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Professor and Head
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School of Medicine
University of Connecticut Health Center

Signature: [Signature]

Date: August 8, 1973

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THE TOXICOLOGY AND METABOLISM OF NICKEl COMPOUNDS

Progress Report

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Abstract of Progress Report
Attachment to U.S. AEC Research Document No. COO-3140-12

"The Toxicology and Metabolism of Nickel Compounds"

The toxicology and metabolism of nickel compounds (e.g. NiCl₂ and Ni₃S₂) have been investigated following their administration to rats and rabbits. Atomic absorption of nickel has also been performed upon a variety of human samples, including urine, feces, serum, sweat and hair. The new knowledge that has been derived from these studies during the past year has included:

1) Development of a mathematical model to describe kinetics of ⁶³Ni(II) metabolism in rats and rabbits in compartmental terms;

2) Demonstration that nickeloplasmin, the serum α₁-macroglobulin which contains nickel, can be labelled with ⁶³Ni in vitro by incubation of (a) non-radioactive serum proteins with (b) ⁶³Ni-containing ultrafiltrates of serum from rabbits which had received i.v. ⁶³NiCl₂. The α₁-macroglobulin is not labelled by incubation with ⁶³Ni(II), in the absence of serum ultrafiltrable constituents. These findings suggest that nickeloplasmin is a ternary complex of α₁-macroglobulin with an ultrafiltrable Ni-complex;

3) Demonstration of pronounced species variation in affinities of serum albumins for ⁶³Ni(II), attributable to genetic differences in amino acid sequence of albumins at a specific locus;

4) Discovery that carcinogenicity of Ni₃S₂ in rats is profoundly inhibited by simultaneous injection of Mn (but not by injection of Cu, Cr, Al), providing a new experimental system for study of metal inter-
actions in carcinogenesis;

(5) Observations that feces and sweat are major routes for elimination of nickel in man; and,

(6) Documentation that intraperitoneal injection of NiCl$_2$ in rats causes prompt development of hyperglycemia.

These findings have all helped to elucidate mechanisms of nickel toxicity, as well as the regulation of nickel metabolism. They have direct relevance to hazards of occupational and environmental exposures of man to nickel compounds.

F. William Sunderman, Jr., M. D.

August 8, 1973
Scientific Accomplishments During the Past Grant Year

I. Binding of $^{63}\text{Ni}$ to Rabbit Serum Nickeloplasmin In Vivo and In Vitro. As specified in last year's Renewal Proposal, one of the principal goals of the research contract was to elucidate the biochemical and physiological properties of nickeloplasmin, the nickel-containing serum macroglobulin. Substantial progress has been made in this regard. Rapid In vivo labelling of nickeloplasmin with $^{63}\text{Ni}$ was accomplished as early as 2 hours after a single intravenous injection of $^{63}\text{NiCl}_2$ in rabbits. Labelling of nickeloplasmin with $^{63}\text{Ni}$ was also achieved In vitro by mixing (a) serum ultrafiltrates from rabbits which had received i.v. $^{63}\text{NiCl}_2$, with (b) concentrated proteins obtained by ultrafiltration of sera collected from the same rabbits prior to injection of $^{63}\text{NiCl}_2$. $^{63}\text{Ni}$-nickeloplasmin preparations which were labelled In vivo or In vitro yielded a single protein band on analytical acrylamide gel electrophoresis, and were identified as an $\alpha_1$-macroglobulin by immunodiffusion and immunoelectrophoresis. Nickeloplasmin was not labelled In vitro after incubation of dialyzed serum proteins with $^{63}\text{NiCl}_2$, nor after...
incubation of crude serum macroglobulins with $^{63}$NiCl$_2$. These findings suggest that $^{63}$Ni-nickeloplasmin is a stable ternary complex of serum $\alpha_1$-macroglobulin with an ultrafiltrable $^{63}$Ni-constituent of serum. The obvious significance of this observation is that serum nickeloplasmin might play a role in the transport of a specific nickel-complex, analogous to the role of transcobalamin in the transport of cyanocobalamin. This speculation is particularly exciting, inasmuch as earlier studies in our laboratory on the dietary essentiality of nickel have recently been confirmed by other workers. A manuscript (submitted herewith) which describes the rapid labelling of nickeloplasmin with $^{63}$Ni in vivo and in vitro has been accepted for publication in Bioinorganic Chemistry$^1$, and an abstract (submitted herewith) of our oral presentation of this investigation at the Federation Meetings has been published in Federation Proceedings$^2$.

II. Compartmental Analysis of $^{63}$Ni(II) Metabolism in Rats and Rabbits. To elucidate the toxicology and metabolism of nickel compounds, it is necessary to understand the kinetics of Ni(II) distribution and elimination. A compartmental model was formulated in order to facilitate predictions of Ni(II) kinetics. The kinetics of $^{63}$Ni(II) metabolism was studied in rats and rabbits following a single intravenous injection of $^{63}$NiCl$_2$. In both species, $^{63}$Ni(II) was rapidly cleared from plasma or serum during the first 2 days after the injection, and it disappeared at a much slower rate from 3 to 7 days. Measurements of $^{63}$Ni(II) distribution and excretion in both species suggested that $^{63}$Ni(II) is diluted within a volume composed of two compartments, and that $^{63}$Ni(II) is elim-
inated by first-order kinetics. A mathematical model has been developed which describes $^{63}$Ni(II) kinetics in quantitative terms. The two-compartment model of $^{63}$Ni(II) kinetics has been tested and verified by its ability correctly to predict the concentrations of $^{63}$Ni in serum or plasma of rodents following continuous or repeated injections of $^{63}$NiCl$_2$. A manuscript (submitted herewith) which describes the mathematical model of $^{63}$Ni(II) kinetics has been accepted for publication in Research Communications in Chemical Pathology and Pharmacology$^3$, and an abstract (submitted herewith) of our oral presentation of this topic will be published in the Proceedings of the Second International Symposium on Trace Element Metabolism in Animals$^4$. A portion of this work was performed by a graduate student, Joel Becker, in fulfillment of his research requirement for the degree of Master of Science. A copy of Mr. Becker's thesis is also submitted$^5$.

III. Role of Serum Albumin in Transport and Detoxification of Nickel.

In the Renewal Proposal which was submitted last year, it was proposed to test the hypothesis that species differences in the partitions of serum protein-bound and ultrafiltrable nickel are attributable to species variations in the association constants of albumin for Ni(II). This hypothesis has been tested and confirmed. Equilibrium dialysis was employed in order to compare the in vitro binding of $^{63}$Ni(II) by purified serum albumin from five mammalian species. These measurements have shown that the $^{63}$Ni(II) affinities of canine and porcine serum albumins are substantially less than the affinities of human, rabbit and rat serum albumins. The relatively diminished binding of nickel to dog and
and porcine albumin is the result of the absence of a histidine residue at the third locus from the N-terminus of these albumins. Based upon the observed species differences in nickel affinity of albumins, it is predicted that dogs and pigs may have enhanced susceptibilities to nickel toxicity. A manuscript (submitted herewith) which describes this investigation has been published in Research Communications in Chemical Pathology and Pharmacology.

IV. Study of Ultrafiltrable Ni-Receptors in Serum. Three new methods have been developed for the separation of $^{63}$Ni-binding ultrafiltrable constituents of serum and urine: (1) thin-layer chromatography on powdered cellulose plates in a solvent system of isopropanol: water; (2) thin-layer chromatography on microcrystalline cellulose plates in a solvent system of dioxane: pyridine: water; and (3) thin-layer electrophoresis on powdered cellulose plates in ammonium carbonate buffer at pH 7.4. In each of these three systems, five distinct $^{63}$Ni-binding constituents have been detected by autoradiography in ultrafiltrates of sera obtained from rabbits at 30 min. after i.v. injection of $^{63}$NiCl$_2$. The radiodense bands on the autoradiographs correspond to ninhydrin-positive bands on the stained thin-layer plates. One of the major $^{63}$Ni-binding constituents has tentatively been identified as L-aspartic acid. The other constituents are yet to be identified, but it is apparent that L-histidine is not a major $^{63}$Ni-binding agent.

This investigation represents a major component of our research effort. It is anticipated that within six months this investigation will have reached the stage of drafting an initial publication. It is hoped that
the identification of ultrafiltrable Ni-receptors may open new avenues to chelation therapy of poisoning by nickel and other metals.

V. Study of Nickel Carcinogenesis. Fischer rats in five experimental groups were given a single intramuscular injection of penicillin suspension containing carcinogenic Ni$_3$S$_2$ dust (2.5 mg) alone, or in combination with equimolar amounts of aluminum, copper, chromium or manganese dusts. Rats in five control groups were treated identically, excepting that the Ni$_3$S$_2$ dust was omitted. After 24 months, the incidence of sarcomas at the injection site was 63% in the group which received the combination of Ni$_3$S$_2$ and manganese dusts, compared with incidences of 96-100% in the groups which received Ni$_3$S$_2$ alone or in combination with aluminum, copper or chromium dusts (P < 0.001). No sarcomas occurred at the injection site in control groups which did not receive Ni$_3$S$_2$. The finding that addition of equimolar amounts of manganese dust to Ni$_3$S$_2$ dust significantly depresses Ni$_3$S$_2$-induced tumorigenesis provides a new experimental system for investigations of metal interactions in carcinogenesis. A manuscript (submitted herewith) which describes this investigation has been submitted for publication in Cancer Research$^7$, and an abstract (submitted herewith) of our oral presentation has been published in the Proceedings of the American Association for Cancer Research$^8$. Considerable effort during the past year has been directed to preparing a comprehensive review of nickel carcinogenesis in man and experimental animals. This review article (submitted herewith) has been published in Annals of Clinical and Laboratory Science$^9$. 
VI. Nickel Analyses in Biological Materials. Atomic absorption spectrometry has been employed in order to establish the normal values for nickel elimination in feces, to serve as a basis for evaluations of environmental or occupational exposures to nickel. A manuscript (submitted herewith) which reports the measurements of nickel excretion in feces of healthy adults has been published in Clinical Chemistry\textsuperscript{10}.

A surprising discovery has been that the sweat is a major route for the excretion of nickel and other metals in man. Measurements of nickel, copper, zinc and lead have been performed in sweat obtained from healthy adults during sauna bathing. An oral presentation of our findings was given at the meetings of the American Association of Clinical Chemists (abstract submitted herewith)\textsuperscript{11}, and a manuscript on this investigation (submitted herewith) has been submitted for publication in Clinical Chemistry\textsuperscript{12}.

A thorough evaluation has been made of the analytical methods for nickel in biological materials by (a) non-flame atomic absorption spectrometry, and by (b) anodic-stripping voltametry. Our experience has indicated that non-flame atomic absorption spectrometry using the graphite tube furnace is the most sensitive method which is now available for nickel analysis. A manuscript is submitted herewith which reviews measurements of nickel and other trace metals in biological materials by atomic absorption spectrometry. This manuscript, which has been accepted for publication in Human Pathology, includes a preliminary description of our new non-flame atomization technique for atomic absorption analysis of nickel\textsuperscript{13}.
VII. Hyperglycemia in Rats induced by NiCl₂. Dr. Clary of the National Institute of Occupational Health and Safety in Cincinnati has recently noted immediate hyperglycemia in rats following intraperitoneal injection of NiCl₂ (personal communication to FWS). Dr. Clary's findings were immediately confirmed and extended in our own laboratory. Measurements of serum glucose have been performed in Fisher rats at intervals of 0, 0.5, 1, 2, 3 and 4 hours after i.p. injections of NiCl₂ in dosages of 0.005, 0.0125, 0.025, 0.050, 0.075, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 mg Ni/100 gm body weight. (The LD₅₀ for NiCl₂ is 0.8 mg/100gm). At the 0.4 mg/100 gm dosage, the mean concentration of serum glucose becomes increased from 91 mg/dl at 0 hours to 235 mg/dl at ½ hour! There is prompt return of serum glucose to normal levels by 4 hours. Our findings to date are summarized in Table 1. The mechanism of the NiCl₂-induced hyperglycemia has not yet been established, but is being intensively studied in our laboratory. This study may indicate an important and heretofore unrecognized manifestation of nickel toxicity in animals and man.

VIII. Compliance with Contract Requirements. The specific objectives of this year's research, as itemized in last year's Renewal Proposal were:

(1) Identification of ultrafiltrable Ni-receptors in serum and urine. Substantial advances have been made in this effort. Three independent methods have been developed for the separation of serum ultrafiltrable ⁶³Ni-receptors, and one of the serum ⁶³Ni-binding constituents has been tentatively identified as L-aspartic acid. Intensive work in this area is currently in progress, (see Section IV above).
## Table I

*Studies of the Effect of i.p. NiCl₂ upon Serum Glucose* \(^{a}\)

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Dosage of NiCl₂ (mg Ni/100g)</th>
<th>No. of Rats</th>
<th>Serum Glucose Concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>1 (Controls)</td>
<td>0</td>
<td>21</td>
<td>91±14</td>
</tr>
<tr>
<td>2</td>
<td>0.005</td>
<td>11</td>
<td>88±9</td>
</tr>
<tr>
<td>3</td>
<td>0.025</td>
<td>8</td>
<td>91±9</td>
</tr>
<tr>
<td>4</td>
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<td>8</td>
<td>95±8</td>
</tr>
<tr>
<td>5</td>
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<td>8</td>
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<td>0.1</td>
<td>7</td>
<td>89±4</td>
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<td>7</td>
<td>92±3</td>
</tr>
<tr>
<td>8</td>
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<td>7</td>
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<td>0.6</td>
<td>8</td>
<td>84±9</td>
</tr>
<tr>
<td>11</td>
<td>0.8</td>
<td>8</td>
<td>92±12</td>
</tr>
</tbody>
</table>

\(^a\) Fischer rats, ♂, fasting for 16 hr before i.p. injection of NiCl₂ in isotonic NaCl soln, (Control rats received injections of NaCl soln).

\(^b\) Serum glucose in blood from tail at indicated interval after i.p. injection of NiCl₂, (The values are the mean ± standard deviation).

\(^c\) p<0.05 \hspace{1cm} \(^d\) p<0.01 \hspace{1cm} \(^e\) p<0.001 vs Group 1 at the same time interval after i.p. injection.
(2) Elucidation of biochemical and physiological properties of serum nickeloplasmin. Rapid labelling of nickeloplasmin with $^{63}\text{Ni}$ has been accomplished in vivo and in vitro (see Section I above). Nickeloplasmin appears to be a ternary complex of $\alpha_1$-macroglobulin with an ultrafiltrable nickel complex.

(3) Study of the role of serum albumin in the transport and detoxification of nickel compounds. Species differences in affinities of serum albumins for nickel have been demonstrated by equilibrium dialysis studies (see Section III above).

(4) Nickel analyses in biological materials from human sources. Measurements of nickel in feces and sweat have been reported, and an improved method for nickel analysis has been developed by non-flame atomic absorption spectrometry (see Section VI above).

(5) Study of nickel carcinogenesis using $^{63}\text{Ni}_3\text{S}_2$. The International Nickel Company has recently provided us with a sample of $^{63}\text{Ni}_3\text{S}_2$ for use in our studies of nickel carcinogenesis. Investigations of $^{63}\text{Ni}_3\text{S}_2$ metabolism will be initiated during the next year. In the meantime, a compartmental analysis of $^{63}\text{Ni}$(II) metabolism has been completed in rats and rabbits, which establishes a conceptual framework for investigations of the metabolism of carcinogenic nickel compounds (see Sections II and V above).

The principal investigator (Dr. Sunderman) has devoted approximately 20% of his time and effort to this project. His effort will be continued at this level throughout the contract year.

IX. Significance of the Research, and its Relevance to National Priorities. The Committee on Biological Effects of Atmospheric Pollutants
(BEAP) of the National Academy of Sciences has recently approved the Report and Recommendations of its Panel on Nickel. (The Principal Investigator is Chairman of the Panel on Nickel, and a Member of the BEAP Committee). The Committee has recognized the potential health problems caused by environmental and occupational exposure to nickel, and has formally recognized that "further research is needed in the following areas: (a) clarification of the role of nickel in nutrition, with particular emphasis upon its possible dietary essentiality, (b) elucidation of the molecular binding sites for nickel which are physiologically significant, and/or are involved in the detoxification and elimination of nickel compounds, (c) determination of the mechanism and clinical importance of pathological alterations of nickel concentrations in body fluids and tissues. Improvements are required in the sensitivity, precision and accuracy of methods for nickel analysis in biological materials." In addition, the BEAP Committee has recommended programs of environmental and industrial monitoring of nickel; epidemiological investigations of nickel carcinogenesis; studies of the relative toxicity of nickel compounds, and investigations of the pathogenesis of nickel dermatitis. Insofar as the Principal Investigator is aware, AEC Research Contract No. AT(11-1)-3140 is currently the only research program in the United States which is specifically focused primarily upon the toxicology and metabolism of nickel compounds.

References

1. Decsy, M. I. and Sunderman, F. W., Jr.: Binding of $^{63}$Ni to rabbit serum $\alpha_1$-macroglobulin in vivo and in vitro. Bioinorganic Chemistry, in press.


