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THE TOXICOLOGY AND METABOLISM OF NICKEL COMPOUNDS

Progress Report

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DISCLAIMER

Abstract of Progress Report

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"The Toxicology and Metabolism of Nickel Compounds"

The toxicology and metabolism of nickel compounds ($NiCl_2$, Ni_3S_2, NiS, and Ni powder) were investigated in rats and hamsters. The new knowledge has included:

(1) Demonstration that hyperglucagonemia is primarily responsible for the acute hyperglycemic effect of parenteral Ni(II) in rats.

(2) Demonstration that parenteral injection of Ni(II) in rats produces acute nephropathy with proteinuria and amino aciduria, and with ultrastructural lesions of renal glomeruli and tubules.

(3) Confirmation of the inhibitory effect of manganese upon the carcinogenicity of Ni_3S_2 after intramuscular injection in rats, and elucidation of the effects of manganese upon the rates of excretion of nickel, and upon the acute histological reactions produced by Ni_3S_2.

(4) Discovery that the antidotal efficacy of triethylenetetramine (TETA) in acute Ni(II) poisoning in rats is substantially greater than that of other chelating agents, including α-lipoic acid, diethyldithiocarbamate, d-penicillamine, and glycylglycyl-L-histidine-N-methylamide.

(5) Observation that the acute renal toxicity of Ni(II) is suppressed by administration of TETA, but that the hyperglycemic and hyperglucagonemic responses to Ni(II) are not prevented by TETA.

(6) Confirmation that marked erythrocytosis is induced in rats by a single intrarenal injection of Ni_3S_2, and elucidation of the time-response and dose-response relationships for the Ni-induced erythrocytosis.

F. William Sunderman, Jr., M.D.
August 8, 1975

Notice

ERDA RESEARCH DOCUMENT NO. COO-3140-34

Progress Report for Energy Research and Development Administration Contract

"The Toxicology and Metabolism of Nickel Compounds"

August 8, 1975

Principal Investigator: F. William Sunderman, Jr., M.D., Professor and Head, Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, Connecticut 06032.

Scientific Accomplishments During the Past Grant Year:

A. Study of Chelation Therapy of Acute Ni(II)-Toxicity. A major accomplishment of this contract year has been the discovery that triethylenetetramine (TETA) is highly effective as an antidote for acute Ni(II) toxicity in rats. TETA has been found to be superior to α-lipoic acid, diethyldithiocarbamate, d-penicillamine and glycylglycyl-L-histidine-N-methylamide in efficacy for the prevention of death following *ip* injection of Ni(II) in rats. Illustrative data are given in Tables 1-3. TETA has been found to have relatively low toxicity in rats. The acute (7 day) LD_{50} for TETA after *ip* injection in rats has been found to be 2.4 mmol/kg (S.E.M. ± 0.1). A study is in progress in our laboratory on the effect of *im* injection of TETA upon the tissue distribution and rates of excretion of $^{63}Ni(II)$ following *ip* administration to rats. Preliminary results of this study indicate that TETA therapy profoundly diminishes the concentration of ^{63}Ni in target tissues (*e.g.* cardiac muscle), and that TETA markedly increases the excretion of ^{63}Ni in bile and urine. It appears that TETA is superior to other chelating agents for therapy of acute nickel poisoning. As soon as a safety hood has been installed, this investigation will be extended to study the

Table 1

Effect of Intramuscular Injection of Chelating Drugs upon Acute Mortality of Rats after Intraperitoneal Injection of $NiCl_2$

Drug	Dosage of Drug[a] [µmol/kg]	Mortality after *ip* $NiCl_2$[b] [136 µmol Ni/kg]		[408 µmol/kg]	
Sham Injection	0	25/35	(71%)	35/35	(100%)
α-Lipoic Acid	680	16/25	(64%)	24/25	(96%)
Diethyldithiocarbamate	680	2/25[d]	(8%)	17/25[d]	(68%)
Glycylglycyl-L-histidine-N-methylamide	680	0/10[d]	(0%)	4/10[d]	(40%)
d-Penicillamine	680	0/25[d]	(0%)	5/25[d]	(20%)
Triethylenetetramine[c]	680	0/25[d]	(0%)	0/25[d]	(0%)

[a] The drugs were injected into the gluteal muscles within 1 min prior to *ip* injection of $NiCl_2$

[b] Acute mortality ratios within 1 week after injection

[c] LD_{50} = 2.4 mmol/lg (S.E. ± 0.1) based upon acute mortality within 1 week after *im* injection in 180 rats

[d] $p < 0.001$ *vs* sham-treated controls (by χ^2 test)

Table 2

Effect of Intraperitoneal Injection of Chelating Drugs upon Acute Mortality of Rats after Intraperitoneal Injection of $NiCl_2$

Drug	Dosage of Drug[a] [μmol/kg]	Mortality after ip $NiCl_2$[b] [136 μmol Ni/kg]		[408 μmol/kg]	
Sham Injection	0	20/25	(80%)	25/25	(100%)
α-Lipoic Acid	680	16/25	(64%)	25/25	(100%)
Diethyldithiocarbamate	680	15/25	(60%)	21/25	(84%)
d-Penicillamine	680	2/25[d]	(8%)	22/25	(88%)
Triethylenetetramine	680	8/25[c]	(32%)	16/25[c]	(64%)

[a] The drugs were injected ip at 1 hr prior to ip injection of $NiCl_2$

[b] Acute mortality ratios within 1 week after injection

[c] $p < 0.01$ *vs* sham-treated controls (by χ^2 test)

[d] $p < 0.001$ *vs* sham-treated controls (by χ^2 test)

Table 3

Effect of Intramuscular Injections of Triethylenetetramine and d-Penicillamine upon Acute Mortality of Rats after Intraperitoneal Injection of $NiCl_2$

Drug	Dosage of Drug[a] [µmol/kg]	Dosage of $NiCl_2$ [µmol/kg]	Mortality[b]
Sham Injection	0	816	20/20 (100%)
d-Penicillamine	1360	0	0/20 (0%)
d-Penicillamine	1360	816	15/20 (75%)[c]
Triethylenetetramine	1360	0	0/20 (0%)
Triethylenetetramine	1360	816	7/20 (35%)[d]

[a] The drugs were injected into the gluteal miscles within 1 min prior to ip injection of $NiCl_2$

[b] Acute mortality ratios within 1 week after injection

[c] $p < 0.05$ *vs* sham-treated controls (by χ^2 test)

[d] $p < 0.001$ *vs* sham-treated controls (by χ^2 test)

antidotal efficacy of TETA in acute $Ni(CO)_4$ poisoning. An abstract (copy appended) describing the superiority of TETA as an antidote for Ni(II)-toxicity has been submitted for presentation at the International Symposium on Trace Metals which will be held in Toronto in October, 1975 (1). A manuscript describing this research should be completed within the next six months.

B. Investigations of Nickel-Induced Hyperglycemia. As specified in last year's renewal proposal, a principal goal of the present research contract has been the elucidation of the mechanisms of nickel-induced hyperglycemia. This goal has been largely accomplished. Measurements of immunoreactive glucagon were performed upon plasma obtained from rats at intervals from 0.5 to 4 hr after *ip* injection of Ni(II) in dosages of 34 and 68 μmol/kg body wt. The injections of Ni(II) produced prompt increases in plasma glucagon concentrations which closely paralleled the increases in plasma glucose, and which preceded hyperinsulinemia. At 0.5 hr after *ip* Ni(II) (68 μmol/kg), plasma glucagon averaged 0.75 ± 0.24 ng/ml (*vs* 0.20 ± 0.05 in control rats, $p < 0.001$), and plasma glucose concentrations averaged 236 ± 17 mg/dl (*vs* 137 ± 11 in control rats, $p < 0.001$). Plasma concentrations of glucagon and glucose in Ni(II)-treated rats returned to control values within 2-4 hr following the *ip* injection. These findings confirm and extend previous research in our laboratory and demonstrate that hyperglucagonemia is responsible, at least in part, for the acute hyperglycemic effect of Ni(II). A manuscript (copy appended) which describes these findings has been accepted for publication in Toxicology and Applied Pharmacology (2).

Measurements of $K_3Fe(CN)_6$-linked α-ketoglutarate dehydrogenase

activity have been performed in hepatic mitochondria of rats sacrificed at intervals from 0.5 to 24 hr after a single *ip* injection of $NiCl_2$ in dosage of 68 μmol/kg, and following 5 repeated daily injections of $NiCl_2$ in dosage of 34 μmol/kg. Although marked hyperglycemia was found in these rats, there was no significant inhibition of the α-ketoglutarate dehydrogenase activity. Thus, in this respect, the acute toxicity of Ni(II) differs from that of Co(II). Impairment of α-ketoglutarate dehydrogenase activity does not appear to be involved in the pathophysiological mechanism of Ni(II)-induced hyperglycemia. This important negative finding will be published in a review paper on acute Ni(II)-toxicity.

An investigation has been performed of the effect of *im* injection of triethylenetetramine (TETA) upon the hyperglycemia, hyperglucagonemia and hyperinsulinemia which develops in rats following *ip* injection of Ni(II). The data in Table 4 demonstrate that TETA does *not* prevent Ni(II)-induced hyperglycemia and hyperglucagonemia, although TETA does partially suppress the hyperinsulinemia in Ni(II)-treated rats.

C. Investigation of Nickel-Induced Fanconi Syndrome. Another principal goal of this year's contract has been to investigate the acute nephropathy that develops in rats following *ip* injection of $NiCl_2$. Ni(II) was found to cause proteinuria after *ip* administration to rats at a dosage (34 μmol/kg) which was insufficient to produce amino aciduria. The proteinuria was probably the result of glomerular injury, since electron microscopy consistently revealed fusion of foot processes of glomerular epithelial cells. At a higher dosage of Ni(II) (51 μmol/kg), significant histidinuria occurred without any increase in the urinary

Table 4

Effect of Intramuscular Injection of Triethylenetetramine (TETA) upon Hyperglycemia, Hyperglucagonemia and Hyperinsulinemia Induced in Rats by Intraperitoneal Injection of $NiCl_2$

Experimental Groups[a]	Dosage of Ni(II) [μmol/kg]	Dosage of TETA [μmol/kg]	Plasma Glucose[b] [mg/dl] 0.5 hr	Plasma Glucose[b] [mg/dl] 1.0 hr	Plasma Glucagon[b] [ng/ml] 0.5 hr	Plasma Glucagon[b] [ng/ml] 1.0 hr	Plasma Insulin[b] [μU/ml] 0.5 hr	Plasma Insulin[b] [μU/ml] 1.0 hr
Sham Injections[c]	0	0	121±7 [40]	120±10 [40]	0.21±0.09 [20]	0.20±0.07 [20]	19±4 [20]	20±7 [20]
TETA alone	0	680	139±15[e] [40]	144±15[e] [40]	0.24±0.11 [20]	0.32±0.16[c] [20]	27±9[c] [20]	23±7 [20]
Ni(II) alone	68	0	215±33[e] [40]	230±31[e] [40]	1.08±0.41[e] [20]	0.95±0.26[e] [20]	51±16[e] [20]	59±15[e] [20]
Ni(II) + TETA	68	680	216±22[e] [40]	196±20[e] [40]	1.05±0.32[e] [20]	1.16±0.39[e] [20]	31±7[ef] [20]	28±9[df] [20]

[a] Each experimental group contained 80 rats. Forty rats were bled at 0.5 hr and 40 rats were bled at 1.0 hr after administration of Ni(II) *ip* and/or TETA *im*. Controls received sham *ip* and *im* injections. Glucose was measured in all of the plasma samples. Plasma samples from half of the rats in each group were used for radioimmunoassay of glucagon, and plasma samples from the remaining rats were used for radioimmunoassay of insulin.

[b] Each value is the mean ± standard deviation. The number of rats studied is indicated within brackets.

[c] Analyses of plasma samples from 40 rats prior to sham injection yielded the following baseline, fasting values: glucose = 89 ± 6 mg/dl; glucagon = 0.18 ± 0.06 ng/ml, and insulin = 19 ± 5 μU/ml.

[d] $p < 0.05$ *vs* sham-treated controls (by "t" test).

[e] $p < 0.01$ *vs* sham-treated controls (by "t" test).

[f] $p < 0.01$ *vs* Ni(II)-treated rats (by "t" test).

excretion of other amino acids. At dosages of 68 and 85 mol/kg, Ni(II) caused greatly increased excretions of most α-amino acids, presumably mediated by inhibition of amino acid transport systems of the renal tubules. Amino acid and protein excretions consistently returned to normal by the fifth day after *ip* injection of Ni(II), at all of the dosages that were studied. A paper (abstract appended) that describes these findings was presented at the 1975 Annual Meeting of the Society of Toxicology (3), and a manuscript (submitted herewith) has been accepted for publication in Toxicology and Applied Pharmacology (4).

As a continuation of this avenue of research, the antidotal effect of triethylenetetramine (TETA) upon Ni(II)-induced nephropathy has recently been investigated. As shown in Table 5, *im* administration of TETA (750 μmol/kg) greatly reduced the severity of the proteinuria and amino aciduria produced by *ip* injection of $NiCl_2$ (100 μmol/kg). This study furnishes clear-cut evidence that TETA is therapeutically effective in ameliorating the renal toxicity of Ni(II).

D. Attempts to Fractionate ^{63}Ni-Complexes from Serum and Urine by Preparative High-Pressure Liquid Chromatography. As specified in last year's research proposal, a determined attempt has been made to employ high-pressure liquid chromatography for fractionations of ^{63}Ni(II)-complexes in ultrafiltrates of serum and urine from rabbits after *iv* injections of $^{63}NiCl_2$. A wide variety of fractionation conditions, solvents and stationary phases has been tested, and fractions have been collected for (*a*) measurements of ^{63}Ni by liquid scintillation spectrometry and (*b*) analyses of amino acids by ion-exchange chromatography. Regrettably, these experiments have been unsuccessful.

Table 5

Effect of *im* TETA[a] upon Urinary Excretions of ^{63}Ni, Protein and Amino Acids in Rats after *ip* $^{63}NiCl_2$[b]

Constituent	Excretions of ^{63}Ni (% dose/day), Protein (mg/kg/day) and Amino Acids (umol/kg/day) in Urine[c]								
	Control	[Group A] $^{63}NiCl_2$ alone [N=6]				[Group B] $^{63}NiCl_2$ plus TETA [N=6]			
	Rats [N=9]	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
^{63}Ni	---	39 ± 3	5.1 ± 1.1	1.4 ± 0.4	0.6 ± 0.1	39 ± 7	2.1 ± 0.4[i]	0.4 ± 0.1[i]	0.3 ± 0.1[i]
Total Protein	19 ± 4	71 ± 21[f]	82 ± 11[f]	30 ± 7[e]	24 ± 15	32 ± 4[f,i]	14 ± 2[i]	21 ± 5[g]	19 ± 3
Neutral α-Amino Acids									
Glycine	17 ± 2	38 ± 7[f]	64 ± 17[f]	23 ± 6[d]	20 ± 5	24 ± 3[f,i]	17 ± 1[i]	21 ± 1[f]	22 ± 6
Alanine	16 ± 2	65 ± 18[f]	92 ± 37[f]	21 ± 8	12 ± 4	28 ± 4[f,i]	12 ± 2[i]	15 ± 3	15 ± 2
Valine	2.0 ± 0.3	28 ± 7[f]	45 ± 16[f]	9.5 ± 2.0[f]	3.7 ± 1.1[e]	12 ± 2[f,i]	3.7 ± 0.7[i]	4.7 ± 0.5[f,i]	4.0 ± 0.8[f]
Leucine	2.2 ± 0.4	23 ± 6[f]	36 ± 12[f]	8.3 ± 1.4[f]	3.6 ± 0.7[e]	9.5 ± 2.3[f,i]	2.8 ± 0.7[i]	4.1 ± 1.1[e,i]	2.9 ± 0.5[d]
Isoleucine	1.4 ± 0.3	13 ± 3[f]	23 ± 7[f]	5.0 ± 0.8[f]	2.0 ± 0.3[e]	5.1 ± 1.1[f,i]	1.6 ± 0.4[i]	2.1 ± 0.4[e,i]	1.5 ± 0.3[g]
Serine	8.4 ± 0.8	37 ± 9[f]	64 ± 16[f]	15 ± 3[f]	9.6 ± 2.6	16 ± 2[f,i]	9.2 ± 1.2[i]	11 ± 1[f,h]	9.3 ± 1.1
Threonine	10 ± 1	32 ± 9[f]	52 ± 16[f]	14 ± 3[d]	12 ± 4	17 ± 2[f,i]	8.6 ± 0.9[i]	9.5 ± 0.3[h]	9.1 ± 0.9
Phenylalanine	2.1 ± 0.4	10 ± 3[f]	12 ± 3[f]	4.0 ± 0.6[f]	2.4 ± 0.5	4.8 ± 1.0[f,i]	2.4 ± 0.7[i]	2.9 ± 0.6[d,h]	2.7 ± 0.9
Tyrosine	2.3 ± 0.6	8.0 ± 2.6[f]	15 ± 7[e]	2.4 ± 1.5	1.9 ± 1.0	3.3 ± 0.7[d,i]	2.0 ± 0.4[i]	1.8 ± 0.8	1.9 ± 0.8
Methionine	2.1 ± 0.2	7.0 ± 1.6[f]	11 ± 3[f]	3.0 ± 0.4[f]	2.1 ± 0.4	3.6 ± 0.5[f]	2.1 ± 0.3[i]	2.4 ± 0.2[d,h]	2.3 ± 0.4
Cystine	1.3 ± 0.2	6.0 ± 1.2[f]	11 ± 3[f]	2.3 ± 0.3[f]	1.6 ± 0.3	1.8 ± 0.2[f]	1.2 ± 0.1[i]	1.5 ± 0.2[i]	1.3 ± 0.1[g]
Acidic α-Amino Acids and Amides									
Aspartic Acid	14 ± 2	12 ± 2	11 ± 2[d]	5.4 ± 1.1[f]	6.0 ± 1.1[f]	11 ± 2[d]	14 ± 2[g]	8.3 ± 0.5[f,i]	8.8 ± 2.5[e,g]
Asparagine	6.3 ± 1.2	18 ± 3[f]	20 ± 7[f]	8.6 ± 3.6	7.0 ± 2.1	11 ± 2[f,i]	5.6 ± 1.0[i]	11 ± 3[e]	8.3 ± 1.7[d]
Glutamic Acid	5.6 ± 1.3	42 ± 11[f]	46 ± 10[f]	18 ± 7[e]	14 ± 7[d]	23 ± 5[f,i]	6.8 ± 1.5[i]	11 ± 2[f,g]	16 ± 12
Glutamine	15 ± 2	45 ± 18[e]	69 ± 14[f]	14 ± 4	5.8 ± 2.9[f]	16 ± 3[i]	11 ± 3[i]	12 ± 2[d]	8.2 ± 1.5[f]
Basic α-Amino Acids									
Histidine	5.5 ± 1.0	44 ± 4[f]	17 ± 4[f]	6.1 ± 1.7	4.9 ± 1.1	7.3 ± 1.2[d,i]	5.2 ± 0.8[i]	6.2 ± 0.5	5.3 ± 0.6
Arginine	4.7 ± 0.7	23 ± 6[f]	36 ± 11[f]	9.2 ± 2.0[f]	4.6 ± 0.7	9.2 ± 2.0[f,i]	4.0 ± 1.1[i]	5.3 ± 1.7[h]	5.0 ± 1.2
Lysine	5.9 ± 0.6	44 ± 11[f]	90 ± 24[f]	18 ± 3[f]	7.3 ± 1.4	21 ± 4[f,i]	7.5 ± 0.8[i]	9.2 ± 1.2[f,i]	8.3 ± 1.3[e]
Ornithine	2.1 ± 0.3	5.0 ± 2.0[e]	7.6 ± 2.8[f]	2.8 ± 0.9	2.2 ± 0.6	3.2 ± 0.6[e,g]	2.5 ± 0.3[i]	3.1 ± 0.2[f]	3.2 ± 0.6[e,g]
Citrulline	<1	7.8 ± 2.0[f]	14 ± 5[f]	2.6 ± 0.6[f]	<1	2.8 ± 0.6[f,i]	<1[i]	<1[i]	<1

Table 5 (cont'd)

Substituted and Misc. Amino Acids									
β-Alanine	7.7 ± 1.2	9.2 ± 3.6	3.4 ± 2.3[e]	5.8 ± 3.2	4.6 ± 2.8[d]	6.8 ± 2.0	7.4 ± 1.4[h]	11 ± 1[f,i]	8.2 ± 0.8[h]
γ-Aminobutyric Acid	1.5 ± 0.3	1.8 ± 0.7	1.6 ± 0.2	2.1 ± 0.6	1.8 ± 0.3	1.6 ± 0.3	2.1 ± 0.7	2.6 ± 1.0[d]	2.3 ± 0.3[f,g]
1-Methylhistidine	4.4 ± 2.7	10 ± 7	6.3 ± 2.2	4.7 ± 1.7	6.0 ± 3.5	4.8 ± 2.1	3.2 ± 1.4[h]	4.9 ± 1.6	4.7 ± 1.7
3-Methylhistidine	5.1 ± 0.9	6.9 ± 1.0[e]	4.9 ± 1.2	4.9 ± 1.5	5.0 ± 1.5	5.0 ± 1.0[h]	3.7 ± 0.8[g]	4.9 ± 0.8	4.3 ± 0.6
Sarcosine (N-methylglycine)	3.4 ± 0.5	2.7 ± 0.6[d]	2.8 ± 0.7	3.1 ± 0.9	3.4 ± 0.5	4.1 ± 0.6[d,h]	3.5 ± 0.4[g]	4.7 ± 0.5[f,h]	4.1 ± 1.8

[a] TETA, 750 μmol/kg *im*

[b] $^{63}NiCl_2$, 100 μmol/kg = 1,375,000 cpm/rat

[c] Mean ± S.D.

[d] $p < 0.05$ *vs* corresponding value in control rats

[e] $p < 0.01$ *vs* corresponding value in control rats

[f] $p < 0.001$ *vs* corresponding value in control rats

[g] $p < 0.05$ *vs* corresponding value in rats with $^{63}NiCl_2$ alone

[h] $p < 0.01$ *vs* corresponding value in rats with $^{63}NiCl_2$ alone

[i] $p < 0.001$ *vs* corresponding value in rats with $^{63}NiCl_2$ alone

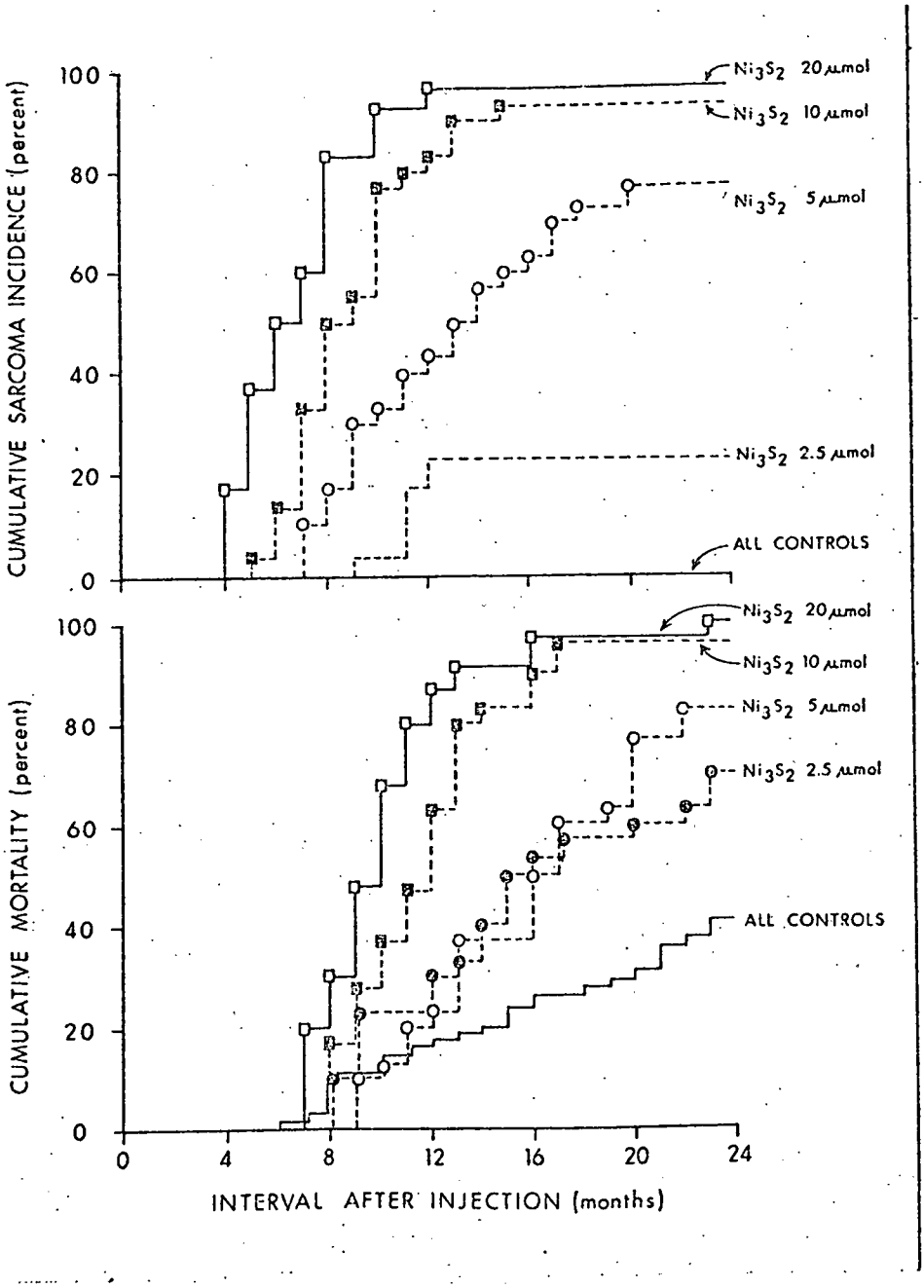
Whereas five distinct ^{63}Ni-complexes could be clearly resolved by thin-layer chromatography of serum ultrafiltrates on cellulose plates (5), we have not been able to accomplish fractionations of ultrafiltrable ^{63}Ni-complexes by high-pressure liquid chromatography. We have concluded that the stationary phases that are currently available for HPLC are not suitable for this application. It should be noted that the stationary phases that have been tested included various coated microbeads of glass that have recently been developed by the Corning Glass Co.

E. Studies of Nickel Carcinogenesis. Our investigation of Mn inhibition of Ni_3S_2 carcinogenesis is nearing completion. During 1972, Ni_3S_2 was administered *im* to 480 ♂ Fischer rats at several dosage levels, alone or in combination with Mn and Cr dusts. The last surviving rats in these experimental groups were killed according to schedule during December, 1974. The gross autopsies and histopathological examinations have been completed.

Dose-response curves for sarcoma incidence at the injection site and cumulative mortality curves are given in Figure 1 for rats which received *im* Ni_3S_2 alone. There were 30 rats in each of these test groups and a total of 180 rats in the combined control groups (as will be described in Table 6). Of the 152 sarcomas that developed at the injection site in rats that received only Ni_3S_2, 82 (54%) were categorized as rhabdomyosarcomas; 39 (26%) undifferentiated or unclassified sarcomas; 25 (16%) fibrosarcomas; 5 (3%) liposarcomas; and 1 (1%) angiosarcoma. Metastases to lungs, mediastinal lymph nodes, liver, or retroperitoneal lymph nodes were found in 60% of sarcom-bearing rats. As shown in Figure 1, the sarcoma incidence was 97% within 1 year in rats which received

Figure 1

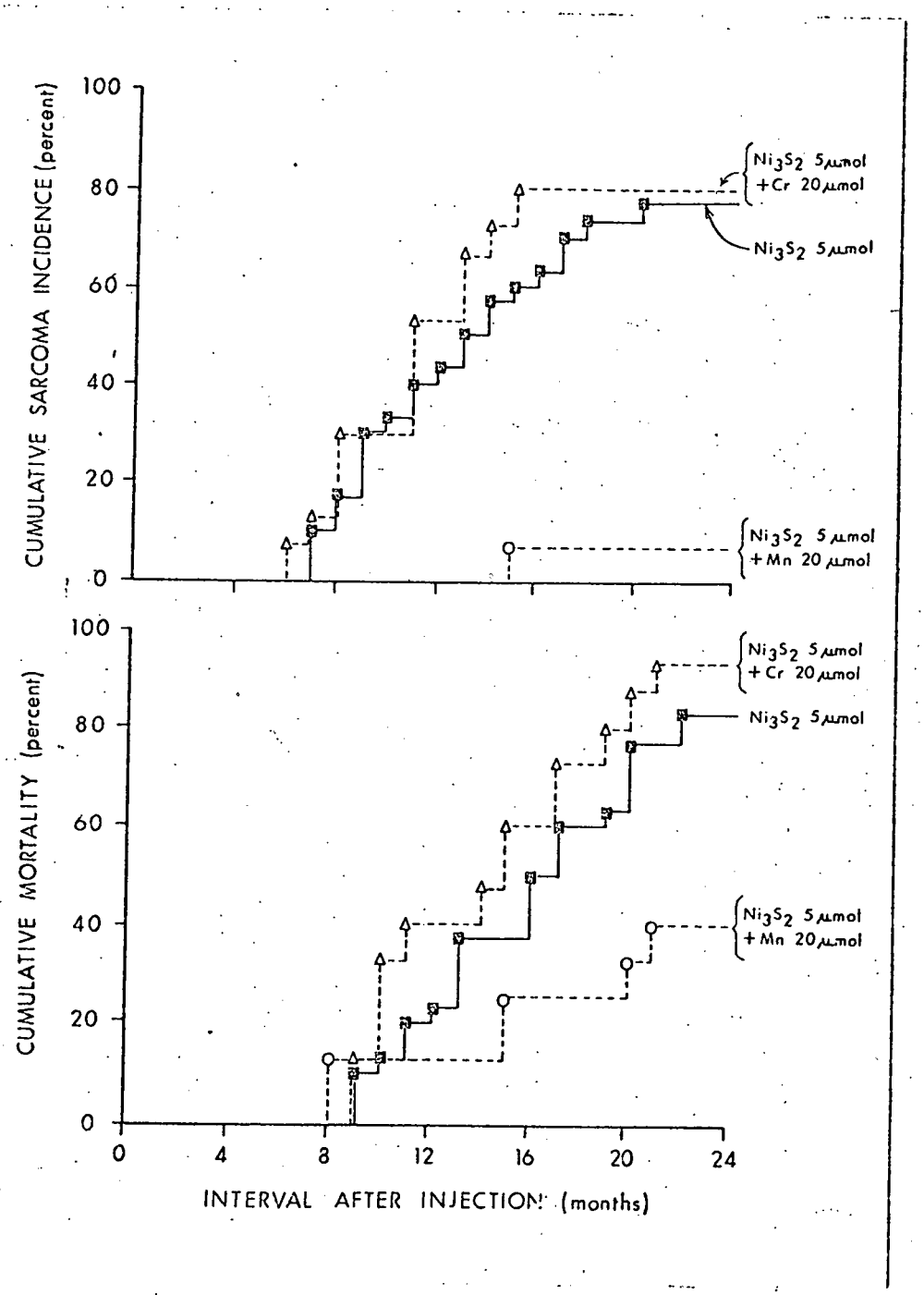
Dose-Response Study of Ni_3S_2 Carcinogenesis



This figure shows the cumulative incidence of sarcomas at the injection site and the cumulative mortality in Groups of 30 ♂ Fischer rats which received a single i.m. injection of Ni_3S_2 in dosage of 2.5, 5, 10 or 20 µmol, and in a combined control group of all 180 rats which did not receive any Ni_3S_2.

Figure 2

Effects of Four-Fold Molar Excess of Cr^0 and Mn^0 dusts upon Ni_3S_2 Carcinogenesis



This figure shows the cumulative incidence of sarcomas at the injection site and the cumulative mortality in a group of 30 ♂ Fischer rats which received a single i.m. injection of 5 µmol of Ni_3S_2, and in two groups of 15 ♂ Fischer rats which received Ni_3S_2 (5 µmol) in combination with 20 µmol of Cr^0 or Mn^0 dusts.

20 moles of Ni_3S_2. Typical dose-response relationships for cumulative sarcoma incidence and cumulative mortality were observed with dosages of Ni_3S_2 ranging from 2.5 to 20 μmoles. No sarcomas developed at the injection site in any of the controls. As expected for Fischer rats, the mortality in the controls reached 42% at 24 months after injection (*i.e.* at 26 months of age).

A summary of the effects of Mn and Cr upon the incidence of sarcomas induced by Ni_3S_2 in all of the experimental groups is given in Table 6. Mn dust consistently produced significant reduction in sarcoma incidences compared to rats that received only Ni_3S_2. Cr dust had no effect.

As an example, in Figure 2 are shown cumulative sarcoma incidence and cumulative mortality curves for rats which received Mn and Cr dusts (20 μmol) and Ni_3S_2 (5 μmol). It may be seen that combined administration of 20 μmoles of Mn with 5 μmoles of Ni_3S_2 reduced the sarcoma incidence from 77 to 7% ($p < 0.001$), whereas a similar administration of Cr dust had no significant effect. Mn dust suppressed the cumulative mortality as well. Thus, in rats which received 5 μmol of Ni_3S_2 alone, the 2 year mortality was 83%, compared to 40% in rats which received Ni_3S_2 plus Mn ($p < 0.01$). The cumulative mortality rate in the group with combined Ni_3S_2 plus Mn did not differ from that found in the combined controls.

Measurements have been performed of ^{63}Ni excretion in urine and feces of a group of 4 rats which received a single *im* injection of $^{63}Ni_3S_2$ (5 μmol) and of a group of 4 rats which received a single *im* injection of $^{63}Ni_3S_2$ (5 μmol) in combination with Mn dust (20 μmol).

Table 6

Effects of Mn and Cr upon the Incidence of Ni_3S_2-Induced Sarcomas in Fischer Rats

Experimental Category			Sarcoma Incidence at Injection Site Ni_3S_2 Dosage (μmol)				
Group	Metal	(μmol)	0	2.5	5	10	20
A	none	0	0/60	7/30	23/30	28/30	29/30
B	Mn	10	0/15	0/15	3/15[b]	8/15[a]	
C		20	0/15	0/15	1/15[b]		
D		40	0/15	1/15			
E		80	0/15				
F	Cr	10	0/15	4/15	8/15	14/15	
G		20	0/15	7/15	12/15		
H		40	0/15	7/15			
I		80	0/15				
All Controls[c]			0/180				

[a] $p < 0.01$ *vs* Group A, by χ^2 test

[b] $p < 0.001$ *vs* Group A, by χ^2 test

[c] All rats which did not receive any Ni_3S_2

Table 7

Effect of Mn^{0} upon Kinetics of ^{63}Ni Excretion in Urine and Feces of Fischer Rats after Intramuscular Injection of $^{63}Ni_3S_2$ [a]

Parameter	Experimental Groups		Units
	A	*B*	
No. of Rats	4	4	
$^{63}Ni_3S_2$ dosage	5	5	µmol
Mn dosage	0	20	µmol
Size of $Pool_1$ (C_1)	63 ± 8	55 ± 12	% of dose
Size of $Pool_2$ (C_2)	27 ± 6	30 ± 6	% of dose
Size of $Pool_3$ (C_3)	11 ± 2	15 ± 8	% of dose
Half-life of $Pool_1$ (Θ_1)	14 ± 1	15 ± 1	days
Half-life of $Pool_2$ (Θ_2)	60 ± 24	55 ± 18	days
Fecal ^{63}	9.0 ± 2.7	10.5 ± 6.5	% of total ^{63}Ni excretion

[a] Based upon the following equation:

$$^{63}\text{Ni Retention (\% of dose)} = C_1 e^{\frac{-0.693}{\Theta_1} t} + C_2 e^{\frac{-0.693}{\Theta_2} T} + C_3$$

Urine and feces were collected for 8 consecutive weeks and again at 14 weeks and 20 weeks. The results of this study are illustrated in Figure 3. These data indicate that the concurrent administration on Mn did not significantly influence the rate of excretion of ^{63}Ni. These data have been subjected to compartmental analysis, assuming a three-compartment model, and the results are given in Table 7. There were no significant differences in the pool sizes or half-lives of the 3 compartments. This study indicates that the inhibitory effect of Mn dust upon Ni_3S_2 carcinogenesis is not attributable to an effect of Mn upon the rates of excretion or volumes of distribution of ^{63}Ni derived from $^{63}Ni_3S_2$.

A serial sacrifice study was performed in which groups of 3 rats were killed at intervals from 1 day to 10 weeks after a single *im* injection of Ni_3S_2 (5 μmol) and/or Mn dust (20 μmol) (singly or in combination). Histopathological studies revealed that concurrent injection of Mn dust did not significantly alter the acute pathological reactions to Ni_3S_2. Atomic absorption spectrometry analyses of Ni were performed upon the entire thigh muscles of rats which were killed at intervals of 1, 6, 8 and 10 weeks after the injection. The results of these analyses (Table 8) indicate that Mn did not significantly affect the retention of Ni derived from Ni_3S_2.

Table 8

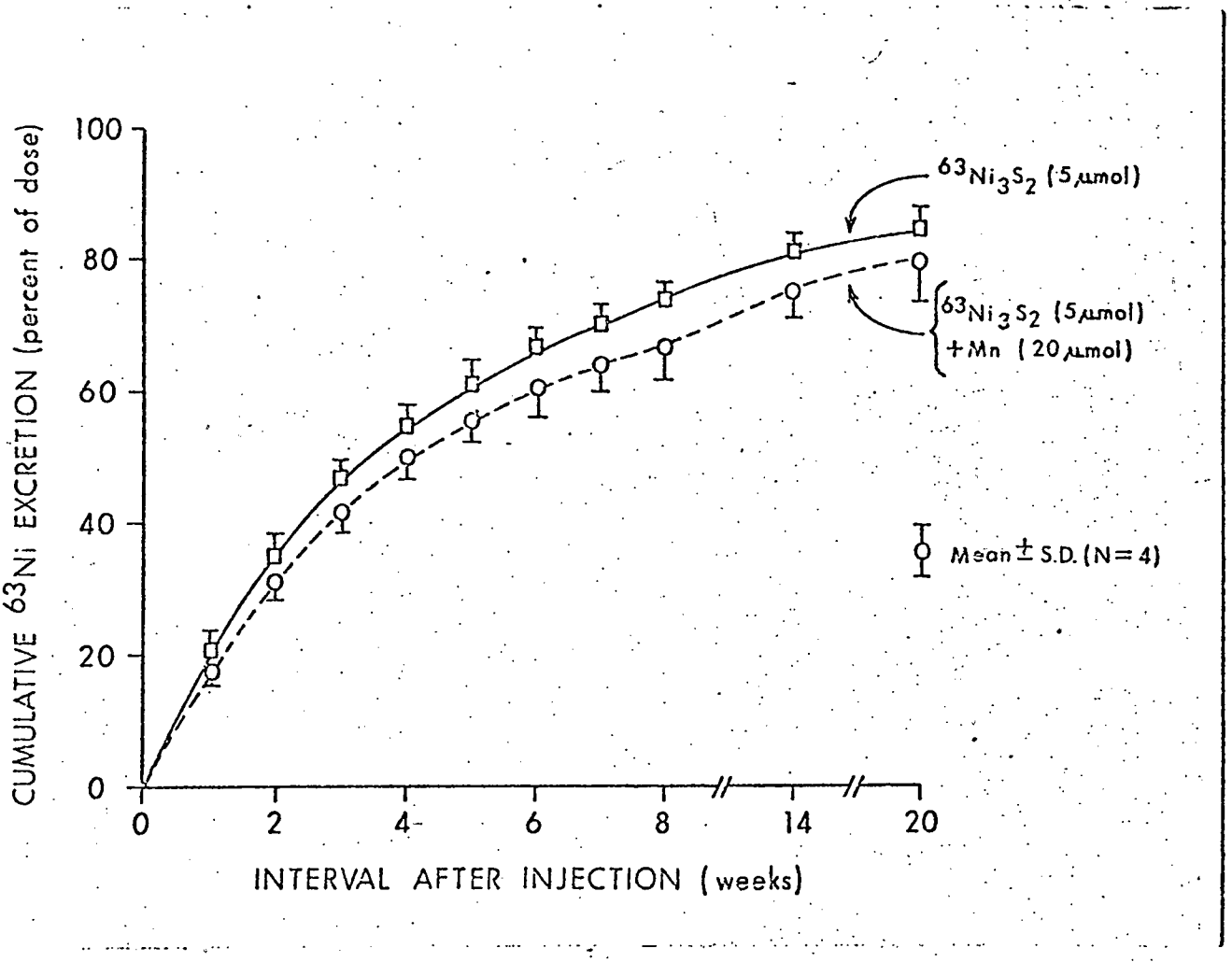
Ni in Thigh Musculature of Fischer Rats after Injection of Ni_3S_2 and Mn Singly or in Combination[a]

Group	No. of Rats	Compound	Dosage (μmol)	Ni at Injection Site (% of dose) *Time after injection*			
				1 wk	*6 wk*	*8 wk*	*10 wk*
A	12	Ni_3S_2	5	64(63-65)	25(22-29)	22(21-24)	18(12-23)
C	12	Ni_3S_2 plus Mn	5 20	60(41-80)	24(20-27)	20(18-22)	17(14-19)

[a] Three rats in each group were killed at each interval

Figure 3

Effect of Mn^0 Dust upon the Excretion of ^{63}Ni derived from $^{63}Ni_3S_2$ in Fischer Rats



This figure shows the cumulative excretion of ^{63}Ni in urine and feces of a group of 4 ♂ Fischer rats which received a single im injection of 5 µmol of $^{63}Ni_3S_2$, and in a group of 4 ♂ Fischer rats which received $^{63}Ni_3S_2$ (5 µmol) in combination with 20 µmol of Mn^0 dust.

A paper (abstract submitted herewith) which described these studies was presented at the 1975 Annual Meeting of the American Association for Cancer Research (6). Some of the preliminary results of this study have been included in a review paper on metal carcinogenesis (submitted herewith) that has been accepted for publication in Advances in Modern Toxicology (7). A manuscript which describes this research is currently being drafted and will shortly be submitted for publication in Cancer Research.

As specified in last year's renewal proposal, carcinogenesis studies have been initiated in Syrian hamsters to determine (*a*) whether epidermoid cancers can be induced in the mucosa of the oropharynx by topical administration of Ni_3S_2; and (*b*) whether cancers of the lung can be induced by intratracheal instillations of Ni_3S_2. In addition, comparisons are under way of the carcinogenicities of Ni powder, Ni_3S_2, NiFeS, and NiS following intramuscular injection in Fischer rats. An abstract (submitted herewith) (8) of a paper that describes the preliminary results of this study has been submitted for presentation at the International Conference on Heavy Metals in the Environment, to be held in Toronto, Canada in October 1975.

F. Studies of Ni_3S_2-Induced Erythrocytosis in Rats. Jasmin and Solymoss (9) have reported that an intrarenal (but *not* an intramuscular) injection of Ni_3S_2 induces pronounced erythrocytosis in rats. This remarkable finding has been confirmed and extended in our laboratory. We are in the process of establishing the time-response and dose-response relationships for Ni_3S_2-induced erythrocytosis. In our *first experiment*, Ni_3S_2 powder (10 mg) in 0.2 ml of NaCl solution was injected into one

kidney of 18 ♀ rats. For comparisons, Ni_3S_2 (10 mg) was also injected *im* into thigh muscles of a group of 24 ♀ rats, and 0.2 ml of NaCl solution was injected into one kidney of a control group of 20 ♀ rats. Hematological studies (RBC, WBC, hematocrit, hemoglobin) have been performed at monthly intervals for 7 months. Data for blood hematocrit in the three groups of rats are given in Table 9. Significant increases in hematocrit ($p < 0.001$) were observed in the group that received intrarenal Ni_3S_2 from 1 to 4 months after the injection. No significant increases in hematocrit were found in the group that received intramuscular Ni_3S_2. The erythrocytosis induced by intrarenal Ni_3S_2 was accompanied by increase in erythrocyte mass (4.9 ± 0.7 ml/100 g in 4 rats at 8 wk *vs* 1.9 ± 1.0 ml/100 g in 4 control rats, ($p < 0.01$), and by diminution in plasma volume (1.8 ± 0.4 ml/100 g in 4 rats at 8 wk *vs* 2.5 ± 0.3 ml/100 g in 4 control rats ($p < 0.05$)). Histological examinations of 4 to 6 rats in each group at 8 wk revealed scarring along needle tracts and black nickel deposits in kidneys of rats that received intrarenal Ni_3S_2 and slight scarring in kidneys of the control group that received intrarenal NaCl. The bone marrow of rats that received intrarenal Ni_3S_2 showed marked hyperplasia with erythrocytic predominance. Concommitant increases in blood erythrocyte counts and hemoglobin concentrations were observed in rats that received intrarenal Ni_3S_2, but not in the other two groups. No significant changes were observed in blood leukocyte or platelet counts in any group.

In our *second experiment*, Ni_3S_2 powder in 012 ml of NaCl solution was injected into one kidney of four groups of ♂ Fischer rats (12 rats per group) in dosages of 0.63, 1.25, 2.5 and 5.0 mg, respectively. A

Table 9

Effect of Intrarenal or Intramuscular Injection of Nickel Subsulfide (Ni_3S_2) upon Blood Hematocrit in Rats

Experimental Groups	Dosage of Ni_3S_2 [mg/rat]	Hematocrit at Intervals after an Injection of Ni_3S_2[a]							
		<1 wk	*1 mo*	*2 mo*	*3 mo*	*4 mo*	*5 mo*	*6 mo*	*7 mo*
Controls[b]	0	41±2 [20]	42±2 [20]	43±3 [20]	41±2 [10]	39±3 [10]	39±2 [10]	41±1 [10]	40±2 [10]
Intrarenal Ni_3S_2	10	38±4[d] [18]	58±7[e] [18]	61±8[e] [18]	58±9[e] [15]	54±7[e] [15]	46±6[e] [15]	43±4 [15]	39±2 [15]
Intramuscular Ni_3S_2	10	40±2 [24]	40±2[c] [24]	41±3[c] [24]	40±2 [22]	40±3 [22]	38±2 [22]	40±3 [20]	41±2 [6]

[a] Hematocrit values are expressed as %; each value is the mean ± standard deviation; the number of rats studied at each interval is indicated within brackets

[b] The control rats were given an intrarenal injection of 0.2 ml of NaCl solution (140 mmol/liter)

[c] $p < 0.05$ *vs* corresponding value in controls (by "t" test)

[d] $p < 0.01$ *vs* corresponding value in controls (by "t" test)

[e] $p < 0.001$ *vs* corresponding value in controls (by "t")test)

Table 10

Dose-Response Relationship for the Effect of Intrarenal Injection of Nickel Subsulfide (Ni_3S_2) upon Blood Hematocrit in Rats

Experimental Groups	Dosage of Ni_3S_2 [mg/rat]	Hematocrit at Intervals after an Injection of Ni_3S_2[a]				
		<1 wk	*1 mo*	*2 mo*	*3 mo*	*4 mo*
Controls[b]	0	40±2 [12]	42±4 [11]	45±2 [11]	39±2 [11]	37±2 [10]
Intrarenal Ni_3S_2	0.63	40±1 [12]	48±6[c] [11]	51±7[c] [11]	50±7[d] [11]	47±8[d] [11]
	1.25	40±2 [12]	51±5[d] [12]	57±6[d] [12]	53±4[d] [12]	50±4[d] [12]
	2.5	38±2 [12]	51±3[d] [12]	54±5[d] [11]	54±5[d] [12]	50±4[d] [12]
	5.0	40±2 [12]	54±5[d] [12]	56±4[d] [11]	59±2[d] [12]	56±4[d] [12]

[a] Hematocrit values are expressed as %; each value is the mean ± standard deviation; the number of rats studied at each interval is indicated within brackets

[b] The control rats were given an intrarenal injection of 0.2 ml of NaCl solution (140 mmol/liter)

[c] $p < 0.05$ *vs* corresponding value in controls (by "t" test)

[d] $p < 0.001$ *vs* corresponding value in controls (by "t" test)

control group of rats received an injection of the saline vehicle. The results to date for monthly measurements of blood hematocrit are given in Table 10. Significant erythrocytosis has been observed from 1 to 4 months after the injection, even at the lowest dosage of Ni_3S_2 (0.63 mg). In general, the degree of erythrocytosis is directly related to the dosage of Ni_3S_2 (Table 10). A paper (abstract appended) which describes our preliminary findings was presented at the 1975 Spring Meeting of the Association of Clinical Scientists (10). These studies are being continued, and it is anticipated that a further paper on this work will be presented at the 1975 Annual Meeting of the American Society of Hematologists in December 1975. A manuscript will thereafter be submitted for publication.

G. Measurements of Nickel in Biological Fluids by Electrothermal Atomic Absorption Spectrometry. As specified in last year's renewal proposal, continued research has been directed to improvement and evaluation of a new analytical method for nickel analysis in biological fluids by means of electrothermal (non-flame) atomic absorption spectrometry. Progressive refinements in techniques of sampling, charring, atomization, background-correction, standardization and computation have been introduced, and the method is now a reliable procedure for measurement of nickel in serum (but not yet in urine). Problems in trace metal analysis by electrothermal atomic absorption have been discussed in a review article (submitted herewith) that has been accepted for publication in the Annals of Clinical and Laboratory Science (11). The new method for analysis of serum nickel has been accepted for presentation at the Annual Applied Seminar of the Association of Clinical Scientists (Philadelphia,

November, 1975). A manuscript that describes the method is currently being drafted and will be submitted for publication in Annals of Clinical and Laboratory Science.

H. Miscellaneous Accomplishments. An improved method for analysis of serum iron has been developed, and a manuscript (submitted herewith) which describes this technique has been accepted for publication in Annals of Clinical and Laboratory Science (12). A review article (submitted herewith) on clinical disturbances of zinc metabolism has been published in the Annals of Clinical and Laboratory Science (13), and a chapter on trace metals (submitted herewith) has been written for publication in a new textbook on Chemical Diagnosis of Disease (14).

On August 6, 1975, we received official notification of ERDA's approval of the supplement to our research contract, which authorizes us to expand this research program in order to investigate the potential health hazards to workers and the general population from nickel exposures derived from coal-gasification technologies. The research which is specified in the supplemental proposal was immediately begun by ordering the requisite animals, radioisotopes and equipment, and initiating reassignments of our technical staff. This reorientation and expansion of our ERDA-sponsored research program should be effectively accomplished by September 30, 1975.

I. Compliance with Contract Requirements. The specific objectives of this year's research, as specified in last year's renewal proposal were:

(1) Investigation of Nickel-Induced Hyperglycemia. This work has been accomplished as specified in Section B of this Progress Report.

(2) Investigation of Nickel-Induced Fanconi Syndrome. This work has been accomplished, as specified in Section C of this Progress Report.

(3) Fractionations of ^{63}Ni-Complexes by Preparative High-Pressure Liquid Chromatography. This work has been pursued with substantial effort but without success, as discussed in Section D of this Progress Report.

(4) Uptake, Protein-Binding and Release of ^{63}Ni by Leukocytes. As a substitute for this project, we have investigated the therapeutic efficacy of triethylenetetramine (TETA) in acute nickel poisoning, since this new avenue of research appeared to be exceptionally important to the accomplishment of one of the specified long-range goals of this research program (*i.e.* the development of new methods for the therapy of nickel poisoning). Our findings on the antidotal effectiveness of TETA are summarized in Section A of this Progress Report.

(5) Nickel Carcinogenesis. The phases of this project which were scheduled to be undertaken during the past year have been substantially accomplished, as specified in Section E of this Progress Report.

(6) Analytical and Environmental Studies: (*a*) Development and evaluation of a method for nickel analysis in serum by electrothermal atomic absorption spectrometry has been almost accomplished as specified in Section G of this Progress Report. (*b*) Studies of nickel exposure of inhabitants of a trailer park in Sumter County, South Carolina have been undertaken in collaboration with the Center for Disease Control and the South Carolina State Department of Health. This environmental problem is now under litigation in the State of South Carolina. (*c*) The

projected interlaboratory comparison of nickel analyses in urine samples has been deferred until the electrothermal atomic absorption method has been adapted for measurements of nickel in urine.

J. Effort of the Principal Investigator. The Principal Investigator devoted 25% of his time and effort to this project during the period from December 1, 1974 to June 30, 1975. Commencing July 1, 1975, the Principal Investigator is now devoting 35% of his time and effort to this project.

K. Significance of the Research and its Relevance to National Priorities. Much environmental concern has been focused upon lead, cadmium and mercury within recent years, and considerable research is currently being directed to the toxicology of these metals in laboratories throughout the United States. The causes for concern regarding environmental pollution and industrial toxicity from nickel and its compounds are equally serious, as indicated by the recent report on nickel by the Committee on Medical and Biological Effects of Environmental Pollutants of the National Academy of Sciences (15). Insofar as the Principal Investigator can ascertain, ERDA Contract No E(11-1)-3140 is currently the only research program in the United States which is specifically focused upon the toxicology and metabolism of nickel compounds.

The relevance of this research to national priorities has been substantially enhanced by the recent 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.* Ni_3S_2) may represent serious, unrecognized hazards to

industrial workers and even to the general population. Detailed discussions of the pertinence of nickel toxicology to the broad area of health-related effects of coal as an energy source have been included in the recent Proposal for a Supplement to this contract (submitted on April 28, 1975), and hence will not be repeated in this Progress Report. In order to investigate the health-related effects of nickel exposures derived from coal-gasification technologies, an expanded scope of this program has recently been approved so that the principal investigator and his fellow-workers are now initiating teratogenicity and mutagenicity testing of nickel compounds, and development of new biochemical indices and analytical methods for detection of nickel exposures. These new data and techniques will become important to national priorities if nickel-catalyzed gasification technologies for coal are adopted.

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