Spectrophotometric Measurements of Serum Nickel

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An ultraviolet spectrophotometric procedure has been developed for the measurement of serum nickel. Samples of serum are lyophilized and the dry residues are subjected to acid digestion. Nickel is separated from interfering elements by chloroform extraction of nickel dimethylglyoximate at alkaline pH. Nickel is converted to the diethyldithiocarbamate complex and is extracted with isoamyl alcohol. The absorbance of nickel bisdiethyldithiocarbamate is measured as the difference between the absorbance maximum (325 m\(\mu\)) and the absorbances at 2 background wave lengths (295 and 355 m\(\mu\)). The concentrations and volumes of the reagents have been adjusted in order to achieve a sensitivity of 0.002 ppm (2 \(\mu\)g./L.). The recovery of 2.5 \(\mu\)g. of nickel added to 8 samples of serum averaged 104% (S.D. \(\pm\) 8), with a range from 91 to 115%. The mean concentration of nickel in serum from 23 normal subjects was 2.2 \(\mu\)g./100 ml. (S.D. \(\pm\) 1.8), with a range from 0.1 to 7.7 \(\mu\)g./100 ml. The median concentration of serum nickel was 1.7 \(\mu\)g./100 ml.

Clinical interest in measurements of serum nickel has been stimulated by the report of elevated concentrations of nickel in sera of patients with myocardial infarction. Using a spectrographic method for semiquantitative estimations of nickel, D'Alonzo and Pell (1) have observed that serum nickel concentrations were greater than 50 \(\mu\)g./100 ml in 19 of 20 patients with acute myocardial infarction. The differences between the concentrations of serum nickel in patients with myocardial infarction and in control subjects were highly significant (\(p = 0.000002\)). Based upon these findings, D'Alonzo and Pell (1) have

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speculated that nickel may either be involved in the etiology of myocardial infarction, or that an enzyme containing nickel may be released into the serum following myocardial infarction.

As a preliminary step in investigation of nickel metabolism in myocardial infarction, a spectrophotometric procedure for measurement of serum nickel has been developed in our laboratory. Previously, the technic of Kinkaid et al. (2) for the determination of nickel in biological materials was modified in order to increase its sensitivity by approximately 10-fold (3). Further refinements in this method for nickel analysis are described in the present paper which have resulted in an additional twofold increase in sensitivity, and have enabled measurements to be made of the concentrations of nickel in serums from normal subjects.

**Materials and Methods**

**Principle**

Samples of serum are lyophilized and the dry residues are subjected to acid digestion. Nickel is separated from interfering elements by chloroform extraction of nickel dimethylglyoximate at alkaline pH. Nickel is converted to the diethyldithiocarbamate complex and is extracted with isoamyl alcohol. The absorbance of nickel bisdiethyldithiocarbamate is measured as the difference between the absorbance at the adsorption maximum (325 mμ) and the mean of the absorbances at 2 background wavelengths (295 and 355 mμ).

**Precautions**

Each batch of reagents is analyzed to ensure that it is not contaminated with nickel. Metal-free water, obtained by distillation and ion exchange, is used for the preparation of solutions. Glassware is cleaned with warm nitric acid and rinsed with metal-free water immediately before use. Analyses are performed in duplicate, and measurements of the recovery of nickel added to serum are included in each group of determinations.

**Reagents**

- **Nitric acid** Reagent grade, s.g. 1.5.
- **Sulfuric acid** Reagent grade, s.g. 1.84.
- **Hydrogen peroxide solution** Reagent grade, 30% (w/v).
- **Ammonium hydroxide solution** Reagent grade, 29% (w/v).
- **Dilute ammonium hydroxide solution** 1% (w/v).
- **Hydrochloric acid** 0.5N.
- **Chloroform**
- **Carbon tetrachloride**
Isoamyl alcohol
Octyl alcohol

Dimethylglyoxime solution  Dimethylglyoxime, 0.25 gm., is dissolved in 50 ml. of absolute ethanol and diluted with water to 250 ml. This solution is stored in the refrigerator and is stable for at least 1 month.

Sodium diethyldithiocarbamate stock solution  Sodium diethyldithiocarbamate trihydrate, 0.2 gm., is dissolved in 100 ml. of water. This solution is prepared each week and stored in the refrigerator.

Sodium diethyldithiocarbamate working solution  A 1:10 dilution (with water) of the stock solution is prepared immediately prior to use.

Citrate buffer A  Diammonium citrate, 200 gm., is dissolved in 600 ml. of water, adjusted to pH 9.0–9.5 with concentrated NH₄OH, and transferred to a 1-L. separatory funnel. Ten ml. of dimethylglyoxime solution are added and the mixture is extracted 4 times with 30 ml. of chloroform. The aqueous solution is filtered through Whatman No. 4 filter paper and diluted to 1 L. with water.

Citrate buffer B  Diammonium citrate, 200 gm., is dissolved in 600 ml. of water, adjusted to pH 9.0–9.5 with concentrated NH₄OH, and transferred to a 1-L. separatory funnel. Stock sodium diethyldithiocarbamate solution, 5 ml., is added and the mixture is extracted with 20 ml. of CCl₄ until the solvent layer is colorless (at least 4 extractions). The aqueous solution is filtered through Whatman No. 4 filter paper and diluted to 1 L. with water.

Nickel stock standard  Metallic nickel, 500 mg., is dissolved, with heating, in 20 ml. of HNO₃ (1:1) and diluted with water to 1 L.

Nickel working standard, (0.5 µg./ml.)  Nickel stock standard, 1 ml., is diluted to 1 L. with 0.5N HCl.

Procedure

Collection of Blood and Separation of Serum

Venepuncture is performed with a No. 19-gauge platinum-ruthenium needle.* Samples of blood (100 ml.) are withdrawn using all-glass 50-ml. hypodermic syringes. The blood is dispensed into 50-ml. centrifuge tubes, and is allowed to clot at room temperature for 30 min. before the serum is removed. Special care should be taken to minimize hemolysis.

Lyophilization and Digestion

Transfer 20-ml. samples of serum to 50-ml. Kjeldahl digestion flasks. The serum is frozen by immersing the flasks in a mixture of dry ice

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*Supplied by the Hamilton Co., Whittier, Calif.
and acetone, and the flasks are connected to the manifold of a lyophilization apparatus. When lyophilization is complete, the flasks are removed from the manifold. Add 20 ml. of HNO₃ to the dried serum samples, as well as to 2 empty digestion flasks ("blank" samples) and to 2 digestion flasks containing 1.0 μg of nickel ("standard" samples). Pyrex beads are dropped into each flask to prevent bumping, and 0.5 ml. of octyl alcohol is added to prevent foaming. The flasks are heated on a Kjeldahl digestion apparatus, and are boiled down to a volume of 1–2 ml. Additional octyl alcohol is introduced dropwise, as necessary, to prevent foaming. The flasks are allowed to cool. Add 3 ml. of concentrated H₂SO₄ and continue the digestion. Whenever charring occurs, 4 drops of concentrated HNO₃ are added immediately so that the color of the digestion mixture returns from black to yellow. When the contents of each flask reach a volume of 1–2 ml., 4 drops of 30% H₂O₂ solution are added in order to bleach the yellow color. If charring occurs after the addition of H₂O₂, 4 drops of HNO₃ are added as before. With repeated additions of H₂O₂ and HNO₃ the digestion mixture eventually becomes colorless. Boiling is continued for at least 15 min. after the last addition of H₂O₂.

**Nickel Extraction**

Each digestion flask is cooled to room temperature. Add 5 ml. of citrate buffer A and again cool the flask to room temperature. The contents of the flask are adjusted to pH 8.5 by addition of concentrated NH₄OH. The measurement of pH in the Kjeldahl flask is facilitated by the use of a probe-type pH electrode. Add 3 ml. of dimethylglyoxime solution. The contents of the flask are mixed with a "Vortex" rotatory mixer and then are allowed to stand for 5 min. Add 3 ml. of chloroform and shake the contents of the flask for 1 min. The flask is allowed to stand for approximately 10 min. in order to separate the aqueous and chloroform phases. Using a Pasteur pipet, the chloroform phase is transferred to a 50-ml. Teflon-stoppered centrifuge tube. Extraction of the aqueous phase is repeated with 2 additional 3 ml. volumes of chloroform. Add 20 ml. of dilute NH₄OH solution to the combined chloroform extracts. The contents of the centrifuge tube are mixed with the Vortex mixer. After centrifugation at 1500 rpm for 10 min., the upper (NH₄OH) phase is aspirated and discarded. The washing of the chloroform extracts is repeated with 2 additional 20-ml. volumes of dilute NH₄OH solution. Add 20 ml. of 0.5N HCl to the combined chloroform extracts. The contents of the centrifuge tube are mixed with the Vortex mixer. After centrifugation at 1500 rpm for 10 min., the lower (chloroform) phase is aspirated and discarded. Add 2 ml.
of carbon tetrachloride ($\text{CCl}_4$) to the HCl extract. The mixture is centrifuged and the lower ($\text{CCl}_4$) phase is aspirated and discarded. Add 5 ml. of citrate buffer B to the centrifuge tube and adjust the contents to pH 8.5 by addition of concentrated $\text{NH}_4\text{OH}$. Add 2 ml. of sodium diethylthiocarbamate working solution. The contents of the tube are mixed with the Vortex mixer and allowed to stand for 15 min. Add 3 ml. of isoamyl alcohol; the tube is stoppered, and the contents are mixed for 1 min. After centrifugation at 1500 rpm for 15 min., the lower (HCl) phase is aspirated and discarded, and the isoamyl alcohol phase is transferred to a quartz spectrophotometer cuvet of 1-cm. pathlength and 2-ml. capacity.

Spectrophotometry and Computations

Spectrophotometry is performed in a Beckman Model DK-2A ratio-recording spectrophotometer using isoamyl alcohol as the reference solution. The absorbance curve is recorded from 290 to 360 m$\mu$. The corrected absorbance of nickel bisdiethylthiocarbamate is computed graphically as illustrated in Fig. 1, or is calculated by the following equation:

$$A_{\text{corr.}} = A_{325 \text{ m}\mu} - \frac{A_{295 \text{ m}\mu} + A_{355 \text{ m}\mu}}{2}$$

Experimental Studies and Results

Collection of Blood

No differences were observed between the concentrations of serum nickel in duplicate samples of blood which were obtained via platinum-ruthenium needles and via Teflon needles. Likewise, no differences were found between the concentrations of serum nickel in duplicate samples of blood which were collected in all-glass syringes and in plastic transfusion bags. In blood samples which were grossly hemolyzed, the concentrations of serum nickel were as much as 1.5 $\mu$g./100 ml. higher than in control samples. The concentration of nickel in a sample of serum which was allowed to remain in contact with the blood clot for 8 hr. was 0.8 $\mu$g./100 ml. higher than in a control sample of serum which was separated from the blood clot 30 min. after collection. On the basis of these findings, it would appear to be important to avoid hemolysis and prolonged contact of serum with the blood clot.

Digestion of Serum

The acid-digestion procedure was facilitated by prior lyophilization of the serum samples. Constant attention was necessary during the acid digestion in order to avoid charring and to prevent loss of the
sample through bumping or foaming. It usually required 12–14 hr. to complete the digestion. Although the digestion was more rapid when perchloric acid (HClO₄) was employed instead of H₂O₂, HClO₄ was not selected for routine use owing to the possible hazard of explosion.

**Extraction of Nickel Dimethylglyoximate**

According to Kinkaid (2), Stary (4), and Sandell (5), separation of nickel from the other metals which are present in biological materials may be accomplished by chloroform extraction of nickel dimethylglyoximate. Although iron and copper are potential sources of interference, inclusion of the citrate ion in the buffer keeps ferric phosphate in aqueous solution, and traces of copper dimethylglyoximate which are soluble in chloroform may be removed by repeated extractions with dilute NH₄OH (5). In the present investigation, no interference in the determination of nickel was observed when ferric or cupric ions were added to serum samples in quantities equivalent to 200 μg./100 ml. of serum. Maximum recovery of nickel was accomplished by 3 extractions.

![Fig. 1. Spectral absorption curves of nickel bisdiethydithiocarbamate. (See text).](image-url)
of the nickel dimethylglyoximate with 3 ml. of chloroform followed by a single re-extraction of the chloroform with 20 ml. of 0.5N HCl.

**Extraction of Nickel Bisdiethyldithiocarbamate**

The optimum concentration of diethyldithiocarbamate ion was studied by including sodium diethyldithiocarbamate trihydrate in the final reaction mixture in quantities ranging from 0.1 to 10 mg. Greatest sensitivity was achieved by use of 0.4 mg. of sodium diethyldithiocarbamate, rather than 10 mg., as previously described (3). Our findings indicate that the sensitivity of measurements of nickel by the ultraviolet spectrophotometric technic, which was previously employed in our laboratory, was limited by the presence of too great an excess of diethyldithiocarbamate ion, resulting in unnecessarily high "blank" absorbance at 325 m. The optimum volume of isoamyl alcohol for extraction of the nickel bisdiethyldithiocarbamate was found to be 3 ml. Increasing the volume of the isoamyl alcohol extract to 4 ml. diminished the sensitivity of analysis, owing to dilution. Additional extractions with isoamyl alcohol did not improve the sensitivity or precision of the method.

**Spectrophotometric Measurements**

The precision of replicate analyses was improved by use of the Allen technic of correcting for variability in "base line" absorbance (6). The most satisfactory results were obtained when absorbance meas-
Measurements were made at the absorption peak of nickel bisdiethylidithiocarbamate (325 mμ) and at 2 background wavelengths which were equidistant on either side of the peak (295 and 355 mμ, respectively). As shown in Fig. 1, a straight line could be drawn through the absorbance readings of “blank” samples at 295, 325, and 355 mμ.

As illustrated in Fig. 2, the calibration chart of absorbance versus nickel concentration was linear with quantities of nickel ranging from 0.5 to 2.5 μg. per sample. It may be noted from the relative slopes of the calibration curves that the determination of nickel by the present procedure is more sensitive than by the previous spectrophotometric procedure (3), or by the atomic absorption procedure (7) which is used in our laboratory for measurements of nickel in urine.

Sensitivity, Precision, and Recovery

Under the described conditions, the gram atom absorptivity at 325 mμ was 37,400. The limit of detection, expressed on the basis of a corrected absorbance of 0.01 absorbance unit, was 0.04 μg. of nickel per sample. With 20-ml. samples, an absorbance reading of 0.01 was approximately equivalent to 0.2 μg. of Ni per 100 ml. of serum (i.e., 2 parts per billion). The range of variation of duplicate analyses of 23 normal serums was 0.0–0.4 μg. Ni per 100 ml., and the S.D. of replicate measurements was ± 0.20 μg. Ni per 100 ml. The coefficient of variation of replicate measurements of nickel in normal serum was 9.1%. The mean recovery of 2.5 μg. of Ni added to eight 20-ml. samples of serum was 104% with a S.D. of ± 8% and a range of recovery from 91 to 115%.

Measurements of Serum Nickel

A frequency-distribution curve for the concentration of nickel in serums from 23 normal persons is portrayed in Fig. 3. The subjects were 9 male and 14 female technologists, age 20–35 years, who had no occupational exposure to nickel. The mean concentration of serum nickel was 2.2 μg./100 ml. (S.D. = ± 1.8), with a range of 0.1–7.7 μg./
100 ml. The median concentration of serum nickel was 1.7 \( \mu \text{g.} /100 \text{ ml.}\) The quantity of nickel in 1 sample was barely detectable, with a corrected absorbance value of 0.005. The concentration of nickel in this serum was estimated to be 0.1 \( \mu \text{g.} /100 \text{ ml.}\)

**Discussion**

A résumé of published values for the normal concentration of nickel in plasma and serum is given in Table 1. The mean concentration of serum nickel which was observed in the present investigation is lower than the mean concentrations which have been obtained by spectrographic technics. All of the investigators have found that the concentrations of nickel in normal serum or plasma were near the limits of detection of their analytical methods. In contrast to the limit of detection of 2 parts per billion by the present procedure, the limit of detection by the spectrographic method of Paixao and Yoe was 20 parts per billion (11), and that of Herring et al. was 7 parts per billion (12). Butt et al. (13) did not indicate the limit of detection of nickel by their spectrographic technic; however, they stated that nickel was not detected in 18 of their 48 serums.

Published values for the normal concentration of nickel in whole blood and erythrocytes are summarized in Table 2. Cluett (8), Paixao and Yoe (11), and Butt et al. (13) have reported that the mean con-

**Table 1. Résumé of Normal Concentrations of Nickel in Plasma and Serum Using Various Methods**

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Subjects (No.)</th>
<th>Range (ng./100 ml.)</th>
<th>Mean (ng./100 ml.)</th>
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<tr>
<td><strong>PLASMA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Cluett (8)</td>
<td>1956</td>
<td>1</td>
<td></td>
<td>1.2</td>
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<tr>
<td>Koch et al. (9)</td>
<td>1956</td>
<td>—</td>
<td>1.0–8.5</td>
<td>3.0†</td>
</tr>
<tr>
<td>Monacelli et al. (10)</td>
<td>1956</td>
<td>12</td>
<td>1.0–6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Paixao &amp; Yoe (11)</td>
<td>1959</td>
<td>39</td>
<td>0.0–18.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Herring et al. (12)</td>
<td>1960</td>
<td>109</td>
<td>0.0–27.0</td>
<td>6.0</td>
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<tr>
<td><strong>SERUM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Butt et al. (13)</td>
<td>1964</td>
<td>48</td>
<td>—</td>
<td>5.3–6.2†</td>
</tr>
<tr>
<td>Gofman et al. (14)</td>
<td>1964</td>
<td>39</td>
<td>0.0–18.0</td>
<td>—</td>
</tr>
<tr>
<td>Present study</td>
<td>1966</td>
<td>23</td>
<td>0.1–7.3</td>
<td>2.2§</td>
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</table>

*All authors used a spectrographic method, with these exceptions: spectrophotometric method used by Cluett and in the present study; X-ray spectroscopic method used by Gofman et al.  
†S.D., ± 1.9.  
‡Range of mean (Ni not detected in 18 serums).  
§S.D., ± 1.8.
Table 2. Résumé of Normal Concentrations of Nickel in Whole Blood and Erythrocytes Using Various Methods*  

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Subjects (No.)</th>
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<th>Mean</th>
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<td>2.0-9.0</td>
<td>4.1</td>
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<tr>
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<td>—</td>
<td>—</td>
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<td>1963</td>
<td>153</td>
<td>0.9-9.8§</td>
<td>4.2‡</td>
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<td>Butt et al. (13)</td>
<td>1964</td>
<td>47</td>
<td>—</td>
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**WHOLE BLOOD**

**ERYTHROCYTES**

<table>
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<th>Subjects (No.)</th>
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<td>40</td>
<td>0.0-16.0</td>
<td>5.1</td>
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<tr>
<td>Herring et al. (12)</td>
<td>1960</td>
<td>106</td>
<td>0.0-31.0</td>
<td>5.3</td>
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</table>

*All authors used a spectrographic method except Cluett who used a spectrophotometric method.
†Calculated from values for plasma and erythrocytes.
‡S.D., ± 2.8.
§Ninety-fifth percentile.

Concentrations of nickel in whole blood or erythrocytes were higher than in serum or plasma. Although the normal concentrations of nickel in whole blood or erythrocytes have not been established in our laboratory, our observation of increased concentrations of nickel in hemolyzed serum supports the view that the concentration of nickel is higher in erythrocytes than in serum. Contrary findings have been reported by Herring et al. (12), who noted a slightly lower mean concentration of nickel in erythrocytes than in plasma. Herring and associates did not detect any significant differences between the nickel concentrations in blood from males and females or from Negros and Caucasians.

In a previous investigation (7), measurements were made of the concentrations of nickel in purified preparations of human serum proteins obtained by continuous-flow electrophoresis and by cold-ethanol fractionation. The highest concentrations of nickel were consistently found in the beta globulin fractions. Our conclusion that a nickel-binding globulin is present in human serum has recently been confirmed by Himmelhoch et al. (17). Fractions of dialyzed human serum obtained by gradient chromatography on DEAE-cellulose columns were analyzed for nickel and other metals by emission spectrography. Himmelhoch et al. identified a nickel-binding protein which was distinctly separated from the proteins which bound iron, zinc, and manganese. Based upon the presence of nickel in a single protein fraction, Himmelhoch and his associates speculated that serum nickel may be a constituent of a specific metalloprotein.
Although the spectrophotometric method for the measurement of serum nickel is intended primarily for use in investigations of nickel metabolism in myocardial infarction, the technic should also be valuable in the diagnosis of poisoning from nickel carbonyl and other toxic nickel compounds. It is anticipated that measurements of serum nickel may furnish an index of the severity of acute nickel poisoning, and serve as a guide to the efficacy of chelation therapy. In addition, the procedure may provide a means of detecting chronic exposure to inhalation of nickel, a condition which is associated with a high incidence of cancer of the respiratory tract (1c). Measurements of serum nickel in patients with myocardial infarction and in experimental animals exposed to nickel carbonyl are currently being undertaken in our laboratory and will be described in subsequent publications.

References