrocytosis by intrarenal injection of nickel subsulfide. (Abstract), Ann. Clin. Lab. Sci. (in press).

(27B) Sunderman, F.W., Jr. and Downs, J.R.: Assay of heme oxygenase activity in kidney microsomes by gas chromatography of released carbon monoxide. (Abstract), Clin. Chem. (in press).

(28) Sunderman, F.W., Jr.: Effects of xenobiotics on heme oxygenase activity. In: The Use of Laboratory Test Results: Variations Due to Drug Intake (G. Siest, Ed.), Edition Martinus Nijhoff, Luxembourg (in press).

A series of investigations has been conducted on the pronounced erythrocytosis that develops in rodents following intrarenal injection of αNi_3S_2 (22-27). Significant differences were observed between the susceptibilities of four rat strains to αNi_3S_2-induced erythrocytosis (24). Guinea pigs were found to develop erythrocytosis after ir injection of αNi_3S_2, but mice, squirrels, gerbils, and hamsters did not develop any hematological response (27A). Several nickel compounds were tested in rats by ir injection in equal dosages (7 mg Ni/rat). The compounds included $NiTi_2$, NiTe, NiSb, NiAs, αNi_3S_2, Ni_3Se_2, crystalline βNiS, amorphous NiS, and Ni dust. Blood hematocrit values became significantly increased only in rats that received αNi_3S_2, crystalline βNiS, Ni_3Se_2, and Ni dust (24,27A). Administration of αNi_3S_2 to rats by im, intrahepatic, or intrasplenic routes, and administration of $NiCl_2$ to rats by ip infusion did not induce erythrocytosis (26,27). Intrarenal injection has been the only effective route. αNi_3S_2-induced erythrocytosis can be partially suppressed by simultaneous injection of Mn dust (23) or ip infusion of sodium diethyldithiocarbamate (27A). αNi_3S_2-induced erythrocytosis is associated with increased plasma concentrations of erythropoietin (25). These studies have identified physiological mechanisms and factors which affect αNi_3S_2-induced erythrocytosis in rats, and have established the species-specificity, compound-specificity, and route-specificity of the experimental model. This experimental model furnishes an attractive avenue to explore the role of erythropoietin in the regulation of erythrocyte production. To determine whether nickel induction of renal heme oxygenase activity is involved in the control of erythropoietin production. a sensitive assay for heme oxygenase activity has been developed (27B). This method is currently being used to monitor stimulation of heme oxygenase by nickel compounds. The background literature on effects of xenobiotic agents on heme oxygenase activity has been summarized in a review article (28).

F. Metabolism, Toxicity, and Chelation of Nickel

(29) Kasprzak, K.S. and Sunderman, F.W., Jr.: Mechanisms of dissolution of nickel subsulfide in rat serum. Res. Commun. Chem. Pathol. Pharmacol. 16:95-108, 1977.

(30) Onkelinx, C. and Sunderman, F.W., Jr.: Mathematical modelling of nickel[II] metabolism. In: Nickel in the Environment (J.O. Nriagu, Ed.), Wiley Interscience, New York (in press).

(31) Kasprzak, K.S. and Sunderman, F.W., Jr.: Radioactive ^{63}Ni in biological research. Pure Appl. Chem. 51:1375-1389, 1979.

(32) Horak, E., Zygowicz, E.R., Tarabishi, R., Mitchell, J.M., and Sunderman, F.W., Jr.: Effects of nickel chloride and nickel carbonyl upon glucose metabolism in rats. Ann. Clin. Lab. Sci. 8: 476-482, 1978.

(33) Horak, E. and Sunderman, F.W., Jr.: Nephrotoxicity of nickel carbonyl in rats. Ann. Clin Lab. Sci. (in press).

(34) Baselt, R.C., Sunderman, F.W., Jr., Mitchell, J.M., and Horak, E.: Comparisons of antidotal efficacy of sodium diethyldithiocarbamate, D-penicillamine and triethylenetetramine upon acute toxicity of nickel carbonyl in rats. Res. Commun. Pathol. Pharmacol. 18:677-688, 1977.

(35) Shen, S.K., Williams, S., Onkelinx, C., and Sunderman, F.W., Jr.: Use of implanted minipumps to study the effects of chelating drugs on renal ^{63}Ni clearance in rats. Toxicol. Appl. Pharmacol. 51:209-217, 1979.

The metabolism of αNi_3S_2 was elucidated by in vitro study of the mechanism of dissolution of $^{63}\alpha Ni_3S_2$ in rat serum. The αNi_3S_2 was slowly oxidized to crystalline nickel monosulfide (β NiS), which subsequently underwent further oxidation to yield soluble Ni[II] complexes and relatively insoluble particles of nickel hydroxide ($Ni(OH)_2$) (29). The kinetics of ^{63}Ni in rodents have been summarized (30), and applications of ^{63}Ni in biological research have been reviewed (31). Hyperglucagonemia was identified as the factor that induces hyperglycemia in rats following administration of $NiCl_2$ and $Ni(CO)_4$ (32). Exposure of rats to inhalation of $Ni(CO)_4$ was shown to induce nephrotoxicity, manifested by proteinuria and aminoaciduria (33). The relative efficacy of chelating agents for therapy of $Ni(CO)_4$ poisoning was studied in rats; d-penicillamine and sodium diethyldithiocarbamate were both effective antidotes, but triethylenetetramine was ineffective (34). The effects of chelating drugs on renal ^{63}Ni clearance was investigated in rats; d-penicillamine and triethylenetetramine increased ^{63}Ni clearance, whereas sodium diethyldithiocarbamate did not affect ^{63}Ni clearance (35). A high-resolution procedure has been developed for separation of ^{63}Ni-binding proteins in rat kidney cytosol by agarose gel electrophoresis and isoelectric focusing with detection of ^{63}Ni by autoradiography. This technique is being employed to identify proteins that are involved in renal uptake and tubular secretion of $^{63}Ni[II]$.

G. Other Studies of Nickel Toxicology

(36) Bernacki, E.J., Parsons, G.E., and Sunderman, F.W., Jr.: Investigation of exposure to nickel and lung cancer mortality. Ann. Clin. Lab. Sci. 8:190-194, 1978.

The possible association between exposure to nickel-containing compounds and lung cancer mortality was assessed in workmen at an aircraft engine factory in Hartford, Connecticut. This case-control study was limited to men who died prior to retirement from work, and hence the possible latent period for development of lung cancer was restricted. The proportion of deaths from lung cancer in the cohort of nickel-exposed workers was equal to that in a control cohort of workers who had minimal occupational exposures to nickel.

GRADUATE STUDENTS TRAINED DEGREES GRANTED, AND POST DOCTORAL TENURES COMPLETED

A. Graduate Students

(1) Sidney Hopfer, Ph.D., Doctorate in Pathobiology, University of Connecticut, 1979. Dr. Hopfer's doctoral thesis was concerned with the induction of erythrocytosis in rats by intrarenal injection of nickel subsulfide. He collaborated in papers #16, 23-26.

B. Medical Students

(1) Edward Trudeau, M.D., University of Connecticut, 1978. Dr. Trudeau worked on this program during a three-month student research elective. He collaborated in paper #13.

(2) Ramsay Tarabishi, M.D., University of Connecticut, 1979. Dr. Tarabishi worked on this program during a three-month student research elective. He collaborated in paper #32.

(3) Steven Williams, M.D., University of Connecticut, 1980. Dr. Williams worked on this program during a three-month student research elective. He collaborated in paper #35.

C. Posdoctoral Tenures

(1) Thomas Grove, Ph.D., Doctorate in Biochemistry, Case-Western Reserve University, 1977. Dr. Grove was a post-doctoral fellow in clinical chemistry who worked on this program during a three-month elective rotation. He collaborated in paper #2A.

(2) Elizabeth Zygowicz, Ph.D., Doctorate in Biochemistry, University of Illinois, 1976. Dr. Zygowicz was a post-doctoral fellow in clinical chemistry who worked on this program during a three-month elective rotation. She collaborated in paper #7.

(3) Max Costa, Ph.D., Doctorate in Pharmacology, University of Arizona Medical School, 1978. From February 1979 to January 1980, Dr. Costa was the recipient of an NIH Post-Doctoral Fellowship in Cancer Research in the Department of Laboratory Medicine and Institute of Materials Science of the University of Connecticut, with Dr. Sunderman as Major Advisor. Partial support for Dr. Costa's experiments was provided by this DOE contract. He collaborated in paper #14.

CURRENT STATUS OF KNOWLEDGE

When this program was initiated 12 years ago, there was practically no research on nickel metabolism or toxicology being performed in other laboratories. Within the past decade, there has been steady increase in research on nickel in laboratories throughout the world. Scientific developments and governmental regulations have recently generated an explosive expansion of interest in biological effects of nickel, as manifested by the three international conferences on nickel metabolism and toxicology that have taken place during 1978-1980. The most important recent contributions to knowledge of nickel metabolism and toxicology from other laboratories have been the following:

A. Analytical Chemistry

(37) Flora, C.J. and Nieboer, E.: Determination of nickel by differential pulse polarography at a dropping mercury electrode. Anal. Chem. 52:1013-1020, 1980.

(38) Chen, J.R., Francisco, R.B., and Muller, T.E.: Legionnaires' disease: Nickel levels. Science 196:906-908, 1977.

(39) Dornemann, A. and Kleist, H.: Bestimmung von Nanospuren Nickel in biologischer Matrix. Fresenius Z. Anal. Chem. 300:197-199, 1980.

Flora and Nieboer (37) found that addition of dimethylglyoxime to ammoniacal tartrate or citrate buffers enhanced by a factor of 15 the sensitivity of derivative polarography on nickel at a dropping mercury electrode. By means of dimethylglyoxime sensitized differential pulse polarography, Flora and Nieboer (37) detected nickel concentrations as low as 2 to 3 µg/litre in buffered reaction mixtures. At the Swansea Conference on Nickel Toxicology in September 1980, Nieboer will report excellent agreement between analyses of nickel in human urine samples by dimethylglyoxime-sensitized polarography and by the IUPAC reference method for electrothermal atomic absorption spectrophotometry. Chen et al. (38) employed particle-induced x-ray emission spectrometry for measurements of nickel concentrations in tissue specimens obtained at autopsy from patients who died of Legionnaires' desease and from control patients. The correlation coefficient was 0.936 between nickel concentrations obtained by Chen et al. (38), and those obtained by Sunderman (5) using electrothermal atomic absorption spectrophotometry. The results obtained by Flora and Nieboer (37) and Chen et al. (38) using completely different analytical methods have provided independent support

of the accuracy of results obtained by atomic absorption spectrophotometry. Dornemann and Kleist (39) have reported a two-step isolation procedure for assay of nickel in biological samples. After wet oxidation, iron is removed by extraction with cupferron into chloroform; then nickel is extracted as hexamethylenedithiocarbamidate into a mixture of diisopropyketone and xylene, and nickel is measured by electrothermal atomic absorption spectrophotometry. This procedure may facilitate analysis of nickel in whole blood, liver, kidney, and other tissues which are rich in nickel. The technique is currently being evaluated at the University of Connecticut.

B. Biochemistry

(40) Diekert, G., Klee, B., and Thauer, R.K.: Nickel, a component of factor F-430 from methanobacterium thermoautotrophicum. Arch. Microbiol. 124:103-106, 1980.

(41) Whitman, W.B. and Wolfe, R.S.: Presence of nickel in factor F-430 from methanobacterium bryantii. Biochem. Biophys. Res. Commun. 92:1196-1201, 1980.

Diekert et al. (40) and Whitman and Wolfe (41) have independently discovered that nickel is an essential component of coenzyme F-430, which is requisite for methanogenesis in bacteria. Factor F-430 is the first nickel-containing biologically active compound of low molecular weight to be reported. The structure of F-430 has not yet been established, but it has physical properties similar to vitamin B_{12} (40). This discovery has stimulated considerable interest in molecular biochemistry, because of the speculation that F-430 may prove to be a vitamin.

C. Nutrition

(42) Anke, M., Kronemann, H., Groppel, B., Hennig, A., Meissner, D. and Schneider, H.J.: The influence of Ni-deficiency on growth, reproduction, life expectancy, and different biochemical parameters in goats and mini-pigs. (Abstract), In: Proceedings Internationales Arsen- und Nickelsymposium (M. Anke and H.J. Schneider, Eds.), Karl-Marx University Press, Leipzig (in press).

(43) Schnegg, A. and Kirchgessner, M.: Zur Essentialitat des Nickels fur das tierische Wachstum. (Abstract), In: Proceedings Internationales Arsen- und Nickelsymposium (M. Anke and H.J. Schneider, Eds.), Karl-Marx University Press, Leipzig (in press).

(44) Nielsen, F.H.: Nickel deprivation in the rat: Its effect on the absorption of ferric ions. (Abstract), In: Proceedings Internationales Arsen- und Nickelsymposium (M. Anke and H.J. Schneider, Eds.), Karl-Marx University Press, Leipzig (in press).

Investigations in three laboratories have independently established the nutritional essentiality of nickel for mammalian species, including

goats, swine, and rats (42-44). Nickel appears to be involved in iron absorption, carbohydrate metabolism, and osteogenesis, but the exact physiological role of nickel remains to be established. The nutritional requirements for nickel is estimated to be approximately 0.5 ppm in food of man and animals (42).

D. Cardiac Physiology

(45) Rubanyi, G. and Kovach, A.G.B.: The effect of nickel ions on myocarcial contractility and coronary flow in the isolated rat heart. (Abstract), In: Proceedings Internationales Arsen- und Nickelsymposium (M. Anke and H.J. Schneider, Eds.), Karl-Marx University Press, Leipzig (in press).

(46) Prasad, C.M., Nair, K.G., and Sneth, U.K.: Antiarrhythmic action of nickel chloride. (Abstract), In: Proceedings of the Swansea Conference on Nickel Toxicology (S.S. Brown and F.W. Sunderman, Jr., Eds.), Academic Press, New York (in press).

Rubanyi and Kovach (45) showed that Ni[II] in concentrations as low as 6 µg/litre significantly inhibits myocardial contractility and increases coronary arterial resistance in the isolated, perfused rat heart. Prasad et al. (46) found that Ni[II] effectively antagonizes digitalis-induced cardiac arrhythmias in intact rats and in isolated heart preparations. Rubanyi and Kovach (45) and Prasad et al. (46) have deduced that Ni[II] competes with Ca[II] for entrance into cardiac cells and increases the intracellular concentrations of malic and oxaloacetic acids. These observations have renewed interest in the clinical significance of hypernickelemia that often occurs in patients following myocardial infarction, as demonstrated 10 years ago by research at the University of Connecticut.

E. Teratogenesis

(47) Lu, C-C., Matsumoto, N., and Iijima, S.: Teratogenic effects of nickel chloride on embryonic mice. Teratology 19:137-142, 1979.

(48) Gilani, S.H. and Marano, M.: Congenital abnormalities in nickel poisoning in chick embryos. Arch. Environ. Contamin. Toxicol. 9:17-22, 1980.

(49) Schneider, H.J., Anke, M., and Klinger, G.: The nickel status of human beings. (Abstract), In: Proceedings Internationales Arsen- und Nickelsymposium (M. Anke and H.J. Schneider, Eds.), Karl-Marx University Press, Leipzig (in press).

Lu et al. (47) observed increased resorptions, decreased fetal weight, and cerebral, ocular, palatine, and skeletal anomalies in mice following ip injection of $NiCl_2$ to pregnant dams on days 7 to 11 of gestation. Gilani and Marano (48) reported microphthalmia, exencephaly, everted viscera, ectopia cordis, skeletal anomalies, and internal hemorrhages in chicks following injections of $NiCl_2$ into the air sac of the egg on

days 0 to 4 of incubation. These findings suggest that $Ni(CO)_4$ may not be uniquely teratogenic; divalent nickel compounds may also pose teratogenic hazards. Until recently there was no evidence of human teratogenic risks from exposure to nickel compounds, but Schneider et al. (49) has reported increased nickel concentrations in tissues of a malformed human infant that died 13 days after delivery; and they speculated that prenatal exposure to nickel might have been a teratogenic factor.

G. Carcinogenesis

(50) DiPaolo, J.A. and Casto, B.C.: Quantitative studies of in vitro morphological transformation of Syrian hamster fetal cells by inorganic metal salts. Cancer Res. 39:1008-1013, 1979.

(51) Rivedal, E. and Sanner, T.: Synergistic effects on morphological transformation of hamster embryo cells by nickel sulfate and benzo(a)pyrene. Cancer Letters 8:203-208, 1980.

(52) Cuckle, H., Doll, R., and Morgan, L.G.: A study of the mortality of men working with soluble nickel compounds. (Abstract), In: Proceedings of the Swansea Conference on Nickel Toxicology, (S.S. Brown and F.W. Sunderman Jr., Eds.), Academic Press, New York (in press).

(53) Burges, D.C.E.: Mortality study of nickel platers. (Abstract), In: Proceedings of the Swansea Conference on Nickel Toxicology, (S.S. Brown and F.W. Sunderman Jr. Eds.), Academic Press, New York (in press).

DiPaolo and Casto (50) and Rivedal and Sanner (51) have independently reported findings similar to Costa et al. (14) that in vitro exposures of Syrian hamster fetal cells to αNi_3S_2 or $NiSO_4$ result in morphological transformation. Rivedal and Sanner (51) observed that when cells were exposed to combinations of $NiSO_4$ and benzo(a)pyrene, the transformation frequencies were much higher than when the compounds were tested separately. Rivedal and Sanner's observation of mutagenic synergism between nickel sulfate and benzo(a)pyrene is consistent with our previous observations of carcinogenic synergism between αNi_3S_2 and benzo(a)-pyrene in rats. At the Swansea Conference on Nickel Toxicology in September 1980, Cuckle et al. (52) will report a significant increase in lung cancer mortality in workers in a Welsh chemical plant which manufactures soluble nickel salts, and Burges (53) will report a significant increase in gastric cancer in nickel electroplating workers. 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.

H. Molecular Toxicology

(54) Maines, M.D. and Kappas, A.: Nickel mediated alterations in the activity of hepatic and renal enzymes of heme metabolism and heme dependent cellular activities. In: Clinical Chemistry and Chemical Toxicology of Metals, (S.S. Brown, Ed.), Elsevier Press, Amsterdam, 1977, pp. 75-81.

(55) Eybl, V., Kontensky, J., Sykora, J., Mertyl, F., and Svacinova, J.: The interaction of nickel and cadmium in experimental intoxication. (Abstract), In: Proceedings Internationales Arsen- und Nickelsymposium (M. Anke and H.J. Schneider, Eds.), Karl-Marx University Press, Leipzig (in press).

Maines and Kappas (54) discovered that sc administration of $NiCl_2$ to rats causes a prompt ten-fold induction of heme oxygenase activity in renal microsomes. The effect of $NiCl_2$ upon heme oxygenase in kidney was much more pronounced than in liver. These observations stimulated the principal investigator's interest in the molecular mechanisms and toxicological importance of this phenomenon. Eybl et al. (55) have recently observed that sc injection of $NiCl_2$ markedly diminished phenobarbital-induced sleeping time in rats; they have postulated an inhibitory effect of nickel on hepatic mixed function oxidase activities.

I. Nephrology

(56) Webster, J.D., Parker, T.F., Alfrey, A.C., Smythe, W.R., Kubo, H., Neal, G., and Hull, A.R.: Acute nickel intoxication by dialysis. Ann. Intern. Med. 93:631-633, 1980.

(57) Stein, G., Schneider, H.J., and Stadie, G.: The influence of chronic renal insufficiency and of different tumors of the urogenital system on the Ni-level of indicator organs. (Abstract), In: Proceedings Internationales Arsen- und Nickelsymposium (M. Anke and H.J. Schneider, Eds.), Karl-Marx University Press, Leipzig (in press).

Webster et al. (56) reported nickel intoxication in 23 dialyzed patients with renal insufficiency, caused by leaching of nickel from a stainless-steel heating tank. Stein et al. (57) observed substantial increases in cerebral nickel concentrations in patients with chronic renal insufficiency, most of whom were being treated with extracorporeal hemodialysis. 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.

PRESENT DIVISION OF FEDERAL SUPPORT FOR THE OVERALL RESEARCH PROGRAM

Financial support for research in the principal investigator's laboratory comes from two sources:

(1) Department of Energy Contract No. EV-03140 on "The Toxicology and Metabolism of Nickel Compounds"; and

(2) National Institute of Environmental Health Sciences Grant No. ES-01337, on "Nickel Carcinogenesis"; which was renewed and funded on June 1, 1979 for 3 years. The annual budget during the current grant year is $72,149; the total budget during the three-year period is $213,925.

The NIESH grant is focused specifically on the carcinogenicity of nickel compounds and it provides only minor support for the other studies in the investigator's laboratory. The DOE grant supports studies of the analysis, metabolism, toxicology, teratogenicity, and hematological effects of nickel compounds and it provides minor support for the carcinogenesis studies. The two research programs are distinct, independent, and complementary; there is no significant overlap of financial support. The principal investigator is a full-time salaried employee of the University of Connecticut; he does not receive any remuneration from grants or contracts.

PLANS FOR CONTINUATION OF PRESENT OBJECTIVES AND NEW OBJECTIVES

The following is a broad outline of our plans for continuation of the research during the next three years. Specific experiments and methods are described in the Renewal Proposal that accompanies this report.

A. Analyses of Nickel in Body Fluids and Tissues

An accurate, reliable method for analysis of nickel in whole blood and tissues will be developed, and the method will be evaluated by the IUPAC procedures for attaining international concensus. The method will then be promulgated as an IUPAC reference procedure in order to promote the uniformity of nickel analyses in whole blood and tissues that are being performed on a world-wide basis. The validity of nickel analyses in whole blood as an index of occupational and environmental exposures to nickel compounds will be critically assessed. Nickel analyses will be performed upon tissues obtained at autopsy from persons who die from sudden trauma in order to establish reference values. Nickel concentrations will be determined in body fluids, excreta, and tissues of patients with acute myocardial infarction, burns, hepatic cirrhosis, chronic renal insufficiency, and other diseases in which disturbances of nickel metabolism have been reported, in order to elucidate the pathophysiological alterations of nickel metabolism.

B. Studies of the Metabolism of ^{63}Ni-Labelled Compounds

Separation and speciation of ultrafiltrable ^{63}Ni-binding ligands in rat and rabbit serum and urine will be investigated by high-performance liquid chromatography and liquid scintillation counting. Fractionation and characterization of ^{63}Ni-binding macromolecules in rat kidney will be continued by column chromatography, agarose gel electrophoresis, and isoelectric focusing with detection of ^{63}Ni by autoradiography. These studies will elucidate the macromolecules and metabolites that are involved in renal uptake, tubular transport, and urinary excretion of ^{63}Ni[II]. Subsequent studies will be focused upon transport of ^{63}Ni[II] across the placental and bloodbrain barriers. Phagocytosis, solubilization and elimination of particulate ^{63}Ni-labelled compounds (e.g., $^{63}\alpha Ni_3S_2$, amorphous ^{63}NiS) by leukocytes and macrophages will be intensely investigated. If coenzyme F-430 from bacteria can be suitably labelled with ^{63}Ni and purified in sufficient quantities, intestinal absorption, tissue distribution, kinetics, and excretion

of ^{63}Ni-F-430 will be compared to the corresponding parameters for ^{63}Ni[II].

C. Embryotoxicity, Teratogenicity, and Mutagenicity

The teratogenicity of divalent nickel compounds (e.g., $NiCl_2$) will be tested in hamsters. The effects of $Ni(CO)_4$ on male fertility in rats will continue to be studied. Research in progress on transplacental carcinogenicity of αNi_3S_2 in rats will be completed.

D. Carcinogenesis Studies

To establish the relationships of chemical composition, crystal structure, and physical properties of nickel compounds to their relative carcinogenic activities, a series of investigations will be performed. Some of these studies are already in progress. The carcinogenicities of $NiTi_2$, NiTe, NiAs, Ni_3As_2, NiFe, Ni_3Se_2, NiSe, αNi_3S_2, βNiS, amorphous NiS, NiSb, and Ni dust are being tested by im injection in male Fischer rats. These compounds were prepared or evaluated by the Institute of Materials Science at the University of Connecticut, and their purity and x-ray diffraction patterns have been established. Particle size distributions have been determined by our laboratory with scanning electron microscopy. The solubilities of these compounds in rat serum and rat muscle cytosol will be determined, as well as the susceptibility of the compounds for phagocytosis and solubilization by macrophages. The capabilities of the compounds to induce erythrocytosis following ir injection in rats will be measured, to test the possibility that erythropoietin release from rat kidney following ir injection of nickel compounds may by a manifestation of neoplastic transformation (i.e., an early oncofetal marker of renal neoplasia).

E. Hematological Effects of Nickel Compounds

Experiments will determine whether or not αNi_3S_2-induced erythrocytosis in rats is mediated by increased erythrogenin activity in kidney homogenates. Since there is uncertainty and dispute about the methods for assay of erythrogenin activity, careful preliminary evaluation and standardization of an erythrogenin assay will be essential. However, this effort is necessary in order to establish the mechanisms whereby ir injection of αNi_3S_2 stimulates erythropoietin activity in rat plasma. The gas chromatographic technique for assay of heme oxygenase activity that has been developed in our laboratory will be used to explore the mechanisms whereby nickel compounds induce heme oxygenase activity in rat kidney microsomes, and to determine the relationship of this phenomenon to the renal toxicity of nickel.

SIGNIFICANCE IN BIOLOGY AND MEDICINE, AND NEED FOR FUTURE INVESTIGATIONS

Nickel compounds and alloys are ubiquitous in civilized human communities; the potential for nickel toxicity, carcinogenicity, and possible teratogenicity is widespread from occupational and environmental exposures. Our nation's energy programs, by placing more reliance upon coal

and solar heat as energy sources, enhance the potential hazards from nickel toxicity, owing to the presence of nickel in coal fly ash, the use of nickel catalysts in hydrogenation processes, and the importance of nickel components in storage batteries. Knowledge of nickel metabolism and toxicology is rapidly expanding, attributable in part to the impetus of research conducted at the University of Connecticut under the auspices of this research contract. Despite the progress to date, there are abundant important, interesting, and challenging areas for future investigations.