Radioactive nickel chloride (\textsuperscript{63}NiCl\textsubscript{2}) was injected i.v. into rabbits in dosage of 0.24 mg Ni/kg body wt. \textsuperscript{63}Ni was rapidly cleared from the serum (T\textsubscript{1/2} \approx 8.2 h) during the period from 1 h to 2 days after the injection, but slowly (T\textsubscript{1/2} \approx 95 h) during the period from 4 to 7 days after the injection. During 24 h after injection of \textsuperscript{63}NiCl\textsubscript{2}, an average of 90% of serum \textsuperscript{63}Ni was bound to albumin and 10% was ultrafiltrable. Chromatography on Sephadex G-25 demonstrated the presence of five distinct \textsuperscript{63}Ni complexes (Fractions I to V) in serum ultrafiltrates. During 24 h after injection of \textsuperscript{63}NiCl\textsubscript{2}, an average of 78% of the administered dose of \textsuperscript{63}Ni was excreted in the urine. Chromatography of the urine on Sephadex G-25 separated three \textsuperscript{63}Ni complexes, which appeared to have identical chromatographic mobilities as serum Fractions II, III, and V. The chemical identities of the ultrafiltrable \textsuperscript{63}Ni complexes in serum and urine have not been established, although one (Fraction V) resembles Ni histidine in its chromatographic mobility on Sephadex G-25. This study demonstrates that ultrafiltrable nickel in serum and urine does not exist primarily as free Ni(II), but instead as Ni complexes, and suggests that ultrafiltrable Ni receptors play an important physiological role in nickel homeostasis by serving as diffusible vehicles for the extracellular transport and renal excretion of nickel.

Additional Keyphrases: Sephadex chromatography • ultrafiltrable Ni receptors and Ni homeostasis • myocardial infarction, stroke, burns, uremia, cirrhosis • detoxification • atomic absorption spectrometry • electrophoresis on cellulose acetate • ultrafiltration • radioisotopes • absence of free Ni\textsuperscript{2+} from biol. fluids • nickeloplasmin

Previous investigations have shown that abnormal concentrations of serum nickel are frequently decreased in patients with chronic uremia and hepatic cirrhosis (4, 5). Measurements by atomic absorption spectrometry have demonstrated that nickel exists in normal human and rabbit sera in three distinct forms: (a) ultrafiltrable nickel; (b) albumin-bound nickel, and (c) in a nickel-containing macroglobulin, which has been named "nickeloplasmin" (5-7). In the present study, \textsuperscript{63}NiCl\textsubscript{2} has been administered intravenously to rabbits in order to determine the in vivo binding of \textsuperscript{63}Ni(II) to constituents of serum and urine. As will be described, this study indicates that most of the serum \textsuperscript{63}Ni(II) becomes bound to albumin and to ultrafiltrable Ni receptors. Furthermore, this study shows that ultrafiltrable Ni complexes play a major role in detoxification and urinary excretion of nickel.

Materials and Methods

The experimental animals were 18 albino rabbits (12'6', 6'9) of the New Zealand strain, weighing 3.2 to 3.6 kg. The rabbits were fed Purina laboratory rabbit chow with supplemental fresh vegetables. Radioactive nickel chloride (\textsuperscript{63}NiCl\textsubscript{2}, specific activity = 5.96 mCi/mg Ni; New England Nuclear Corp., Boston, Mass. 02118) was administered in a single intravenous injection of 0.24 mg of Ni per kilogram of body weight (equivalent to 1.43 mCi/kg). The injection vehicle was sterile NaCl solution (8.5 g/liter), adjusted to pH 7.0. For each experiment, the requisite volume (1.5 to 2 ml) of \textsuperscript{63}NiCl\textsubscript{2} dissolved in NaCl solution was slowly injected into a marginal ear vein of an unanesthetized rabbit, using a 5-ml syringe with a 24-gauge needle. During 24 h following the administration of \textsuperscript{63}NiCl\textsubscript{2}, the rabbit was kept in a metabolism cage in order to collect the urine. During this period, the rabbit was given water freely, but food was withheld so that the urine collection would not be contaminated with particles of food. At intervals ranging from 1 h to 7 days after the injection of \textsuperscript{63}NiCl\textsubscript{2}, blood samples (1 to 5 ml) were collected from a marginal vein of the contralateral ear, using a 5-ml syringe with 22-gauge needle. In certain experiments in which it was necessary to obtain 20 to 30 ml of blood, the rabbit was exsanguinated by cardiac puncture. Serum samples were discarded if any hemolysis was detected. For measurements of biliary excretion of \textsuperscript{63}Ni, two rabbits were

\textsuperscript{63}Ni Complexes in Rabbit Serum and Urine after Injection of \textsuperscript{63}NiCl\textsubscript{2}

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anesthetized with pentobarbital, and polyethylene cannulas were inserted into the common bile ducts. When the rabbits had awakened from anesthesia, \(^{63}\text{NiCl}_2\) was injected intravenously, and bile was collected for 5 h. All of the studies of \(^{63}\text{Ni}\)-complexes were initiated without delay, as soon as the specimens of serum, urine, or bile were obtained. Unless otherwise noted, the fractionation procedures were performed in a cold room at 4°C.

**Atomic absorption spectrometry of serum nickel** was performed by the method of Nomoto and Sunderland (1), with use of a Model 403 atomic absorption spectrometer (Perkin-Elmer Corp., Norwalk, Conn. 06882).

**Electrophoresis** of serum proteins was performed on 2.5 × 10 cm membranes of cellulose acetate, using a horizontal-type electrophoresis cabinet (A. H. Thomas Co., Philadelphia, Pa. 19105). The conditions of electrophoresis were: (a) 5-ml sample volume; (b) sodium barbital buffer, pH 8.6, 0.075 mol/liter; (c) current of 120 V for 2 h; and (d) drying of membranes at 85°C for 10 min. Autoradiography of unstained electrophoresis membranes was performed by placing the dried membranes in x-ray cassettes that were loaded with polyester base x-ray film (“Gaestar”; Ansco Co., Rochester, N. Y.). The exposure periods were 18 or 90 days. After the autoradiograms were developed, the electrophoresis membranes were stained with Ponceau S dye, as described by Savory et al. (8).

**Ultrafiltration** of serum was performed at 25°C with a pressure dialysis apparatus (Model MC-8 with PM-10 membrane; Amicon Corp., Lexington, Mass. 02173). The specific gravities of the sera, ultrafiltrates, and residues were determined at 25°C with a microcyphonometer, so that concentrations of \(^{63}\text{Ni}\) per milliliter of solution could be converted to concentrations per gram of water. Serum ultrafiltrable \(^{63}\text{Ni}\) was computed as described by Sunderland (9) for measurements of serum ultrafiltrable Ca and Mg.

**Column chromatography** of serum on Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J. 08854) was performed in a chromatographic column (diameter, 2.5 cm; bed height, 85 cm) equipped for ascending migration, with Tris-HCl buffer (pH 7.4, 0.1 mol/liter). The serum volume was 2 ml and the fraction volumes were 2 ml, collected by use of an “Escargot” volumetric fractionator (Gilson Medical Electronics, Inc., Middleton, Wis. 53562). Effluent was monitored at 280 nm with an absorptiometer (Gilson). Serum ultrafiltrates and urine samples were chromatographically fractionated on columns of Sephadex G-25 (Pharmacia) (2.5 × 85 cm column equipped for ascending migration) with use of Tris-HCl buffer (pH 7.4, 0.1 mol/liter).

The sample volume was 5 ml and the fraction volumes were 3 ml. Computations of void volumes (V₀), bed volumes (Vₑ) and partition coefficients (Kₑ) for the chromatographic fractionations on Sephadex G-200 and G-25 were performed as described by Fischer (10).

**Test for formation of \(^{63}\text{Ni}\)-complexes in vitro.** The capacities of various pure compounds to form stable complexes with \(^{63}\text{Ni}\)(II) in dilute solutions at physiological pH were tested by the following procedure. To 4.5 ml of Tris-HCl buffer (pH 7.4, 0.1 mol/liter) were added 0.5 ml of an aqueous solution of the compound to be tested (1 mmol/liter) and 75 \(\mu\)l of \(^{63}\text{NiCl}_2\) solution (3.33 mmol/liter). The concentrations of the compound to be tested and of \(^{63}\text{Ni}\)(II) in the final mixture were \(1 \times 10^{-4}\) and \(5 \times 10^{-5}\) mol/liter, respectively. The mixture was incubated for 3 h in a water bath at 37°C, and then was used for chromatography on a column of Sephadex G-25, as described above. Under these conditions, \(^{63}\text{Ni}\) complexes were eluted from the column at characteristic partition coefficient (Kₑ) values, whereas free \(^{63}\text{Ni}\)(II) became firmly adsorbed to the Sephadex G-25 and could be eluted only after addition of ethylenediaminetetraacetic acid (5 ml of Tris-HCl buffer, pH 7.4, containing 0.01 mole of EDTA per liter). The following chromatographically pure compounds were tested by this procedure: (a) L-histidine-HCl; (b) L-lysine-HCl; (c) L-cysteine-HCl; (d) L-aspartic acid (all obtained from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan); (e) L-histidyl-L-glutamic acid (Schwarz/Mann Biochemicals, Orangeburg, N. Y. 10962); (f) DL-histidyl-DL-histidine; (g) folic acid; (h) adenosine-triphosphate, disodium salt (all obtained from Nutritional Biochemicals Corp., Cleveland, Ohio 44128); and (i) carnosine and (j) urocanic acid (both obtained from Calbiochem, Inc., San Diego, Calif. 92112). This same procedure was also used to form Ni-histidine-[\(^{14}\text{C}\)], using [\(^{14}\text{C}\)]-L-histidine (New England Nuclear), and nonradioactive NiCl₂ (Fisher).

**Scintillation counting.** Samples of serum, urine, bile, ultrafiltrates and chromatographic fractions were prepared for scintillation counting by adding 0.1 ml of each specimen to vials containing 12 ml of scintillation fluid (Beckman Instruments, Inc., Fullerton, Calif. 92634), consisting of 8 g of “butyl-PPD” [2-(4′-t-butyphenyl)-5-(4′-biphenyl)1,3,4-oxazol], and 150 ml of Beckman “BioSolve No. 3” per liter of toluene. The scintillation vials were allowed to remain at 25°C for at least 24 h before scintillation counting. Radioactivity was measured with a Model CPM-100 liquid scintillation spectrometer (Beckman) within a counting error of ±2%. The counting efficiency of each sample was determined by addition of an internal standard of \(^{63}\text{NiCl}_2\). Computations of the percentages of administered \(^{63}\text{Ni}\) that were retained within the serum volume were based on an average serum volume of 38.8 ml/kg body weight in rabbits (11). Computations of percentages of administered \(^{63}\text{Ni}\) that were excreted in bile were based on an average biliary secretion rate of 4.9 ml/hour per kilogram body weight in rabbits (12).
Results

Comparisons of serum Ni analyses by atomic absorption vs. measurements of serum $^{63}$Ni concentrations by scintillation counting. Serum samples were obtained from a rabbit before $^{63}$NiCl$_2$ was injected and at 1.5, 3, 6, 12, and 24 h afterward. The total nickel concentrations in these sera were 8.5, 1090, 920, 720, 540, and 180 $\mu$g/liter, respectively, as determined by atomic absorption spectrometry. In comparison, the $^{63}$Ni concentrations in these sera were <1, 1070, 890, 750, 590, and 180 $\mu$g/liter, respectively, as estimated by liquid scintillation spectrometry. Excluding the pre-injection determinations, there was no significant difference between these two sets of measurements, based upon a paired-sample t-test (13).

Disappearance of $^{63}$Ni from serum after injection of $^{63}$NiCl$_2$. $^{63}$Ni concentrations were measured in serum samples obtained from rabbits at 1, 2.5, 4, 8, and 12 h and at 1, 2, 3, 4 and 7 days after injection of $^{63}$NiCl$_2$ (Table 1). Serum $^{63}$Ni rapidly diminished from a mean concentration of about 1 mg/liter at 1 h to 120 $\mu$g/liter at 1 day and 1 $\mu$g/liter at the end of 1 week. If we assume that the average serum volume of the rabbit is 38.8 ml/kg (11), the fraction of the injected dose of $^{63}$Ni remaining within the serum volume diminished from 17% at 1 h to 1.9% at 1 day and 0.02% at 1 week. The disappearance curve of $^{63}$Ni from the serum volume (Figure 1) is based upon serial measurements of serum $^{63}$Ni in three rabbits. The disappearance rate was rapid ($T_{1/2} \approx 8.2$ h) during the period from 1 h to 2 days after the injection of $^{63}$NiCl$_2$. The disappearance was much slower ($T_{1/2} \approx 95$ h) during the period from 4 to 7 days after the injection of $^{63}$NiCl$_2$.

Excretion of $^{63}$Ni in urine and bile. Quantitative 24 h collections of urine from the rabbits were difficult to accomplish, because the urine frequently splattered during micturition. Satisfactory urine collections were obtained from three rabbits. In these rabbits, an average of 78% of the administered $^{63}$Ni was recovered in urine during the 24 h after the injection of $^{63}$NiCl$_2$ (range, 74% to 85%). In specimens of bile collected from two rabbits during 5 h after injection of $^{63}$NiCl$_2$, the concentrations of $^{63}$Ni were 840 and 960 $\mu$g/liter. If biliary secretion rate is assumed to be 4.9 ml/hour per kilogram body weight (12), the biliary excretion of $^{63}$Ni in the two rabbits during the 5 h after injection amounted to 8.6 and 9.8% of the administered dose of $^{63}$NiCl$_2$.

Electrophoresis of serum protein-bound $^{63}$Ni. Fractionations by cellulose acetate electrophoresis were performed on sera obtained from rabbits at intervals ranging from 1 h to 1 week after injection of $^{63}$NiCl$_2$. Figure 2 illustrates a typical electrophoretic fractionation of serum, in this instance collected 24 h after the injection. Throughout the period from 1 h to 1 week, protein-bound $^{63}$Ni was predominantly associated with serum albumin. The autoradiograph in Figure 2 shows a faint band of radiodensity that is associated with serum $\alpha_2$-globulin. This $^{63}$Ni-containing fraction, which migrated with $\alpha_2$-globulin, may represent $^{63}$Ni incorporated into serum ceruloplasmin, or it may represent binding of $^{63}$Ni to serum ceruloplasmin, as has been reported to occur in vitro by McKee and Frieden (14).

Measurements of protein-bound and ultrafiltrable $^{63}$Ni. Column chromatography on Sephadex G-200 was used to fractionate sera collected from four rabbits 2 or 24 h after injection of $^{63}$NiCl$_2$. A typical fractionation is shown in Figure 3. The serum $^{63}$Ni was separated into two components: (a) a major peak (about 92% of total serum $^{63}$Ni), which was associated with serum albumin; and (b) a minor peak (about 8% of total serum $^{63}$Ni), which was eluted from the column more slowly than serum albumin. Thus, in two rabbits that were studied 2 h after injection, 91.2 and 92.0% of serum $^{63}$Ni was recovered in the albumin-containing fractions, and 8.7 and 7.7% was recovered in protein-free fractions with elution volumes greater than the bed volume ($V_b$) of the Sephadex G-200 column. In two rabbits that were

**Table 1. Serum $^{63}$Ni after I.V. Injection of $^{63}$NiCl$_2$**

<table>
<thead>
<tr>
<th>Period after injection, h</th>
<th>No. rabbits tested</th>
<th>Mean $^{63}$Ni</th>
<th>Range of $^{63}$Ni</th>
<th>Fraction of dose in serum volume $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>1070</td>
<td>990-1180</td>
<td>17</td>
</tr>
<tr>
<td>2.5</td>
<td>7</td>
<td>850</td>
<td>560-1050</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>750</td>
<td>680-810</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>620</td>
<td>500-800</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>450</td>
<td>320-680</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>No. rabbits</th>
<th>Mean $^{63}$Ni</th>
<th>Range of $^{63}$Ni</th>
<th>Fraction of dose in serum volume $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>120</td>
<td>80-200</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>17</td>
<td>9-27</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>4.2</td>
<td>2.6-6.3</td>
<td>0.068</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2.2</td>
<td>1.5-3.3</td>
<td>0.035</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1.2</td>
<td>0.8-2.0</td>
<td>0.020</td>
</tr>
</tbody>
</table>

$^a$ $^{63}$NiCl$_2$ injected in dosage of 0.24 mg Ni/kg body wt.
$^b$ Assuming serum volume = 38.8 ml/kg (11).

![Fig. 1. Disappearance curve of $^{63}$Ni from serum after i.v. injection of $^{63}$NiCl$_2$, based upon serial measurements in three rabbits](image-url)
studied at 24 h after injection, 93.3 and 92.0% of serum $^{63}$Ni was recovered in the serum albumin-containing fractions, and 5.6 and 7.8% was recovered in the protein-free fractions.

The percentages of serum $^{63}$Ni that were recovered in the protein-free fractions after chromatography on Sephadex G-200 corresponded approximately to the percentages of serum ultrafiltrable $^{63}$Ni as determined by pressure dialysis across an Amicon PM-10 membrane, which retains proteins with molecular weights greater than about 10,000. For example, measurements of the same two sera mentioned above, which were collected at 2 h after injection of $^{63}$NiCl$_2$, yielded percentages of ultrafiltrable $^{63}$Ni of 8.1 and 9.4%, respectively. In eight sera that were collected during the interval from 2 to 8 h after injection of $^{63}$NiCl$_2$, ultrafiltrable $^{63}$Ni averaged 10% of total serum $^{63}$Ni concentrations (range, 8 to 13%). Data in Table 2 illustrate the importance of perfoming the ultrafiltration as soon as possible after blood is collected and serum separated. When ultrafiltration was repeated after the sera had been stored at 4°C for 2 to 13 days, significant increases were observed in the proportions of ultrafiltrable $^{63}$Ni.

Fractions of ultrafiltrable serum $^{63}$Ni and urine $^{63}$Ni. Column chromatography on Sephadex G-25 was used to fractionate serum ultrafiltrates and urine specimens collected from five rabbits at intervals from 2 to 24 h after injection of $^{63}$NiCl$_2$ (Figure 4). Five $^{63}$Ni-containing constituents were consistently separated in the chromatographic fractions of the serum ultrafiltrates. These $^{63}$Ni complexes have been designated as Fractions I to V. Three $^{63}$Ni-containing constituents were found in the urine, which appeared to have identical chromatographic mobilities as serum Fractions II, III, and V. The major urinary $^{63}$Ni peak, Fraction II, corresponded in its chromatographic mobility to a relatively minor peak in the chromatographic pattern of serum ultrafiltrates, suggesting that this $^{63}$Ni complex is rapidly cleared from the serum and excreted in the urine. Thus, Fraction II may well play a major role in the metabolism and urinary excretion of nickel. Fractions I and IV were detected only in serum ultrafiltrates, and were apparently not excreted in urine.

Ionic $^{63}$Ni(II) was not detected either in serum ultrafiltrates or in urine samples. The recovery of $^{63}$Ni in the chromatographic fractions eluted from Sephadex G-25 averaged 90% of the total $^{63}$Ni placed upon the column. Therefore, ionic $^{63}$Ni(II), if it was present, comprised less than 10% of the $^{63}$Ni in serum ultrafiltrates and urine samples.

Formation of Ni complexes in vitro. Figure 5 illustrates the chromatographic elution patterns obtained when Ni-histidine complexes were prepared in vitro with (a) [14C]-L-histidine and nonradioactive NiCl$_2$, and (b) $^{63}$NiCl$_2$ and nonradioactive L-histidine. Ni-histidine-[14C] and $^{63}$Ni-histidine were both eluted from the Sephadex column at an elution volume ($K_{av}$) of 0.87. After $^{63}$Ni-histidine had been eluted, 5 ml of Tris-HCl buffer, pH 7.4, containing 0.01 mole of EDTA per liter, was added to the column, and a small residuum of $^{63}$Ni(II) was recovered as $^{63}$Ni-EDTA. Figure 5 (bottom) shows the elution profile we observed when $^{63}$NiCl$_2$ was added directly to the chromatographic column of Sephadex G-25, in the

![Fig. 2. Electrophoretic fractionation of proteins in serum obtained from a rabbit 24 h after i.v. injection of $^{63}$NiCl$_2$.](image)

![Fig. 3. Column chromatographic fractionation on Sephadex G-200 of serum obtained from a rabbit 2 hours after i.v. injection of $^{63}$NiCl$_2$.](image)

![Table 2. Effect of Storage at 4°C on Ultrafiltrable $^{63}$Ni in Rabbit Sera](table)

<table>
<thead>
<tr>
<th>Storage period, a</th>
<th>No. sera tested</th>
<th>Mean % Range</th>
<th>$P$ vs. Initial value c</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1 days</td>
<td>8</td>
<td>10</td>
<td>8–13</td>
</tr>
<tr>
<td>2–3</td>
<td>4</td>
<td>14</td>
<td>12–15</td>
</tr>
<tr>
<td>6–13</td>
<td>4</td>
<td>20</td>
<td>15–22</td>
</tr>
</tbody>
</table>

a Sera obtained 2 to 8 h after injection of $^{63}$NiCl$_2$.

b Interval between collection of serum and ultrafiltration.

c Paired-sample t-test.

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The formed complexes, with the exception of L-histidine. The $^{63}$Ni became firmly adsorbed to the Sephadex G-25, and was quantitatively recovered only after the addition of buffered EDTA solution.

Chromatographic fractionations on Sephadex G-25 were also used to test whether or not various pure compounds would form stable complexes with $^{63}$Ni(II) in dilute solutions at pH 7.4. Under the experimental conditions described in the "Materials and Methods" section, $^{63}$Ni complexes were formed with L-histidyl-L-glutamic acid and DL-histidyl-DL-histidine, in addition to L-histidine, as previously mentioned. With these three compounds, >75% of the $^{63}$Ni added to the column was recovered in chromatographic peaks with characteristic partition coefficients ($K_{av} = 0.50, 0.79,$ and 0.87, respectively), and <25% of the $^{63}$Ni was recovered in the $^{63}$Ni-EDTA peak. None of the other compounds that were tested formed stable $^{63}$Ni complexes under the reaction conditions. The compounds that failed to form complexes with $^{63}$Ni(II) included: L-alanine, L-cysteine, L-aspartic acid, folic acid, ATP, carnosine, and urocanic acid. With these compounds, >95% of the $^{63}$Ni added to the chromatographic column was recovered in the $^{63}$Ni-EDTA peak.

In Table 3, the partition coefficients ($K_{av}$) of serum ultrafiltrable $^{63}$Ni fractions on Sephadex G-25 at pH 7.4 are compared with those of the $^{63}$Ni-histidine and $^{63}$Ni-histidyl-peptide complexes. It may be noted that the partition coefficient of $^{63}$Ni-histidine corresponds approximately to that of Fraction V in serum.

### Discussion

Wase et al. (15), Payne (16), Sunderman and Selin (17), Chen et al. (18), and Hoffmann and Fiedler (19) have previously administered soluble nickel salts to rodents by parenteral routes, and have observed that the injected nickel is rapidly cleared from the serum and excreted in the urine and feces. For example, Sunderman and Selin (17) injected $^{63}$NiCl$_2$ intravenously into five rats in a dosage of 1 mg Ni/kg, and they recovered 87 ± 3% of the administered $^{63}$Ni in urine during the succeeding 24 h. During four days after the injection of $^{63}$NiCl$_2$, a total of 90 ± 4% of the administered $^{63}$Ni was excreted in urine, 3 ± 1% in feces (17). Consistent with these previous reports, in the present study an average of 78% (range, 74–85%) of $^{63}$Ni administered intravenously to rabbits as $^{63}$NiCl$_2$ was excreted in urine within 24 h. The present finding that a major proportion of serum $^{63}$Ni was bound to albumin agrees with previous observations by Chen et al. (18) and Nomoto et al. (6).

The present study furnishes the first direct evidence that ultrafiltrable nickel in serum or urine does not exist in free ionic form as Ni$^{+2}$, but instead occurs in the form of Ni complexes. In this investigation, we observed five distinct ultrafiltrable Ni complexes in serum ultrafilters and three in urine samples. One of these complexes, Fraction II, appears to be especially important in the urinary excretion of nickel. The chemical identities of the ultrafiltrable Ni complexes have not yet been estab-
lished, although one of the peaks (Fraction V) resembles Ni-histidine in its tendency to be eluted slowly from Sephadex G-25 columns (20, 21).

Attempts to identify the nickel-binding constituents of Fractions I to V by means of thin-layer chromatography and autoradiography are in progress in our laboratory. Several possible ligands are being considered on the basis of their tendencies to form Ni complexes in vitro. Table 4 lists the nonprotein constituents of serum that have been reported by various investigators to bind Ni\(^{+2}\) in vitro. These materials include ultrafiltrable molecules such as amino acids, peptides, sulfolipids, phospholipids, nucleotides, and pyrrolic compounds. Several workers have recently suggested that L-histidine may be involved in the metabolism of copper (52, 53) and zinc (54–56).

Nomoto et al. (6) found that an average of 44% of nickel in normal rabbit serum is present in serum nickeloplasmin, an \(a_2\)-globulin with a molecular weight of \(7 \times 10^6\). They observed that serum nickeloplasmin did not bind Ni\(^{+2}\) in vitro after equilibrium dialysis. Consistent with their observations, in the present study, only a trace of \(^{63}\)Ni became associated in vivo with serum \(a_2\)-globulins after a single intravenous injection of \(^{63}\)NiCl\(_2\). In contrast, in another investigation that is in progress in our laboratory, incorporation of \(^{63}\)Ni into serum nickeloplasmin has been demonstrated in rabbits that received repeated intravenous injections of \(^{63}\)NiCl\(_2\) in a daily dosage of 4.5 \(\mu\)g Ni/kg for 14 to 21 consecutive days (Nomoto, S., Decsy, M. I., Murphy, J. R. and Sunderman, F. W., Jr., Incorporation of \(^{63}\)Ni into rabbit serum nickeloplasmin. Manuscript in preparation).

On the basis of the present study, we concluded that ultrafiltrable Ni receptors serve as diffusible vehicles for the extracellular transport and renal excretion of Ni\(^{+2}\), and we propose that these Ni receptors may play an important physiological role in nickel homeostasis. We believe that identification of the ultrafiltrable Ni-binding constituents in biological fluids of experimental animals may prove to be a vital step towards understanding the pathological alterations of nickel metabolism that occur in common diseases of man (5).

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