Nickel Concentrations in Human Parotid Saliva* †

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ABSTRACT

Measurements of nickel were performed by electrothermal atomic absorption spectrometry upon specimens of parotid saliva from 38 healthy adults. The mean concentration of nickel in saliva was 1.9 ± 1.0 μg Ni per liter (range = 0.8 to 4.5). There was no significant correlation between the concentrations of salivary nickel and protein. No significant differences were observed between the mean concentrations of nickel in saliva samples from (1) men vs women; (2) health center employees vs naval recruits; (3) caries-free subjects vs caries-prone subjects; or (4) non-smokers vs cigarette smokers.

Introduction

The presence of toxic metals in saliva has been recognized for more than two decades, since Mongelli-Sciannameo and Dantin-Gallego detected lead in saliva from patients with chronic plumbism. In 1965, Sunderman reviewed the oral manifestations of human poisoning from arsenic, bismuth, lead and mercury, and he noted that a complaint of "metallic taste" was occasionally the initial symptom of intoxication from these metals. Sunderman suggested that the oral manifestations of metal poisoning might be attributable in part to secretion of metals into the saliva. He speculated that the darkly pigmented "Burtonian" lines that develop near the gingival margins might result from precipitation of insoluble metallic sulfides at sites where soluble metal complexes come into contact with hydrogen sulfide that is formed from decomposition of food debris and plaque in areas of poor dental hygiene.
During the past decade, investigators have measured the concentrations of various trace metals in human saliva, but there have not been any previous measurements of the concentrations of salivary nickel. In the present study, nickel analyses have been performed upon specimens of parotid saliva from healthy subjects, and the effects of dental caries and cigarette smoking upon salivary nickel concentrations have been evaluated. In a future study, the authors plan to compare the concentrations of nickel in specimens of saliva, serum and urine from workers in nickel industries in order to determine whether or not analysis of nickel in saliva can furnish a reliable index of occupational exposures to nickel.

Materials and Methods

Subjects

Two groups of volunteers were studied. Group A comprised 20 healthy employees of the University of Connecticut Health Center (14 men, age 20 to 49 years and six women, age 18 to 35 years). Several of the subjects in Group A had multiple dental fillings, but none of the subjects had severe carious lesions. Group B comprised 18 healthy male naval recruits, (age 17 to 25 years) at the Great Lakes Naval Station, Chicago, IL. The naval recruits were selected for study from a population of approximately 1,000 men whose teeth were evaluated for dental caries by clinical examination and by "bite-wing" radiographs. Nine of the subjects in Group B were completely free of carious lesions of the teeth, and nine of the subjects had severe dental caries with 15 or more teeth that were decayed, missing or filled.

Smoking histories were obtained from all of the 38 subjects in Groups A and B. There were 22 non-smokers and 16 persons who smoked from 10 to 40 cigarettes per day. None of the subjects had occupational exposures to nickel compounds. All of the subjects abstained from eating and smoking for at least one hour prior to collection of saliva.

Saliva Collection

Samples of saliva from the parotid glands were collected by the Lashley technique with stringent precautions to minimize nickel contamination. Teflon collection devices ("Lashley cups") were attached to 15 cm lengths of polyethylene tubing. The collection equipment (Lashley cups and polyethylene tubing) was washed with non-toxic detergent, soaked over-night in nitric acid (4 moles per liter) and rinsed with distilled, demineralized water. The collection equipment was handled with polypropylene gloves to avoid nickel contamination from sweat on the fingers. The Lashley cup was placed over the right Stenson's duct and was attached by gentle suction. Salivary flow was stimulated by having the subject suck on a sour lemon candy ball. The first ml of saliva that passed through the polyethylene collection tubing was discarded, and samples of 8 to 10 ml were then collected in an acid-washed polypropylene test-tube. The duration of collection was measured with a stop-watch in order to estimate the salivary flow-rate. The saliva sample was immediately centrifuged at 900 x g for 10 min in order to sediment cellular debris. The supernatant saliva was frozen at -14° until the time of analysis.

Preparation of Samples for Nickel Analysis

The samples were prepared for nickel analysis by a modification of the method

* Teflon Lashley cups were obtained from Mr. Joseph Loomis of the Human Performance Laboratory, 119 Noll Building, Pennsylvania State University, University Park, PA 16802.
† "Intra-Medic" tubing, PE-150, Becton-Dickinson Co., East Rutherford, NJ 07073.
of Zachariasen et al.\textsuperscript{18} Saliva samples (3 ml) were placed in duplicate 25 ml Pyrex Erlenmeyer flasks. Into three sets of duplicate Erlenmeyer flasks were placed, respectively, 3 ml of blank and standard solutions containing 0, 2.5 and 5 \( \mu \)g Ni per liter. Exactly 2.5 ml of perchloric acid\textsuperscript{\dagger} and 2.5 ml of nitric acid\textsuperscript{§} were added to each flask and the flasks were placed on a cold heating plate in a perchloric acid fume hood. The flasks were gently heated at a low temperature for 30 min in order to avoid loss of sample by foaming. The temperature was gradually increased until the boiling point was reached. After two to three hrs, when the volumes of the digestion mixture were one to two ml, the flasks were cooled. Precisely 1.5 ml of nitric acid and 1.5 ml of perchloric acid were added to each flask and the digestion was continued for approximately two hrs, until the volumes of solution were 0.3 to 0.5 ml. The contents of the flasks were not permitted to evaporate to dryness.

After the contents of the flasks were cooled to room temperature, 0.1 ml of hydrochloric acid\textsuperscript{\textdagger} was added to each flask and the wall of the flask was washed down with 3 ml of water. Precisely 0.1 ml of diammonium hydrogen citrate solution\textsuperscript{¶} and 0.2 ml of sodium dimethylglyoxime solution\textsuperscript{**} were added to each flask. Concentrated ammonium hydroxide\textsuperscript{\textdoubleprime} was added dropwise to each flask with constant mixing until the pH reached 8.5, as monitored with a pH meter. Then the pH was adjusted to 9.0 by dropwise addition of dilute ammonium hydroxide solution (prepared by diluting the concentrated solution 1:10 with water). One ml of methylisobutylketone \textsuperscript{††} (MIBK) was added to each flask, and the flask was sealed by stretching paraffin film across the mouth. The flasks were shaken with a vortex rotary mixer for one min. By use of a Pasteur pipet, each supernatant MIBK extract was transferred to a two ml polypropylene tube, and the tube was sealed with a polypropylene stopper.

** Atomic Absorption Spectrometry of Nickel **

Measurements of nickel were performed by use of an atomic absorption spectrometer\textsuperscript{§§} fitted with (1) “intensatron” nickel hollow cathode lamp, (2) optical baffle system, (3) electrothermal graphite tube atomizer (Model HGA-2100), (4) temperature programming accessory for “ramp-mode”, (5) deuterium background corrector, (6) constant voltage transformer, (7) air and argon tanks and regulators and (8) 10 inch strip-chart recorder (1 mv full scale).

The spectrometer and accessories were adjusted to the following instrumental settings: (1) nickel lamp current: 25 ma; (2) wavelength: 232 nm; (3) slit setting: position 3; (4) recorder chart speed: 6 cm per min; (5) air flow for deuterium lamp: 15 meter units (maximum); (6) argon flow for electrothermal atomizer: 20 meter units; (7) drying cycle: 10 sec from 25° to 120° in ramp mode, and 10 sec at 120°; (8) pyrolysis cycle: 20 sec from 120° to 1200° in ramp mode, and 15 sec at 1200°; and (9) atomization cycle: 8 sec at 2600° C. Samples of 25 \( \mu \)l of MIBK extracts of digested saliva, blanks and nickel standards were pipetted into the graphite tube.

\textsuperscript{\dagger} Perchloric acid, 70 percent w/v, “supra-pur” grade, E. Merck Co., Darmstadt, West Germany.

\textsuperscript{§} Nitric acid, 65 percent w/v, “supra-pur” grade, E. Merck Co., Darmstadt, West Germany.

\textsuperscript{\dag} Hydrochloric acid, 30 percent w/v, “supra-pur” grade, E. Merck Co.

\textsuperscript{¶} Ammonium citrate dibasic, Fisher Scientific Co., Fair Lawn, NJ 07410; 100 g per liter of water.

\textsuperscript{**} Dimethylglyoxime, sodium salt, Baker Chemical Co., Phillipsburg, NJ 08865; 10 g per liter of absolute ethanol.

\textsuperscript{\textdoubleprime} Ammonium hydroxide, 25 percent w/v, “supra-pur” grade, E. Merck Co.

\textsuperscript{††} Methylisobutylketone, “certified” grade, Fisher Scientific Co.

\textsuperscript{§§} Model PE-403, Perkin-Elmer Corp., Norwalk, CT 06852.
### TABLE I

Measurements of Nickel Concentrations in Parotid Saliva from Healthy Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Flow-rate of Saliva (ml/min)*</th>
<th>Protein Conc. (g/liter)*</th>
<th>Nickel Conc. in Saliva (μg Ni/liter)*</th>
<th>Nickel Conc. in Saliva (ng Ni/mg prot.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(A)</em> Health Center Employees in Connecticut</td>
<td>14 ♂</td>
<td>1.0±0.3</td>
<td>3.0±0.6</td>
<td>2.5±1.3</td>
<td>0.9±0.6</td>
</tr>
<tr>
<td></td>
<td>6 ♀</td>
<td>0.6±0.2</td>
<td>3.5±1.1</td>
<td>1.5±0.5</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td></td>
<td>20 ♂</td>
<td>0.9±0.4</td>
<td>3.1±0.8</td>
<td>2.2±1.2</td>
<td>0.8±0.5</td>
</tr>
<tr>
<td><em>(B)</em> Naval Recruits in Chicago, Illinois</td>
<td>9 ♂ (Caries-free)</td>
<td>0.7±0.5</td>
<td>2.5±1.6</td>
<td>1.9±0.7</td>
<td>0.9±0.5</td>
</tr>
<tr>
<td></td>
<td>9 ♂ (Caries-prone)</td>
<td>0.7±0.5</td>
<td>2.0±1.0</td>
<td>1.6±0.4</td>
<td>1.1±1.0</td>
</tr>
<tr>
<td></td>
<td>18 ♂ (Combined)</td>
<td>0.7±0.5</td>
<td>2.3±1.3</td>
<td>1.7±0.6</td>
<td>0.9±0.9</td>
</tr>
<tr>
<td><em>(A &amp; B)</em> All of the Subjects</td>
<td>16 ♂ + ♀ (Non-smokers)</td>
<td>0.9±0.4</td>
<td>3.5±0.3</td>
<td>1.8±1.0</td>
<td>0.8±0.5</td>
</tr>
<tr>
<td></td>
<td>22 ♂ + ♀ (Smokers)</td>
<td>0.7±0.4</td>
<td>2.5±1.0</td>
<td>2.0±1.1</td>
<td>1.0±0.8</td>
</tr>
<tr>
<td></td>
<td>38 ♂ + ♀ (Combined)</td>
<td>0.8±0.5</td>
<td>2.9±0.8</td>
<td>1.9±1.0</td>
<td>0.9±0.7</td>
</tr>
</tbody>
</table>

♦mean ± standard deviation

of the electrothermal atomizer. The temperature program was initiated and the atomic absorption was automatically recorded. The peak heights on the rec-

### TABLE II

Measurements of Nickel in Body Fluids and Excreta from Healthy Adults

<table>
<thead>
<tr>
<th>Body Fluid</th>
<th>No. of Subjects</th>
<th>Nickel Concentration or Excretion Mean ± Std. Dev. Units</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid Saliva</td>
<td>32 ♂</td>
<td>2.0±1.1 μg/liter</td>
<td>Present Study</td>
</tr>
<tr>
<td></td>
<td>6 ♂</td>
<td>1.5±0.5</td>
<td></td>
</tr>
<tr>
<td>Whole Blood</td>
<td>10 ♂</td>
<td>4.5±1.4 μg/liter</td>
<td>Nomoto &amp; Sunderman¹⁴</td>
</tr>
<tr>
<td></td>
<td>7 ♀</td>
<td>5.2±1.1</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>23 ♂</td>
<td>2.6±0.8 μg/liter</td>
<td>Nomoto &amp; Sunderman¹⁴</td>
</tr>
<tr>
<td></td>
<td>17 ♀</td>
<td>2.7±0.7</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>14 ♂</td>
<td>2.6±1.3 μg/day</td>
<td>Nomoto &amp; Sunderman¹⁴</td>
</tr>
<tr>
<td></td>
<td>12 ♀</td>
<td>2.2±0.8</td>
<td></td>
</tr>
<tr>
<td>Sweat</td>
<td>33 ♂</td>
<td>52±36 μg/liter</td>
<td>Hohnadel et al²⁹</td>
</tr>
<tr>
<td></td>
<td>15 ♀</td>
<td>131±65</td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td>13 ♂</td>
<td>240±90 μg/kg</td>
<td>Nechay &amp; Sunderman¹³</td>
</tr>
<tr>
<td></td>
<td>7 ♀</td>
<td>190±40</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>10 ♂ + ♀</td>
<td>258±126 μg/day</td>
<td>Horak &amp; Sunderman¹⁰</td>
</tr>
</tbody>
</table>
order tracing were measured with a ruler, and the concentrations of nickel in saliva were estimated by reference to a calibration curve that was prepared with each run.

Measurements of the recovery of nickel added to saliva were performed with each run. The mean recovery of 5 µg per liter of added nickel was 5.05 µg per liter, (range = 4.65 to 5.70 µg per liter, N = 3), equivalent to 101 percent recovery, (range = 93 percent to 114 percent). The within-the-run precision of duplicate analysis was 9 percent (coefficient of variation).

**Protein Analysis**

Measurements of protein concentrations in 0.2 ml samples of saliva were performed by the biuret technique of Savory et al.15

**Results**

The flow-rates of parotid saliva from the right Stenson’s duct in the entire group of 38 subjects averaged 0.8 ± 0.5 ml per min, (range = 0.14 to 2.0). The concentrations of total protein in saliva samples from the 38 subjects averaged 2.9 ± 0.8 g per liter, (range = 1.2 to 4.5). The concentrations of nickel in saliva from the 38 subjects averaged 1.9 ± 1.0 µg Ni per liter, (range = 0.8 to 4.5). Expressed on the basis of protein concentration, the nickel concentration in the 38 samples of saliva averaged 0.9 ± 0.7 ng Ni per mg of protein, (range = 0.23 to 3.49). There was no significant correlation between the concentration of salivary nickel and protein. No significant differences were observed between the mean concentrations of nickel in saliva samples from (1) men vs women; (2) health center employees vs naval recruits; (3) caries-free subjects vs caries-prone subjects; or (4) non-smokers vs cigarette smokers, based upon statistical comparisons by Student’s “t” test of the data listed in table I.

**Discussion**

The concentrations of nickel in parotid saliva that were obtained in the present study are compared in table II with concentrations of nickel in other body fluids and excreta, as determined in previous studies from the authors’ laboratory. The mean concentration of nickel in saliva is less than one-half of the mean concentration of nickel in whole blood and is slightly lower than the mean concentration of nickel in serum. The authors speculate that measurements of nickel in parotid saliva may prove to have practical advantages over measurements of nickel in blood, serum, urine, feces, hair or sweat for monitoring occupational exposures to nickel. The technique of saliva collection is (1) relatively rapid, (2) non-invasive, (3) non-invasive and (4) easily accomplished with precautions against nickel contamination. (5) In addition, it can be performed repeatedly without affecting the subject.

**Acknowledgments**

The authors are grateful for the technical assistance of Ms. Carol Beck, Mr. Robert Dick and CPO Gerald Mickel, USN, and for the cooperation of the volunteers who participated in this study. In addition the authors are grateful for the help and cooperation of the Commanding Officer and Staff of the Naval Dental Research Institute, Great Lakes Naval Base.

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