Abstract of Progress Report

Attachment to U.S. AEC Research Document No. COO-3140-22

"The Toxicology and Metabolism of Nickel Compounds"

The toxicology and metabolism of nickel compounds (NiCl_2 , NiSO_4 , Ni_3S_2) were investigated in rats and rabbits. The new knowledge has included:

(1) Understanding of nickel-induced hyperglycemia, based upon (a) establishment of dose-response relationships, (b) comparison of hyperglycemic responses of divalent ions of first transition metals, (c) observation of effects of adrenalectomy and hypophysectomy, and (d) measurements of serum insulin during Ni(II) -induced hyperglycemia.

(2) Discovery of localization of ^{63}Ni in pituitary of rabbits after injections of $^{63}\text{NiCl}_2$. This finding may have physiological importance in view of recent reports that administration of Ni(II) to rats depresses release of pituitary prolactin.

(3) Demonstration of ultrafiltrable nickel complexes in rabbit serum after $^{63}\text{NiCl}_2$ injection, based upon fractionations of serum ultrafiltrates by thin-layer chromatography and autoradiography.

(4) Improvement of methods for analysis of nickel and other trace metals (a) by non-flame atomization for atomic absorption spectrometry, and (b) by use of teflon combustion bombs for preliminary digestion of solids.

(5) Substantiation of the value of hair sampling in evaluation of environmental exposures to nickel.

(6) Confirmation that Ni_3S_2 carcinogenicity in rats is depressed by simultaneous injection of Mn dust, and demonstration that this effect

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is not the result of increased local inflammatory reaction or increased rates of elimination of nickel in urine or feces.

F. William Sunderman, Jr., M.D.
August 1, 1974

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AEC RESEARCH DOCUMENT NO. C00-3140-22Progress Report for Atomic Energy Commission Contract"The Toxicology and Metabolism of Nickel Compounds"August 1, 1974

Principal Investigator: F. William Sunderman, Jr., M.D., Professor and
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of Medicine, Farmington, Connecticut 06032.

Scientific Accomplishments During the Past Grant Year:

I. Nickel-Induced Hyperglycemia. As specified in last year's renewal proposal, one of the principal goals of the present research contract was elucidation of the mechanisms of nickel-induced hyperglycemia. Substantial progress has been made toward this goal. The effects of Ni(II), administered ip as NiCl_2 or NiSO_4 , upon plasma glucose and serum insulin concentrations have been investigated in Fischer rats. In fasting rats, hyperglycemia ($p < 0.001$) occurred at 0.5 hr after injection of Ni(II) in dosages from 12 to 85 $\mu\text{mol/kg}$. At dosages $\geq 34 \mu\text{mol/kg}$, hyperglycemia persisted for 4 hr after injection of Ni(II). In approximate order of decreasing magnitude and duration, the hyperglycemic responses to equimolar ip injections of divalent ions of selected first transition metals were: $\text{Ni} \geq \text{Co} \geq \text{Cu} \geq \text{Zn} > \text{Cr} > \text{Fe} > \text{Mn}$. The hyperglycemic responses to Ni(II), Co(II) and glucagon were greatly suppressed but were not completely prevented by adrenalectomy and by hypophysectomy. These findings are attributed to interruption of the permissive actions of glucocorticoids upon hepatic gluconeogenesis and glycogenolysis. Adrenergic blockade with phentolamine and propranolol did not affect the hyperglycemic response to Ni(II). Concurrent administration of insulin antagonized the hyperglycemic response to Ni(II).

Concentrations of endogenous immunoreactive insulin in serum were increased ($p < 0.01$) from 0.5 to 3 hr after injection of Ni(II) in dosage of 68 $\mu\text{mol/kg}$. The hyperglycemic response to Ni(II) is apparently caused by stimulation of glucagon release from pancreatic alpha cells, and inhibition of hepatic carbohydrate metabolism. A manuscript¹ (submitted herewith) which describes these studies of Ni(II)-induced hyperglycemia has been submitted for publication in Toxicology and Applied Pharmacology.

II. Distribution of ^{63}Ni in Rabbit Tissues Following Intravenous Injection of $^{63}\text{Ni(II)}$. As a continuation of the compartmental analyses of $^{63}\text{Ni(II)}$ metabolism which were described in last year's progress report, measurements have been made of the distribution of ^{63}Ni in tissues of rabbits following iv administration of $^{63}\text{NiCl}_2$. In rabbits which were killed at 2 hr after a single injection (4 $\mu\text{mol/kg}$), the relative distribution of ^{63}Ni per g of fresh tissue was: kidney > pituitary > serum > whole blood > lung > heart > testis > pancreas > adrenal > duodenum > bone > spleen > liver > muscle > spinal cord > cerebellum > medulla oblongata = hypothalamus. In rabbits which were killed at 24 hr after the last of 34-38 consecutive daily injections (75 nmol/kg/day), the relative distribution of ^{63}Ni was: kidney > pituitary > spleen > lung > skin > testis > serum = pancreas = adrenal > sclerae > duodenum > liver > whole blood > heart > bone > iris > muscle > cornea > cerebellum = hypothalamus > medulla oblongata > spinal cord > retina > lens > vitreous humor. The most notable finding in this study was the localization of substantial concentrations of ^{63}Ni in the pituitary. A manuscript² (submitted herewith) which describes these measurements has already been accepted and rapidly published in Research Communications in Chemical Pathology and Pharmacology.

III. Binding of ^{63}Ni to Ultrafiltrable Constituents of Rabbit Serum.

As indicated in last year's renewal proposal, a major effort during the past year has been the investigation of partitions of ^{63}Ni between serum proteins and ultrafiltrable nickel receptors. The binding of $^{63}\text{Ni(II)}$ to ultrafiltrable constituents of rabbit serum was studied (a) after *in vitro* incubation (2 hr, 37°) of rabbit serum with $^{63}\text{NiCl}_2$ (10 - 100 $\mu\text{mol/liter}$), and (b) at intervals (0.25 - 2 hr) after *in vivo* administration of $^{63}\text{NiCl}_2$ (40 - 160 $\mu\text{mol/kg}$, iv). Serum ultrafiltrates were fractionated by thin-layer chromatography, and the thin-layer chromatograms were visualized by autoradiography and by ninhydrin staining. Five ultrafiltrable ^{63}Ni -complexes were demonstrated, based upon the presence in the autoradiograms of distinct radiodense ^{63}Ni -bands with chromatographic mobilities which correspond to ninhydrin-positive bands. Unbound $^{63}\text{Ni(II)}$ was not detected in serum ultrafiltrates in either the *in vitro* or *in vivo* experiments. In serums from 10 rabbits which were incubated *in vitro* with $^{63}\text{Ni(II)}$ (10 $\mu\text{mol/liter}$), the mean percentage of ultrafiltrable ^{63}Ni was 36% (range = 33 - 38) of total serum ^{63}Ni . In contrast, in serums which were obtained from 10 rabbits at 2 hr after iv injection of $^{63}\text{Ni(II)}$, (40 $\mu\text{mol/kg}$), the mean concentration of total serum ^{63}Ni was 10.8 $\mu\text{mol/liter}$ (range = 6 - 14), and the mean percentage of ultrafiltrable ^{63}Ni was 15% (range = 5 - 21) of total serum ^{63}Ni . The disparity between the percentages of ultrafiltrable ^{63}Ni which were obtained *in vitro* versus *in vivo* was obviated when the *in vivo* experiments were performed in rabbits which had been subjected to bilateral nephrectomy and ligation of the common bile duct. This investigation confirms the existence of several nickel receptors in serum ultrafiltrates, and substantiates the role of ultrafiltrable complexes

in the excretion of nickel. A manuscript³ which describes these investigations (submitted herewith) has been submitted for publication in Clinical Chemistry.

IV. Binding of ^{63}Ni to α_1 -Macroglobulin from Rabbit Leukocytes.

As described in last year's progress report, experiments in our laboratory demonstrated labelling of rabbit serum α_1 -macroglobulin with ^{63}Ni *in vivo* and *in vitro*. However, we were consistently unable to accomplish incorporation of ^{63}Ni into the serum α -macroglobulin fraction in amounts which were comparable to our previous measurements, based upon atomic absorption of ^{59}Ni in the macroglobulin fraction obtained by chromatography of large quantities of pooled rabbit serum. It is now apparent that this discrepancy was the result of differences in the ways in which the small amounts of blood for ^{63}Ni measurements and the large volumes of blood for ^{59}Ni measurements were collected and processed. When small volumes of blood were collected for ^{63}Ni measurements, the blood was centrifuged in small tubes with prompt and complete separation of leukocytes. On the other hand, when liter volumes of pooled blood were processed for ^{59}Ni measurements, there were substantial delays in centrifugation, and the separation of leukocytes was incomplete. Under these circumstances, the nickel-binding α -macroglobulin was released from leukocytes. The following experiments have led us to this conclusion. Peritonitis was induced in rabbits by intraperitoneal injection of shellfish glycogen dissolved in sterile saline solution. After 16 hr, peritoneal exudate was removed and the polymorphonuclear leukocytes were washed and suspended in buffered Hanks' solution. The leukocytes (4×10^7 cells/ml) were incubated for 1 hr at 37° in the presence of $^{63}\text{Ni(II)}$ (50 $\mu\text{mol/liter}$) and

then were washed and reincubated with Ni-free buffer solution. Under these conditions ^{63}Ni was slowly released from the leukocytes in protein-bound form. Column chromatography of the released proteins on Sephadex G-200 has shown that up to 35% of the ^{63}Ni is bound to α_1 -macroglobulin. (The remainder is primarily associated with albumin.) This observation has been confirmed by repeated experiments, and it provides a new experimental system in which to characterize the nickel-binding macroglobulin. Moreover, it may possibly furnish the explanation for the hypernickelemia which we have previously observed in patients following myocardial infarction, stroke and burns, which are all associated with leukocytosis and leukocytolysis *in vivo*. This investigation has only been in progress for four months, and it will probably require six to eight months of further studies before a manuscript will be ready for publication.

V. Studies of Nickel Carcinogenesis. In last year's progress report, we described our finding that addition of equimolar amounts of manganese dust to Ni_3S_2 significantly depresses Ni_3S_2 -induced tumorigenesis following intramuscular injection in rats. This observation has now been confirmed by a repetition of the entire experiment, and substantial progress has been made in characterizing this new experimental system for investigation of metal interactions in carcinogenesis. The histological reactions at the injection site have been evaluated by serial sacrifice of rats at 1 and 2 days and 1, 2, 4, 6, 8 and 10 weeks after injection of (a) Ni_3S_2 alone (1.25 mg), (b) Mn dust alone (1.0 mg), and (c) Ni_3S_2 (1.25 mg) plus Mn dust (1.0 mg). Measurements of the tissue retention of Mn and Ni at the site of injection after the same intervals have also been performed by atomic absorption analysis. These data are currently being evaluated, and the histological reactions are being categorized and reviewed. Our

preliminary conclusions are that concurrent injections of Ni_3S_2 and Mn dust do not affect the tissue retention of either metal, compared to the retention in rats which received only one of the metals. Moreover, there do not appear to be any significant differences between the sequence of morphological reactions at the injection site in the three groups of rats. These findings are entirely consistent with our working hypothesis that manganese may antagonize nickel inhibition of nucleolar RNA-polymerase activity in target cells. A manuscript describing these findings will be completed next year for presentation at the Annual Meeting of the American Association for Cancer Research.

In a related study, $^{63}\text{Ni}_3\text{S}_2$ has been administered to 8 rats by im injection, and liquid scintillation spectrometry has been employed for measurements of (a) the weekly excretion of ^{63}Ni in urine and feces for 8 months after injection, and (b) the distribution of residual ^{63}Ni at the injection site and in various organs and tissues. These data are currently being subjected to compartmental analysis by the computer techniques which have been developed by our collaborator, Professor Onkelinx. The analysis of the kinetics of $^{63}\text{Ni}_3\text{S}_2$ metabolism should be completed within the next 3 months, and will be included in the paper to be presented at the cancer research meetings.

Studies of the carcinogenicity of Ni_3S_2 in experimental animals have heretofore been limited to parenteral routes of administration, and have consistently resulted in the induction of rhabdomyosarcomas, fibrosarcomas or mesotheliomas, depending upon the site of injection. On the other hand, the cancers which occur in nickel refinery workers are generally squamous cell or adenocarcinomas, and are located primarily

in the nose, sinuses, larynx and lungs. In an attempt to develop an experimental system for induction of epidermoid neoplasms with Ni_3S_2 , we have initiated a study in which Ni_3S_2 is painted on the mucosa of the cheek pouch of the Syrian golden hamster. Suspensions of Ni_3S_2 (0.5% and 1% in glycerol) have been applied to the buccal pouch mucosa of unanesthetized hamsters three times each week for 48 treatments (i.e. for 16 weeks) according to the techniques used by Salley (4), Sklar (5) and Homburger (6). This experiment was initiated on March 4, 1974, with 21 hamsters, divided randomly into three groups. Group A received 48 applications of the vehicle alone (glycerol). Group B received 48 applications of 0.5% Ni_3S_2 in glycerol. Group C received 48 applications of 1.0% Ni_3S_2 in glycerol. Inspection of the buccal pouches with an otoscope, and palpation of the cheeks is being performed each week. As of the date of this report, firm, palpable nodules have developed at the sites of the application in three of the seven hamsters in Group C, and no nodules have developed in any of the other hamsters. The mucosa of the buccal pouch of the three affected hamsters has the appearance of leukoplakia. We strongly suspect that these hamsters in Group C may be developing neoplasms, and we intend to perform exfoliative cytological studies and biopsies to confirm or to refute this suspicion. If the results of this pilot study are positive, a larger scale investigation of this new approach to the study of Ni_3S_2 carcinogenesis will be initiated immediately.

VI. Nickel Analyses in Biological Materials by Flameless Atomic Absorption. Considerable effort was directed to the modification of the Jarrell-Ash Model 810 dual-monochromator atomic absorption spectrometer

in order to accommodate the installation of the Perkin-Elmer Model 2000 graphite tube furnace. The marriage of these two instruments was successfully accomplished, so that analyses of nickel could be performed by simultaneous measurements of two nickel wavelengths: 232.0 nm (resonating line) and 231.0 nm (non-resonating line). Atomic absorption of nickel occurs only at 232.0 nm, whereas non-specific background or matrix absorption occurs equally at 232.0 and 231.0 nm, and nickel can be determined as the difference in absorption at the two adjacent wavelengths. Our experiments confirmed the validity of this approach for analysis of "spiked" samples of serum and urine, but regrettably the analytical sensitivity was inadequate to permit detection of nickel concentrations in 50 μ l samples of serum or urine from normal subjects. It was necessary, therefore, to resort to use of the Perkin-Elmer Model 403 atomic absorption spectrometer, with substantial optical improvements of the deuterium background correction system. Our experience with this approach to direct analyses of nickel in serum has been more rewarding. A paper which describes our current technique was presented at the recent European Congress on Clinical Chemistry, and an abstract⁷ (submitted herewith) has been published in the Zeitschrift fur Klinische Chemie. Further analytical studies are needed before this work is ready for publication. It is anticipated that our manuscript on non-flame atomic absorption spectrometry of nickel in body fluids should be completed within the next six months.

Use of the teflon combustion bomb has proven to be a useful adjunct to trace metal analyses of solid materials such as tissues or feces. A manuscript⁸ describing the application of teflon combustion bombs for analysis of copper in liver tissue (submitted herewith) has been accepted for publication in Annals of Clinical and Laboratory Science.

VII. Measurements of Nickel in Hair from Human Subjects Following Ingestion of Nickel from Contaminated Well-Water. In collaboration with Dr. Jeffrey Davis, Division of Epidemiology, South Carolina Department of Health and Environmental Control, we have been investigating residents of a trailer park in which the well-water had accidentally been massively contaminated with nickel from the effluent of a neighboring battery plant. The investigation was not initiated until more than two months after cessation of the contamination, so that measurements of nickel in serum or urine were not considered likely to serve as biological indices of environmental exposure. Accordingly, measurements of nickel were performed in hair samples from inhabitants of the trailer park and from control subjects. A copy of the report submitted to Dr. Davis on June 13, 1974 is appended. As indicated in this report, these measurements revealed significant increases in the concentrations of nickel in hair. Further epidemiological, clinical and analytical studies are currently in progress, and it is anticipated that a report describing this investigation will be completed within the next six months. This study substantiates the role of hair analyses in evaluation of environmental exposures to nickel.

VIII. Aminoaciduria and Proteinuria following Parenteral Injection of Ni(II). A pilot study has been conducted in which (a) measurements of urinary proteins by the biuret reaction and (b) quantitative ion-exchange liquid chromatography of urinary amino acids were performed upon specimens obtained from 12 Fischer rats before and after an injection of NiCl_2 (34 or 68 $\mu\text{mol/kg}$). This study has demonstrated that parenteral administration of Ni(II) promptly induces massive proteinuria and generalized aminoaciduria which persists for three to five days. Such observations

have not previously been reported for Ni(II), although proteinuria and aminoaciduria are well recognized following exposure to Cd(II) and other toxic metals. These findings are directly relevant to the occurrence of proteinuria and aminoaciduria in certain of the residents of the trailer park who were exposed to ingestion of nickel in well-water (see Section VII). A study of the dose-response relationships between ip Ni(II) and induced proteinuria and aminoaciduria is currently being initiated in our laboratory.

IX. Role of Sweating in Elimination of Trace Metals. As described in last year's progress report, investigations in our laboratory have shown that sweating can be a major route for elimination of nickel and other toxic trace metals in man. During this past year, opportunity was presented to measure the excretion of copper in sweat of seven patients with Wilson's disease (hepatolenticular degeneration). Copper was analyzed by atomic absorption spectrometry in sweat samples obtained during sauna bathing (15 min at 93°C). In one patient who was not being treated with penicillamine, the estimated excretion of copper in total body sweat during the sauna bath was 18 µg. In six patients who were receiving oral penicillamine (1 g/day), the estimated excretion of copper in total body sweat during the sauna bath averaged 106 µg (range = 43 to 179). This study indicates that thermal induction of sweating by means of sauna bathing may serve as a therapeutic adjunct to the customary treatment of Wilson's disease by means of low-copper diet and penicillamine. It also provides additional evidence of the importance of sweating in the elimination of trace metals. The manuscript⁹ which describes these measurements (submitted herewith) has been accepted for publication in the Annals of Clinical and Laboratory Science.

Based upon our findings that nickel in serum ultrafiltrates and in urine is bound to ninhydrin-positive constituents, we have postulated that nickel and other trace metals which are present in relatively great concentrations in sweat are probably also bound to ninhydrin-positive constituents. As the initial step in an attempt to identify the nickel-binding constituents of sweat, we have analyzed the amino acids in sweat from normal volunteers. Amino acid analyses were performed by ion-exchange liquid chromatography upon sweat samples obtained from 22 healthy men during sauna bathing. Amino acid analyses were also performed upon plasma (fasting specimens) and urine (24-hour collection) from the same subjects. Serine, ornithine, citrulline, and aspartic acid were present in sweat in mean concentrations which were more than ten times their mean concentrations in plasma. Glutamine, cystine, α -aminobutyric acid and α -aminoadipic acid were present in sweat in mean concentrations which were <0.2 times their mean concentrations in plasma. The concentrations of specific amino acids in sweat bore no apparent relationship to their concentrations in urine. Thus, citrulline was abundant in sweat, whereas only traces of citrulline were found in urine. Conversely, glutamine, taurine, cystine and several other amino acids were abundant in urine but were present in very low or undetectable concentrations in sweat samples. A paper on this subject was presented at the Spring Meeting of the Association of Clinical Scientists¹⁰ (abstract submitted herewith) and a manuscript¹¹ (submitted herewith) which describes this investigation has been submitted for publication in Clinical Chemistry.

X. Miscellaneous Accomplishments. The spectrophotometric method for measurement of serum ceruloplasmin which was previously developed in our laboratory has now been adapted for automation by use of the Abbott bichromatic analysis system. A manuscript¹² (submitted herewith) which describes this automated technique has been accepted for publication in *Annals of Clinical and Laboratory Science*.

A comprehensive, annotated bibliography on alterations of serum copper and ceruloplasmin concentrations has been compiled (submitted herewith). This bibliography was distributed to 400 participants at an Applied Seminar on Laboratory Diagnosis of Cancer which was held in Washington, D.C. in November 1973, under the auspices of the Association of Clinical Scientists. It has served to stimulate clinical interest in the use of serum copper and ceruloplasmin measurements as laboratory aids in assessing prognosis, and monitoring remission and relapse in patients with various forms of cancer.

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

(1) Improvement of analytical methods for nickel in biological fluids and tissues. Substantial advances have been made in this effort. Use of the graphite tube furnace in conjunction with atomic absorption spectrometry has now been refined⁷ so that this method can be used for environmental, diagnostic and toxicological studies. Intensive additional work in this area is currently in progress (see Section VI above). The teflon combustion bomb has been successfully applied for trace metal analysis of tissues.⁸

(2) Investigation of serum protein-bound and ultrafiltrable nickel.

Thin-layer chromatography and autoradiography have substantiated the occurrence of ^{63}Ni -complexes in ultrafiltrates of rabbit serum after iv injection of $^{63}\text{NiCl}_2$, and measurements of the partition of protein-bound and ultrafiltrable ^{63}Ni have been made after addition of $^{63}\text{Ni(II)}$ to serum *in vivo* and *in vitro* (see Section III above). The nickel-binding α -macroglobulin, "nickeloplasmin," has been shown to be released from rabbit polymorphonuclear neutrophils following *in vitro* incubation of the leukocytes with $^{63}\text{Ni(II)}$ (see Section IV above).

(3) Elucidation of the mechanisms of nickel-induced hyperglycemia.

An extensive investigation has been completed in which we have: (a) established the dose-response relationships for the effects of Ni(II) upon plasma glucose in rats; (b) compared the hyperglycemic responses of rats to Ni(II) , Co(II) , and other divalent ions of first transition metals, as well as to glucagon; (c) observed the effects of adrenalectomy and hypophysectomy upon hyperglycemia induced by Ni(II) and glucagon; and (d) measured the changes in serum concentrations of immunoreactive insulin which occur during Ni(II) -induced hyperglycemia in rats (see Section I above).

(4) Study of nickel carcinogenesis using $^{63}\text{Ni}_3\text{S}_2$ for induction of rhabdomyosarcomas. This investigation is actively in progress (see Section V above). Preliminary observations suggest that Ni_3S_2 may be carcinogenic following repeated topical applications to the mucosa of the buccal pouch of hamsters.

(5) Study of the elimination of nickel and other trace metals in sweat. Two investigations which pertain to this topic have been completed (see Section VIII above).

XII. Effort of the Principal Investigator. The Principal Investigator has devoted approximately 20% of his time and effort to this project. His effort will be continued at this level throughout the contract year, and is projected to increase to 25% in the next year (see Renewal Proposal).

XIII. Significance of the Research and its Relevance to National Priorities. The Committee on Medical and Biological Effects on Environmental Pollutants (MBEEP) of the National Academy of Sciences (NAS) has approved the final draft of the Report of its Panel on Nickel. This report, entitled "Nickel,"¹³ is submitted along with this Progress Report, inasmuch as the Principal Investigator was the Chairman of the NAS Panel on Nickel, and since a major proportion of his scientific effort during the past year was devoted to writing several chapters of this report and to editing and revising the other chapters. The Panel on Nickel concluded (on Page 302 of the Report¹³) that: *"Man's use of nickel and nickel-containing materials has been steadily increasing in recent years, and it is therefore probable that nickel concentrations in ground and surface waters and in the atmosphere will continue to increase. Increased amounts of nickel in the biosphere should be viewed with caution. Emissions from the combustion of fossil fuels, principally coal and petroleum, are a major source of atmospheric nickel. Persons who reside in urban areas are exposed to inhalation of nickel, owing to atmospheric contamination from industrial emissions..."* Much environmental concern has been focused upon lead, cadmium and mercury within recent years, and considerable research is 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. However, insofar as the Principal Investigator can ascertain, AEC Research Contract No. AT(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.

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June 13, 1974

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and Environmental Control
2600 Bull Street
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Dear Dr. Davis:

In accordance with our discussions and correspondence, we have been pleased to perform nickel analyses upon the hair samples from residents of Sumter County, South Carolina. These analyses were performed as described in our recent paper (Nechay, M. W. and Sunderman, F. W., Jr.; Annals of Clinical and Laboratory Science, 3:30-35, 1973). The analyses were conducted on a "blind" basis (i.e. the analysts did not have any knowledge of which of the samples were obtained from the control and exposed subjects). Enclosed is a tabulation of the results of these nickel analyses in hair.

The concentrations of nickel in hair of the 11 control subjects (4 ♂, 7 ♀) averaged 0.44 µg/g; standard deviation ± 0.25; range = 0.09 to 0.98.

Massively increased concentrations of nickel in hair were found in all of the exposed subjects who had resided in the trailer park for more than 1 year. In these 8 subjects (4 ♂, 4 ♀), the average concentration of nickel in hair was 13.1 µg/g; standard deviation ± 5.4; range = 5.1 to 18.0. The probability that these elevated values might have occurred by chance is <0.001, computed by Student's "t" test.

Borderline elevations of nickel concentrations were also present in hair from 2 of the 5 members of the Yarborough family. This family had only resided in the trailer park for 5 months.

Inasmuch as you are still in Africa on your field study, I have telephoned Dr. William Gamble and Dr. Richard Gadsden to notify them of these remarkable findings. I look forward to learning the results of Dr. Gadsden's measurements of protein and amino acids in urine samples from these subjects. Needless to say, I believe that our findings indicate the need for thorough clinical and epidemiological evaluation of the subjects who were exposed to nickel.

Jeffrey P. Davis, M.D.

- 2 -

June 13, 1974

Upon your return to South Carolina, I shall be pleased to make a trip to see you and Dr. Gadsden, so that we can prepare a report of our findings.

With best wishes, I am

Sincerely yours,

F. William Sunderman, Jr., M.D.
Professor and Head

FWS:BA

cc: Dr. Richard Gadsden
Dr. William Gamble

Enclosure

Nickel Concentrations in Hair Samples from Residents of Sumter County

<u>Subject</u>	<u>Race, Sex, Age</u>	<u>Population</u>	<u>Duration of Residence in Trailer Park (yr)</u>	<u>Hair Nickel (ug/g)</u>
Ben Oviatt	W ♂ 27	Control	0	0.98
John W. Zion	W ♂ 27	Control	0	0.47
Marlene McGee	W ♀ 26	Control	0	0.46
Lewis Shaw	W ♂ 26	Control	0	0.30
Robert Wallick	W ♂ 31	Control	0	0.50
Margaret Lamb	W ♀ 26	Control	0	0.26
Susan M. Tiede	W ♀ 25	Control	0	0.23
Shirley Field	W ♀ 38	Control	0	0.55
Lula Davenport	W ♀ 40	Control	0	0.09
Wanda Quarles	W ♀ 27	Control	0	0.80
Sandra Scott	W ♀ 25	Control	0	0.26
Control Subjects (4 ♂, 7 ♀): mean = 0.44 ug/g (S.D. ± 0.25)				
Eugenia A. Jones	W ♀ 28	Trailer Park	8	14.8
Robert L. Jones, Sr.	W ♂ 29	Trailer Park	8	18.0
Robert L. Jones, Jr.	W ♂ 5.5	Trailer Park	5.5	8.0
Patricia E. Allen	W ♀ 24	Trailer Park	3	17.9
Patricia K. Allen	W ♀ 16	Trailer Park	3	17.4
David M. Allen	W ♂ 5	Trailer Park	3	17.5
Mary E. Jordan	W ♀ 30	Trailer Park	3	5.8
David M. Jordan	W ♂ 5	Trailer Park	3	5.1
Betty Jane Yarborough	W ♀ 35	Trailer Park	0.4	1.14
Edward Lewis Yarborough	W ♂ 31	Trailer Park	0.4	0.07
Ralph Edward Yarborough	W ♂ 17	Trailer Park	0.4	0.41
Betty Ann Yarborough	W ♀ 14	Trailer Park	0.4	1.41
Edward L. Yarborough, Jr.	W ♂ 7	Trailer Park	0.4	0.33

Subjects who were residents of the Trailer Park for > 1 year: (4 ♂, 4 ♀) mean = 13.1 ug/g (S.D. ± 5.4)
P vs. controls = < 0.001, computed by Student's "t" test