Peptide and carbohydrate complexes of nickel in human kidney

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The predominant renal and urinary forms of nickel consist of low- M_r complexes. Similarities in the nature of these complexes have been found in kidneys of rats exposed parenterally to NiCl_2 and in rat kidneys treated with NiCl_2 *in vitro*. Similar complexes have also been identified after treatment of bovine and human renal soluble fractions with NiCl_2 . The bulk of nickel in all cases is associated with sulphated oligosaccharide fractions containing uronic acids and neutral sugars. This binding is non-specific, and nickel is readily displaced from these fractions by copper. Smaller amounts of nickel are bound to an acidic peptide, which was purified from human kidneys and partially characterized. Nickel was not displaced from this material by copper at physiological pH. These nickel complexes have not been found in plasma, suggesting that ligand exchange occurs during or after glomerular filtration of the metal.

Elemental nickel and its compounds hold a place among metals such as arsenic, chromium and possibly cadmium as established carcinogens in man (Sunderman, 1979). Following inhalational exposure, nickel-refinery workers have developed carcinomas of lung, nasal sinuses, larynx and kidney, and sarcomas have occurred locally in animals with intramuscular implants of nickel compounds (Sunderman *et al.*, 1975; Sunderman, 1981). The carcinogenic potential of various nickel compounds is variable (Sunderman, 1981; Hansen & Stern, 1983), and the mechanisms of nickel carcinogenesis are poorly understood (Costa, 1983; Hansen & Stern, 1983).

Little is known about the biochemistry of nickel in animals. Suggestions that the metal is essential in man have not been substantiated by demonstration of a nickel-deficiency state (Anke *et al.*, 1983). In human serum, nickel is primarily transported bound to the N-terminal of albumin (Lucassen & Sarkar, 1979; Nomoto, 1980; Lau & Sarkar, 1984). After parenteral administration of nickel salts to experimental animals, toxic changes are seen in lung and kidney (Hennigar & Gross, 1977; Shimkin *et al.*, 1978), which may be considered the target organs for nickel toxicity. On short-term parenteral administration of NiCl_2 to mice, most of the dose is rapidly excreted in urine, and any nickel that is retained is accumulated primarily in the kidney (Parker & Sunderman, 1974; Sarkar,

1980). Upon prolonged administration the metal also accumulates in lung (Herlant-Peers *et al.*, 1982), where the existence of several nickel-binding proteins has been demonstrated (Herlant-Peers *et al.*, 1983).

Although several studies have shown that up to 80% of renal cytosolic nickel is in a low- M_r ultrafilterable form (Parker & Sunderman, 1974; Oskarsson & Tjälve, 1979; Sunderman *et al.*, 1981, 1983; Herlant-Peers *et al.*, 1982, 1983; Abdulwajid & Sarkar, 1983), the emphasis has been on identifying the macromolecular forms of the metal. Thus Sunderman's group (Sunderman *et al.*, 1981, 1983) has found five proteins containing nickel in the renal post-microsomal fraction of nickel-exposed rats. A description of the low- M_r nickel complexes has not been forthcoming, despite their predominance in both renal cytosol and urine (Van Soestbergen & Sunderman, 1972; Sunderman *et al.*, 1981, 1983).

In order to understand the renal handling of this rapidly excreted carcinogenic metal, and the factors determining its retention and excretion, we have undertaken to identify the low- M_r complexes of nickel in the renal soluble fractions of rat, cow and man. The experiments reported in the present paper show striking similarities between these complexes in rats exposed to NiCl_2 *in vivo* and material treated with NiCl_2 *in vitro*. The similarities extend to materials of bovine and human origin treated *in vitro*. In all cases 70% of the low- M_r nickel in the soluble fraction is bound to an

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oligosaccharide fraction, containing sulphate and uronic acids as the probable metal-binding components. The remaining 30% is bound to a highly acidic peptide with M_r approx. 4000. This peptide was purified from human kidneys and is described below. These nickel complexes are distinct from the known forms of the metal in serum (Lucassen & Sarkar, 1979), indicating that ligand exchange may occur during or after glomerular filtration.

Materials and methods

Binding studies performed in vivo

Male Sprague-Dawley rats (150–200 g) were obtained from Charles River (St. Constant, Que., Canada), and given rat chow and water *ad libitum*. A solution of NiCl_2 in 0.9% NaCl (120 μg of Ni^{2+} /ml), containing 175 μCi of carrier-free $^{63}\text{NiCl}_2$ (Amersham, Oakville, Ont., Canada)/ml was injected intraperitoneally (1 ml/kg body wt.). The animals were killed by exsanguination under ether anaesthesia at 6 h, and the kidneys were collected on ice for immediate decapsulation and homogenization in cold 0.9% NaCl solution (1:10, w/v). The soluble fraction was obtained as the supernatant obtained by centrifugation at 105 000 g for 60 min. Samples (15 ml) of this fraction were applied to Sephadex G-75 or Bio-Gel P-60 gel-filtration columns.

Binding studies performed in vitro

Rat kidneys were obtained from animals that had not been exposed to nickel. Bovine kidneys were obtained from freshly butchered cattle, collected on ice and stored frozen. Kidneys from four humans were obtained at autopsy and stored frozen. Three of these were from patients (26-year-old female, 62-year-old and 80-year-old males) dying of causes unrelated to renal pathology. The fourth was from a 72-year-old male diabetic. All kidney samples were processed to obtain the soluble fraction in a manner identical with that described above for the studies performed *in vivo*. They were then incubated with 10 μCi of carrier-free $^{63}\text{NiCl}_2$ /100 ml at 4°C overnight. For preparation of the nickel-saturated material, NiCl_2 was included in the incubation mixture, to a final concentration of 10 mM. These labelled samples were then fractionated by gel-filtration chromatography.

Chromatographic techniques

Samples were fractionated on 3 cm $\times$ 90 cm columns packed with either Sephadex G-75 or Bio-Gel P-60, by elution with 1 mM-sodium borate buffer, pH 8.6. In some experiments, the elution was with 50 mM-collidine/HCl buffer, pH 7.8. The permeation-volume fractions of these columns

were separated by h.p.l.c. on silica-based I-60 protein gel-filtration columns (Waters, Milford, MA, U.S.A.) equilibrated with 50 mM-potassium phosphate buffer, pH 7.2, at a flow rate of 1 ml/min. Final purification of the nickel-binding peptide from human samples was performed on a Waters reversed-phase C-18 column with a linear gradient of 0–10% (v/v) acetonitrile in water, formed over 15 min at a flow rate of 1 ml/min.

Miscellaneous methods

Determination of ^{63}Ni β -radiation was done by using a ^{14}C -counting channel in either Beckman LS8100 or Tracor 6895 liquid-scintillation counters. Amino acid and amino sugar analyses were performed on a Durrum D-500 amino acid analyser. For amino acids, samples were hydrolysed *in vacuo* for 18 h at 110°C in the presence of 5.6 M-HCl. Hydrolysis solutions contained norleucine as an internal standard, and dimethyl sulphoxide (1%, v/v) was included in one tube of duplicate samples. For amino sugar determinations, samples were hydrolysed for 6 h in 4 M-HCl *in vacuo* at 110°C. Total protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Total carbohydrate was determined by the phenol/ H_2SO_4 method (Dubois *et al.*, 1956), with galactose standards. Neutral sugars were determined by g.l.c. of trifluoroacetate derivatives of their *O*-methyl glycosides (Zanetta *et al.*, 1972). Total sulphate was assayed by the rhodizonate procedure (Terho & Hartiala, 1971), with Na_2SO_4 standards, and sialic acid was determined by the thiobarbituric acid assay (Warren, 1958). Samples were hydrolysed in 1 M-HCl for 2 h at 100°C for the sulphate assay, and in 0.05 M- H_2SO_4 for 60 min at 80°C for the sialic acid determination (Parthasarathy & Spiro, 1981). Total uronic acids were determined by a scaled-down version of the carbazole method (Bitter & Muir, 1962).

Absorption spectra were recorded on a Beckman Acta M-VI spectrophotometer, in 1 ml quartz cuvettes. Adjustments of pH were by addition of known amounts of conc. NaOH or conc. HCl to the cuvette by a 10 μl Hamilton syringe. For the copper displacement experiments, the absorption coefficient of the nickel-saturated sample was calculated from the known specific radioactivity of the bound ^{63}Ni , and then stoichiometric amounts of copper were added to the cuvette as 5 μl quantities of an appropriate CuCl_2 stock solution.

Results

Nickel distribution in renal soluble fractions

When rats were injected with NiCl_2 containing ^{63}Ni , $1.4 \pm 0.6\%$ of the dose was recovered from

whole kidney homogenates at 6h, nearly 70% of this being found in the post-microsomal fraction. Of this total soluble ^{63}Ni , $62 \pm 2\%$ is found in a low- M_r fraction freely diffusible through Spectrapor cellulose dialysis tubing with a molecular-mass cut-off (M.C.O.) of 2kDa. Thus a large fraction of the ^{63}Ni is obtained at the permeation volume of a Sephadex G-75 gel-filtration column (Fig. 1a). This fraction was freeze-dried, and further resolved on an I-60 protein separation column by h.p.l.c. (Fig. 2a). It consists of a complex mixture of material absorbing at 230nm, but nickel is associated with only two components.

To determine if the same components bound nickel *in vitro*, soluble renal fractions from unexposed rats were incubated with carrier-free $^{63}\text{NiCl}_2$. When this material was eluted from a Sephadex G-75 column, ^{63}Ni was distributed (Fig. 1b) in a manner nearly identical with that seen in the above experiment performed *in vivo*. Upon h.p.l.c. fractionation of the permeation-volume peak, ^{63}Ni is again distributed between two components (Fig. 2b), which contain the same percentages of ^{63}Ni and are eluted at the same times as the components of Fig. 2(a).

Fig. 1. Sephadex G-75 profiles of the soluble fractions of whole rat kidney homogenates showing absorbance at 280nm (—) and ^{63}Ni radioactivity (-----)

(a) Kidneys isolated from rats exposed *in vivo* to $^{63}\text{NiCl}_2$; (b) rat kidney soluble fraction incubated with NiCl_2 *in vitro*. For full experimental details see the text. V_0 and V_p indicate the void volume and the permeation volume respectively.

In order to obtain larger quantities of the low- M_r nickel-binding components, and with the above results being taken as justification for studying nickel complexes formed *in vitro*, the above fractionations were repeated with material obtained from both bovine and human kidneys. Both the ^{63}Ni Sephadex G-75 profiles (Fig. 3) and distribution of ^{63}Ni in the low- M_r fraction, as seen by h.p.l.c. (Fig. 4), are similar in the bovine and human material. Furthermore, these strongly resemble the corresponding separations of the rat material (Figs. 1 and 2). The nickel-containing components eluted from the h.p.l.c. I-60 column at approx. 8.5 and 9.5 min are referred to below as the first and second nickel-binding components respectively.

Characterization of nickel-binding components

Polyacrylamide-gel electrophoresis of the higher- M_r fractions obtained from the Sephadex G-75 columns (Figs. 1 and 3) revealed the presence of ^{63}Ni in at least four different protein bands (results

Fig. 2. Fractionation of low- M_r nickel-binding fractions of rat kidneys by h.p.l.c. on an I-60 protein column. Material is obtained from the permeation-volume fractions of Fig. 1. —, A_{230} ; -----, ^{63}Ni radioactivity. (a) Exposure to $^{63}\text{NiCl}_2$ *in vivo*; (b) exposure to $^{63}\text{NiCl}_2$ *in vitro*. Conditions are as described in the Materials and methods section.

Fig. 3. Sephadex G-75 fractionation of (a) bovine and (b) human renal soluble fractions exposed to $^{63}\text{NiCl}_2$ *in vitro*. Details are as indicated in Fig. 1 legend.

not shown). These species have been discussed by Sunderman and co-authors (see below) and are not pursued further in the present work.

The first nickel-binding component of the low- M_r fraction was found to consist of ninhydrin-reactive material and to be devoid of sugar in all cases. This component from the human kidney was purified by recycling on the I-60 column, followed by passing twice through a reversed-phase C-18 column. The material thus obtained is of high purity and retains ^{63}Ni (Fig. 5). It is not diffusible at an M.C.O. of 1 kDa and is eluted in the void volume of a Sephadex G-10 column ($>700\text{Da}$). Amino acid analysis (Table 1) is consistent with an M_r of 4000. However, it passes slowly through dialysis tubing with an M.C.O. of 2 kDa (as reported by the manufacturer), possibly as a result of heterogeneity of pore size. Notably, the peptide possesses a high content of aspartic acid/asparagine and glutamic acid/glutamine, and is devoid of cysteine, methionine and aromatic residues. Since the peptide migrates as an anion on high-voltage paper electrophoresis at pH 6.5–8.9, it appears to be an acidic peptide with a high content of aspartic acid and glutamic acid residues.

The second nickel-binding fraction was eluted from the I-60 column in a region rich in carbohydrate. Only fractions eluted between 9 and 11 ml (Fig. 4a) contained detectable carbohydrate. Similar carbohydrate patterns from other species are not shown. This material was further purified by

Fig. 4. Fractionation of the low- M_r nickel-binding material of (a) bovine and (b) human kidneys after exposure to $^{63}\text{NiCl}_2$. Details are as indicated in Fig. 2 legend.

recycling on the I-60 column, but the fraction still contained a mixture of materials (Table 2). Attempts to resolve individual components further as intact nickel complexes were unsuccessful. The bovine material is rich in neutral sugars and uronic acids, with small amounts of glucosamine, protein, sialic acid and sulphate. The corresponding human fraction consisted of neutral sugars and uronic acids with small amounts of hexosamines, and was sulphated to a lesser extent. The composition and elution volume of the human fraction are unchanged by ultrafiltration with a UM05 membrane, indicating that ^{63}Ni is bound to oligosaccharide material of M_r greater than 500. However, this material passes through dialysis tubing of M.C.O. 1 kDa. When this h.p.l.c. fraction was passed through a calibrated Bio-Gel P-2 column, nickel was eluted in two major peaks, corresponding to M_r values of 500 and 900. Both fractions tested positive for carbohydrate, sulphate and uronic acid.

Metal-binding properties of the nickel components

Sufficient amounts of neither the first nor the second low-*M_r* nickel-binding components have yet been obtained in pure form to allow detailed analysis of the metal binding. In order to observe the bound nickel spectroscopically, bovine kidney homogenates incubated with excess of NiCl₂ containing ⁶³Ni were fractionated on Sephadex G-75. Under these nickel-saturating conditions, this fraction still showed a ⁶³Ni profile on the I-60 column similar to Fig. 4(a). Its absorption spectrum (Fig. 6a) is characterized by a broad peak in the visible region ($\lambda_{\text{max.}} = 645\text{nm}$, $A_{645} = 3.2\text{cm}^{-1}$ for a 1M-nickel solution) and dominated below 500nm by the rising ligand absorptions. Lowering the pH to 4.0 by addition of 5 μ l amounts of HCl solution causes reversible dissociation of nickel, as evidenced by the appearance of the Ni(OH₂)₆²⁺

spectrum. This hexaquaonickel absorption is lost when the pH is re-adjusted to neutrality by addition of NaOH solution.

Addition to the cuvette of an amount of CuCl₂ equimolar to the bound nickel results in the appearance of a dark-purple colour within the time of mixing at pH 8.5 (Fig. 6b). Addition of copper to a 10-fold molar excess caused no further change, although under these circumstances only 70% of the ⁶³Ni had been displaced. Under these conditions, displaced nickel is precipitated as nickel hydroxides, and only nickel bound to the biological ligands is soluble. The 30% of the ⁶³Ni remaining bound was not displaced by excess copper over 2h when the pH was maintained at neutrality or above. H.p.l.c. of such copper-treated material showed that only the first ⁶³Ni peak of Fig. 4(a) was present, i.e. the retained ⁶³Ni was entirely in the peptide component.

Discussion

The tissue fractionations described here confirm earlier reports that most of the nickel in rat kidneys

Fig. 5. Purification by h.p.l.c. on a C-18 reversed-phase column of the nickel-binding peptide from human kidney —, *A*₂₃₀; ----, ⁶³Ni radioactivity. Conditions are as described in the Materials and methods section. The slight delay in radioactivity compared with absorption is due to the dead volume of 0.12ml between the flow cell of the spectrophotometer and the sample-collection port.

Table 1. Amino acid composition of human low-*M_r* nickel-binding peptide

Values are means $\pm$ S.E.M. for 11 determinations of separate preparations from four different kidneys. No hydroxylysine or hydroxyproline was detected in any sample.

Amino acid	Composition (mol/100 mol)
Asx	9.3 $\pm$ 0.2
Thr	4.0 $\pm$ 0.2
Ser	12.3 $\pm$ 0.6
Glx	15.8 $\pm$ 0.5
Pro	4.8 $\pm$ 0.6
Gly	21.4 $\pm$ 0.8
Ala	9.5 $\pm$ 0.2
Val	5.3 $\pm$ 0.4
Ile	3.2 $\pm$ 0.3
Leu	5.4 $\pm$ 0.4
His	2.1 $\pm$ 0.3
Lys	2.9 $\pm$ 0.2

Table 2. Composition of carbohydrate rich low-*M_r* nickel-binding fractions of human and bovine kidneys

	Bovine (μ g)	Human (μ g)
Total sugar	100	100
Total protein	3.3	0.6
Amino sugars	5 (glucosamine)	(Trace <i>N</i> -acetylglucosamine + <i>N</i> -acetylgalactosamine)
Neutral sugars	70 (glucose)	39 (glucose)
		37 (galactose)
Sulphate	4.7	0.23 $\pm$ 0.10
Uronic acids	15	16 $\pm$ 8
Sialic acid	0.7	—

Fig. 6. Absorption spectra of nickel-saturated and copper-saturated low- M_r binding components of bovine origin (a) Nickel-saturated material prepared as described in the text. ●, Starting material; ○, after lowering the pH to 4.0 with HCl; ▲, after raising the pH back to 8.2 with NaOH; ■, $\text{Ni}(\text{OH}_2)_6^{2+}$ in borate buffer, pH 7.2; □, nickel-saturated material prepared in collidine buffer. (b) Nickel-saturated material (30 mM in nickel) titrated with copper at pH 6.9, as described in the Materials and methods section. ○, Starting material; ●, after addition of CuCl_2 to 10 mM; ▲, to 15 mM; □, to 20 mM; ■, to 25 mM; △, to 35 mM. Above approx. 15 mM- CuCl_2 there is no further increase in copper binding. The subsequent increase in absorption is due to $\text{Cu}(\text{OH}_2)_6^{2+}$ ($\lambda_{\text{max.}} > 700 \text{ nm}$), from which the copper is precipitated as the pH is raised.

is in the post-microsomal fraction, and that most of this is in low- M_r , ultrafilterable forms (Parker & Sunderman, 1974; Sunderman *et al.*, 1981, 1983). This pattern is the same whether nickel exposure occurs *in vivo* or *in vitro*, and holds also for bovine and human kidneys exposed to NiCl_2 *in vitro*. Sunderman *et al.* (1981, 1983) have described five proteins from rat kidney that bind renal nickel. These ranged in M_r from 10000 to > 130000 . Some differences in the distribution of nickel among these components were observed, which depended on whether exposure was *in vivo* or *in vitro*. Several macromolecular components of bovine and human kidney homogenates bound nickel in the present study.

The identical distributions of nickel between low- M_r and high- M_r components in all three species, and between two low- M_r components with identical retention times on h.p.l.c. I-60 columns, indicate that the same components are present in all cases. Thus, in general, in the mammalian kidney soluble nickel is bound primarily to two components, consisting of a peptide and an oligosaccharide fraction. In contrast with the macromolecular forms of the metal, these predominant

forms of nickel are found after exposure *in vivo* and after exposure *in vitro*. After the exposure *in vivo* some nickel is also eluted after the permeation volume of the Sephadex G-75 column. This may be a consequence of filtration of plasma low- M_r nickel complexes that have weak interactions with the column matrix. Alternatively such complexes may only form in kidney *in vivo*.

The choice of buffer in these studies may be critical, and the possibility must be considered that interaction of buffer salts with both metal and biological ligands may alter the nickel distribution pattern. Metal complexes of many commonly used buffer salts form under a variety of conditions, and it has been pointed out (Nakon & Krishnamoorthy, 1983) that Good's buffers, designed to avoid this very problem, may not always achieve their aim. Borate complexes of sugars influence their electrophoretic mobilities (Verma *et al.*, 1977), and paper electrophoresis of acidic peptides in Tris/HCl buffer may cause them to migrate as cationic complexes at pH 8.6 (D. M. Templeton & B. Sarkar, unpublished work). With these considerations in mind, the fractionations reported in the present paper were carried out in both borate and

collidine buffers, which give identical results (not shown). For h.p.l.c., when inorganic buffers of lower pH were desired, phosphate buffers were used. Under these conditions, inorganic complexes of nickel only occur when excess nickel is present, and are easily distinguished as being eluted at the permeation volume of the I-60 column.

Of additional concern in such studies is the possibility of redistribution of metal among low- M_r ligands as equilibria are perturbed during isolation. The use of different buffers, size fractionation techniques and soluble fractions from different species, all with similar results, argues against such redistribution in the present study. Similar elution positions of nickel are seen when the material is prepared with carrier-free isotope and under nickel-saturating conditions. Thus the presence of nickel does not alter the chromatographic behaviour of the ligands on either the Sephadex or h.p.l.c. columns, and a redistribution dependent on nickel concentration is also excluded.

In the present study, the peptide of human origin was purified and characterized. It is as yet of unknown origin, and has an unusually high content (about 25 mol/100 mol) of aspartic acid/asparagine and glutamic acid/glutamine. Electrophoretic behaviour indicates an acidic peptide, suggesting that many or all of these residues are in the acid form. There is no evidence of sulphur ligands in the absorption spectrum of the nickel-saturated bovine low- M_r fraction, consistent with the absence of cysteine and methionine residues from the purified human peptide. The broad absorption at 645 nm is indicative of the ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$ transition of octahedral nickel complexes (Cotton & Wilkinson, 1972), and its nature suggests typical amino and oxo nickel complexes with ligand sets of N_3O_3 , N_2O_4 or O_6 (Blakely *et al.*, 1983). The absence of a feature due to octahedral imidazole-nickel absorption [$\lambda_{max.} = 525\text{--}555\text{ nm}$ for the ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$ transition (Blakely *et al.*, 1983; Formicka-Kozłowska *et al.*, 1983)] indicates that a histidine residue is not involved in binding. One is led to conclude that aspartic acid and glutamic acid provide polycarboxylate chelation of nickel by this peptide.

The failure of excess copper to displace nickel from the peptide at physiological pH over extended time periods is noteworthy, and is in contrast with the expected relative stabilities of copper and nickel complexes (Cotton & Wilkinson, 1972). It is also in contrast with the observed properties of nickel-binding proteins, such as albumin, with respect to copper exchange (Glenon & Sarkar, 1982). Although the function of this peptide remains unknown, this apparent specificity of metal binding may offer a clue to its biological role.

In all three species, most of the nickel found in low- M_r complexes occurs in an oligosaccharide fraction. In human kidney, this consists of at least two nickel-binding components of M_r values corresponding to tri- and penta-saccharides. These contain glucose, galactose, uronic acids and hexosamines, and are partially sulphated. The nature of these sugars suggests an origin in the components of renal glomerular or tubular basement membranes (Kanwar & Farquhar, 1979; Kefalides, 1981). Sulphated uronic acids and hexosamines occur in proteoglycan structures, whereas the sugars of collagen are primarily glucose and galactose. The glomerular basement membrane filters plasma components on the basis of size and charge (Pesce & First, 1979). The charge-dependence of filtration results from the presence of an array of anionic sites in the basement membrane, which is provided by the sulphated hexosamines and uronic acids of the proteoglycan heparan sulphate, embedded in a matrix of type IV collagen (Kanwar & Farquhar, 1979). Oligosaccharide fragments may be released when the glomerular basement membrane turns over (Schurer *et al.*, 1980). Electrostatic interaction of these species with the plasma forms of nickel could be an important first step in the filtration and excretion of such a metal. Displacement of nickel from this fraction by copper, to form copper complexes, occurred readily. This is consistent with the formation of non-specific electrostatic complexes with metal ions. The formation of such complexes could be an important step in the filtration and excretion of metals. Further discussion of the origin and biological function of this nickel-complexing oligosaccharide fraction must await characterization of the intact metal complexes, as well as identification of the filterable forms of the metal in serum and urine.

Conclusions

1. In the mammalian kidney, most of the burden of nickel is in the post-microsomal fraction. About two-thirds of this nickel is bound in low- M_r (<5000) complexes.

2. These complexes are of two types. In all cases about 30% of the low- M_r nickel is bound to a peptide, and the remainder is associated with oligosaccharides, which include neutral sugars, sulphate and uronic acids.

3. The nickel-binding peptide was purified from human kidneys. It has an apparent M_r of 4000, is rich in glutamic acid and aspartic acid and is devoid of aromatic or sulphur-containing amino acids.

4. Displacement of nickel from the oligosaccharides by copper occurs readily. In contrast, the

peptide shows the unusual property that its nickel is not displaced by excess copper at physiological pH over prolonged times.

5. These complexes of nickel have not hitherto been described in plasma.

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