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Aminoaciduria and Proteinuria in Rats after a Single Intraperitoneal Injection of Ni(II)[1]

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Aminoaciduria and Proteinuria in Rats after a Single Intraperitoneal Injection of Ni(II). GITLITZ, P. H., SUNDERMAN, F. W., JR. AND GOLDBLATT, P. J. (1975). *Toxicol. Appl. Pharmacol.* **34**, 430–440. Proteinuria was found in Fischer female rats after a single ip injection of $NiCl_2$ in dosages from 34 to 85 μmol/kg (2–5 mg Ni/kg). Generalized α-aminoaciduria was found after a single ip injection of $NiCl_2$ in dosages of 68 and 85 μmol/kg (4 and 5 mg Ni/kg). Amino acids in plasma were normal or slightly diminished from 1 to 48 hr after injection of Ni(II). Electron microscopy of kidneys of five rats at 48 hr after Ni(II) (68 μmol/kg) consistently revealed fusion of foot processes of glomerular epithelial cells. Focal tubular necrosis was present in the kidney of one of these rats. This study demonstrates that toxic nephropathy with aminoaciduria and proteinuria develops in rats after ip Ni(II). Amino acid and protein excretions consistently returned to normal by Day 5.

van Soestbergen and Sunderman (1972), Onkelinx *et al.* (1973), and Asato *et al.* (1975) administered $^{63}Ni(II)$ to rodents by iv or ip injection, and found that a major fraction of the ^{63}Ni became bound to ultrafiltrable constituents of serum. The ultrafiltrable ^{63}Ni-complexes were rapidly excreted by the kidneys. Wass *et al.* (1954), Smith and Hackley (1968), Parker and Sunderman (1974), and Clary (1975) investigated the distribution of ^{63}Ni in tissues of rodents after parenteral injections of $^{63}Ni(II)$ and reported that the highest concentrations of ^{63}Ni were consistently present in the kidneys. In view of the accumulation of nickel in renal tissue, we suspected that administration of Ni(II) might produce renal toxicity. The biochemical and ultrastructural studies described in this report demonstrate that a single parenteral injection of Ni(II) can produce an acute, reversible nephropathy in rats.

METHODS

The experimental animals were 136 female albino rats of the Fischer strain[2] with an average body weight of 165 g (range, 140–190 g). The rats were housed individually in metabolism cages and were permitted free access to powdered rat chow and water.

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[2] Charles River Breeding Laboratories, North Wilmington, Mass.

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A group of five or six rats was usually tested in each experiment. At 8:30 AM each rat was given an ip injection (4 ml/kg) of the vehicle (sterile NaCl solution, 145 mmol/liter) into which a specified quantity of $NiCl_2$[3] had been dissolved. The dosages of $NiCl_2$ that were tested were 2, 3, 4, and 5 mg Ni/kg, equivalent, respectively, to 34, 51, 68, and 85 μmol/kg.[4] Urine samples (24 hr) were obtained during 2–7 days after the injection of $NiCl_2$. Urine samples (24 hr) were obtained from control rats during 1 or 2 days after an ip injection of the vehicle. The urine samples were collected in flasks that contained 0.3 ml of thymol in isopropanol (20 g/liter) as a preservative. The individual urine samples were diluted to a volume of 10–15 ml. In certain experiments, a single blood sample (1–2 ml) was obtained from the tail vein at an interval of 1, 6, 24, or 48 hr after ip injection of $NiCl_2$ or at an interval of 24 or 48 hr after ip injection of the vehicle. The blood samples were collected in tubes that contained dried sodium heparinate (100 units/tube).

For measurements of amino acids, urine and plasma samples were deproteinized by mixing 10 volumes of each sample with 1 volume of a solution of sulfosalicylic acid. A 300-g/liter sulfosalicylic acid solution was used for urine samples, and a 500-g/liter solution was used for plasma samples. The mixtures of the samples with sulfosalicylic acid were allowed to remain for 10 min at 25°C and then were centrifuged for 10 min at 7000 *g* at 4°C. One volume of each protein-free supernatant fluid was adjusted to pH 2.0–2.2 by adding lithium hydroxide solution (40 g/liter) and was diluted to 1.2 volumes by adding water. The samples were centrifuged at 14,000 *g* for 15 min at 4°C to remove any fine particles that might clog the capillary column of the amino acid analyzer.[5] Amino acids in 40-μl samples were analyzed by automated ion-exchange chromatography, using stepwise elution with five lithium citrate buffers, as described by Gitlitz *et al.* (1974). This method separated and quantitated all of the amino acids which are commonly found in urine and plasma, except for tryptophan, proline, and hydroxyproline. The chromatographic peak of tryptophan was insufficiently resolved from the chromatographic peak of ammonia. Proline and hydroxyproline were not quantitated, because the absorbance maxima of their ninhydrin complexes are located at 440 nm rather than at 590 nm, which was the wavelength that was employed for photometry of the other amino acid–ninhydrin complexes.

Analyses of total protein in urine were performed by the following microadaptation of the biuret method of Savory *et al.* (1968), using reagents which were prepared as described in the original method. Into duplicate test tubes 2 ml of urine and 2 ml of ethanolic phosphotungstic acid reagent were placed. The tubes were allowed to stand in an ice bath for 15 min and then were centrifuged at 1000 *g* for 15 min at 25°C. The tubes were inverted, and the supernatant fluid was drained and discarded. Biuret reagent (1 ml) was added to one of each pair of tubes, and alkaline tartrate reagent (1 ml) was added to the second tube in each pair. The protein precipitates were dissolved by agitation with a rotary mixer, and the tubes were allowed to stand for 20 min at 25°C in order to achieve maximum development of color. The contents of each tube were transferred to spectrophotometer cuvettes (1-cm light path), and absorbances were measured

[3] "Ultrapure" reagent, Alfa Inorganics, Inc., Beverly, Mass.

[4] For comparison, the LD50 for $NiCl_2$ by ip injection in female Fischer rats was 110 (SE $\pm$ 6) μmol/kg, based upon mortality within 2 weeks under identical experimental conditions (E. Horak and F. W. Sunderman, Jr., unpublished observations).

[5] Amino acid analyzer, Model D-500, Durrum Instrument Company, Palo Alto, Calif.

TABLE 1

EFFECT OF PARENTERAL $NiCl_2$ UPON URINARY EXCRETION OF PROTEIN AND AMINO ACIDS

Constituent	Excretion of protein (mg/kg/day) and amino acids (μmol/kg/day) in urine[a, b]									
	Control rats [$N = 39$]	34 μmol/kg [$N = 6$]		51 μmol/kg [$N = 6$]		68 μmol/kg [$N = 10$]		85 μmol/kg [$N = 6$]		Maximum increase vs controls
		Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	
Total protein	19 ± 5	24 ± 3[c]	25 ± 3[c]	35 ± 13[c]	41 ± 13[d]	61 ± 25[d]	76 ± 41[d]	83 ± 32[d]	100 ± 35[d]	5X
Neutral α-amino acids										
Glycine	19 ± 4	19 ± 3	20 ± 3	20 ± 6	24 ± 8	41 ± 16[d]	64 ± 37[d]	58 ± 15[d]	82 ± 39[d]	4X
Alanine	13 ± 4	13 ± 3	14 ± 6	20 ± 13	32 ± 22	59 ± 26[d]	83 ± 51[d]	95 ± 43[d]	110 ± 59[d]	9X
Valine	2.0 ± 0.5	2.7 ± 1.1	3.2 ± 2.8	6.8 ± 6.3	11 ± 9	23 ± 11[d]	36 ± 24[d]	37 ± 17[d]	49 ± 24[d]	24X
Leucine	2.2 ± 1.1	3.3 ± 1.5	3.8 ± 2.3	4.6 ± 4.1	8.2 ± 6.3	17 ± 8[d]	28 ± 18[d]	30 ± 14[d]	41 ± 19[d]	19X
Isoleucine	1.4 ± 0.4	1.8 ± 0.6	2.0 ± 1.3	3.3 ± 2.5	5.4 ± 3.9	11 ± 5[d]	16 ± 10[d]	18 ± 8[d]	25 ± 11[d]	18X
Serine	8.6 ± 1.6	9.4 ± 1.5	9.7 ± 2.9	13 ± 7	17 ± 10	35 ± 14[d]	59 ± 39[d]	51 ± 19[d]	74 ± 35[d]	9X
Threonine	9.4 ± 1.9	9.6 ± 1.5	10 ± 3	14 ± 7	17 ± 8	31 ± 13[d]	47 ± 28[d]	43 ± 17[d]	57 ± 25[d]	6X
Phenylalanine	2.0 ± 0.6	1.8 ± 0.3	1.9 ± 0.4	2.5 ± 0.9	3.5 ± 1.6	7.2 ± 2.9[d]	9.5 ± 5.3[d]	13 ± 4[d]	13 ± 5[d]	7X
Tyrosine	1.9 ± 0.6	2.3 ± 0.5	2.4 ± 1.2	3.5 ± 2.0	4.7 ± 3.0	8.6 ± 3.3[d]	14 ± 8[d]	15 ± 6[d]	19 ± 9[d]	10X
Methionine	3.0 ± 0.8	2.7 ± 0.4	2.7 ± 0.7	4.1 ± 1.5	4.1 ± 1.6	7.8 ± 2.4[d]	11 ± 5[d]	12 ± 4[d]	15 ± 7[d]	5X
Cystine	2.1 ± 0.6	2.2 ± 0.6	1.8 ± 0.7	2.7 ± 0.7	3.4 ± 1.3	5.9 ± 1.8[d]	9.5 ± 5.8[d]	10 ± 3[d]	14 ± 5[d]	7X
Acidic α-amino acids and amides										
Aspartic acid	15 ± 3	15 ± 2	14 ± 3	15 ± 3	13 ± 3	15 ± 3	15 ± 4	15 ± 5	16 ± 5	
Asparagine	7.8 ± 1.8	7.3 ± 2.8	6.8 ± 0.8	7.2 ± 1.9	8.5 ± 3.2	14 ± 5[d]	20 ± 9[d]	15 ± 7	12 ± 7	3X
Glutamic acid	6.7 ± 1.5	7.9 ± 3.0	6.3 ± 1.7	9.7 ± 4.0	12 ± 7	18 ± 6[d]	28 ± 13[d]	36 ± 12[d]	41 ± 17[d]	6X
Glutamine	13 ± 3	13 ± 2	17 ± 5	20 ± 10	25 ± 12	49 ± 21[d]	68 ± 29[d]	78 ± 31[d]	94 ± 41[d]	7X

Basic α-amino acids										
Histidine	5.0 ± 1.0	11 ± 8	5.3 ± 1.2	27 ± 11[d]	7.3 ± 2.1	45 ± 13[d]	14 ± 7[d]	54 ± 18[d]	19 ± 8[d]	11X
Arginine	4.3 ± 1.1	4.8 ± 1.1	4.7 ± 2.2	7.2 ± 4.4	8.2 ± 4.2	21 ± 10[d]	30 ± 18[d]	36 ± 14[d]	42 ± 20[d]	10X
Lysine	5.5 ± 1.5	6.3 ± 2.2	7.7 ± 4.6	14 ± 11	20 ± 15	40 ± 20[d]	68 ± 44[d]	67 ± 26[d]	99 ± 46[d]	18X
Ornithine	1.9 ± 0.5	2.0 ± 0.5	1.8 ± 0.6	2.6 ± 1.3	3.0 ± 1.5	5.5 ± 1.9[d]	7.5 ± 4.4[d]	7.2 ± 3.6[d]	9.5 ± 4.7[d]	5X
Citrulline	<1	<1	<1	<1	<1	6.1 ± 3.1[d]	12 ± 8[d]	12 ± 4[d]	18 ± 9[d]	>18X
Substituted and miscellaneous amino acids										
β-Alanine	7.2 ± 1.4	6.8 ± 1.1	7.3 ± 1.1	6.5 ± 0.8	6.9 ± 1.8	6.1 ± 2.5	6.2 ± 3.1	7.4 ± 2.5	5.0 ± 3.2	
γ-Aminobutyric acid	1.8 ± 0.6	2.0 ± 0.5	1.9 ± 0.4	1.6 ± 0.5	2.0 ± 0.4	2.9 ± 3.0	2.1 ± 1.1	1.5 ± 0.2	1.6 ± 0.4	
1-Methylhistidine	4.2 ± 2.3	2.8 ± 1.5	2.8 ± 1.0	2.7 ± 1.0[c]	3.4 ± 1.6	3.7 ± 1.8	4.3 ± 1.2	6.8 ± 6.4	4.6 ± 3.0	
3-Methylhistidine	4.5 ± 1.2	5.9 ± 2.1	4.7 ± 1.0	4.5 ± 0.6	3.4 ± 1.1	5.8 ± 1.2	3.8 ± 1.0	5.5 ± 1.3	4.3 ± 1.2	
Sarcosine (*N*-methylglycine)	2.7 ± 0.9	3.0 ± 0.4	3.3 ± 0.5	1.5 ± 0.7[d]	2.0 ± 1.3	1.4 ± 0.8[d]	1.6 ± 1.0[d]	2.6 ± 1.6	2.6 ± 1.1	
Taurine	290 ± 73	230 ± 68	330 ± 41	350 ± 94	340 ± 100	190 ± 52[d]	250 ± 90	380 ± 140	300 ± 190	

[a] Injection of $NiCl_2$ ip at the specified dosages.
[b] Mean ± SD.
[c] $p < 0.01$ vs corresponding value in controls, *t* test.
[d] $p < 0.001$ vs corresponding value in controls, *t* test.

at 440 nm in a spectrophotometer.[6] The nonspecific background absorbance of the sample containing alkaline tartrate reagent was subtracted from the absorbance of the paired sample containing biuret reagent, and the concentration of urine protein was calculated as described by Savory *et al.* (1968).

Measurements of urea nitrogen were performed by a conductivimetric urease assay, as described by Horak and Sunderman (1972), using an automated "BUN-analyzer."[7] Measurements of electrolytes, osmolality, and creatinine were performed as previously described by Sunderman and Sunderman (1966, 1970).

Control rats ($N = 5$) were killed at 24 hr after ip injection of the vehicle, and treated rats were killed at 24 hr ($N = 4$), 48 hr ($N = 5$), and 96 hr ($N = 2$) after ip injection of $NiCl_2$ in the dosage of 68 μmol/kg. The kidneys were perfused *in situ* with cold, buffered glutaraldehyde, as described by Ericsson (1966). Samples of renal cortex and medulla were embedded separately and were postfixed, processed, and examined by light and electron microscopy, as described by Goldblatt *et al.* (1970).

RESULTS

In a pilot experiment, two rats were placed in metabolism cages, and 24-hr urine samples were collected during 2 days prior to an ip injection of Ni(II) (85 μmol/kg) and during 7 subsequent days. Practically identical patterns of urinary excretion of protein and amino acids were observed in both of these rats. The mean urinary excretion of protein and of most of the α-amino acids became greatly increased during the first 2 days after the injection of Ni(II), and returned to the baseline (control) values by Days 3 or 4. The maximum urinary excretion of histidine (10 times the control value) occurred during the first day after the injection of Ni(II). The maximum urinary excretion of all of the other α-amino acids and of protein occurred during the second day after the injection of Ni(II). The urinary excretion of valine, citrulline, leucine, isoleucine, and lysine during Day 2 ranged from 16 to 30 times the corresponding control values. The urinary excretion of the other α-amino acids during Day 2 ranged from 2 to 11 times the control values, except for aspartic acid, 1-methylhistidine, and 3-methylhistidine, which were not significantly increased. The excretion of protein and of all of the α-amino acids were within 0.5–1.5 times the control values throughout the period from 4 to 7 days after the injection of Ni(II).

A dose–response study of the effect of ip Ni(II) upon urinary excretion of protein and amino acids was performed in order to establish the thresholds of renal toxicity (Table 1). Administration of Ni(II) in dosage of 34 μmol/kg caused significant increase in the urinary excretion of protein during the 2 days after the injection, but did not produce any significant changes in the urinary excretion of any amino acid. Administration of Ni(II) in dosage of 51 μmol/kg resulted in significant increase (>fivefold) in urinary excretion of histidine, and significant diminutions in the excretion of 1-methylhistidine and sarcosine on the first day after the injection. In dosages of 68 and 85 μmol/kg, Ni(II) injection caused significant increases in the urinary excretion of all of the neutral, acidic, and basic α-amino acids, except for aspartic acid. As in the pilot experiment, the maximum excretion of histidine occurred during Day 1, and the maximum excretion of all of the other α-amino acids occurred on Day 2. No significant changes were found

[6] Spectrophotometer, Model A-25, Beckman Instruments, Inc., Fullerton, Calif.
[7] "BUN analyzer," Beckman Instruments, Inc., Fullerton, Calif.

in the urinary excretion of β-alanine, γ-aminobutyric acid, 1-methylhistidine, or 3-methylhistidine. Diminished excretion of sarcosine and taurine occurred in rats which received ip Ni(II) in the dosage of 68 μmol/kg.

To ascertain whether or not the increased urinary excretion of α-amino acids might be secondary to increases in their plasma concentrations, measurements were made of α-amino acids in plasma samples which were obtained at 1, 6, 24, and 48 hr after injection of Ni(II) in the dosage of 68 μmol/kg. As shown in Table 2, no increases were found in the plasma concentration of any of the α-amino acids. Significant diminutions

TABLE 2

EFFECT OF PARENTERAL $NiCl_2$ UPON PLASMA AMINO ACIDS AND UREA NITROGEN

	Concentrations of amino acids (μmol/liter) and urea nitrogen (mg/100 ml) in plasma[a, b]				
		Interval after ip $NiCl_2$ in dosage of 68 μmol/kg			
Constituent	Control rats [$N = 19$]	1 hr [$N = 5$]	6 hr [$N = 5$]	24 hr [$N = 5$]	48 hr [$N = 5$]
Neutral α-amino acids					
Glycine	230 ± 22	190 ± 21[c]	210 ± 20	240 ± 28	290 ± 56
Alanine	450 ± 45	230 ± 44[d]	390 ± 50	410 ± 60	490 ± 98
Valine	210 ± 25	130 ± 14[d]	160 ± 15[d]	150 ± 18[d]	172 ± 24[c]
Leucine	130 ± 13	100 ± 20[c]	120 ± 14	110 ± 10	120 ± 14
Isoleucine	78 ± 8	62 ± 13[c]	60 ± 6[d]	65 ± 7[c]	67 ± 8[c]
Serine	240 ± 25	160 ± 24[d]	200 ± 11	230 ± 20	270 ± 43
Threonine	220 ± 20	150 ± 16[d]	190 ± 20	190 ± 17	240 ± 34
Phenylalanine	59 ± 5	52 ± 4[c]	67 ± 6	56 ± 7	63 ± 9
Tyrosine	83 ± 16	54 ± 10[d]	35 ± 7[d]	73 ± 9	78 ± 18
Methionine	46 ± 4	38 ± 4[d]	46 ± 2	46 ± 5	58 ± 8
Cystine	43 ± 5	32 ± 6[c]	34 ± 8	30 ± 5[d]	48 ± 8
Acidic α-amino acids and amides					
Aspartic acid	28 ± 5	16 ± 2[d]	24 ± 6	28 ± 4	30 ± 7
Asparagine	50 ± 6	46 ± 9	50 ± 6	60 ± 8	63 ± 10
Glutamic acid	90 ± 16	79 ± 19	100 ± 13	110 ± 8	120 ± 28
Glutamine	510 ± 72	290 ± 38[d]	350 ± 90	340 ± 60[a]	290 ± 48[d]
Basic α-amino acids					
Histidine	75 ± 7	93 ± 18	74 ± 8	60 ± 5[d]	58 ± 3[c]
Arginine	160 ± 17	120 ± 20[d]	130 ± 10[d]	170 ± 19	170 ± 25
Lysine	360 ± 45	290 ± 28[d]	340 ± 55	380 ± 36	400 ± 70
Ornithine	26 ± 6	27 ± 5	20 ± 3	26 ± 4	33 ± 14
Citrulline	73 ± 6	55 ± 8[d]	61 ± 11	61 ± 5[d]	68 ± 13
Other amino acids					
Taurine	130 ± 37	98 ± 8[c]	140 ± 30	140 ± 25	190 ± 78
Urea nitrogen	21 ± 5	16 ± 3	20 ± 12	27 ± 13	28 ± 13

[a] Injection of $NiCl_2$ ip (68 μmol/kg).
[b] Mean ± SD.
[c] $p < 0.01$ vs values in control rats, t test.
[d] $p < 0.001$ vs values in control rats, t test.

in plasma concentration of several α-amino acids were noted at 1 and 6 hr, and the plasma concentration of a few α-amino acids remained diminished at 24 and/or 48 hr after the injection of Ni(II). Measurements of urine volume, osmolality, and electrolyte excretions were performed upon serial 24-hr collections of urine from six rats on the day before ip injection of Ni(II) (68 μmol/kg), and on the first and second days after the injection. As shown in Table 3, the injection of Ni(II) was accompanied by mild diminutions in

TABLE 3

URINE VOLUME, OSMOLALITY, AND ELECTROLYTE EXCRETION FOLLOWING IP INJECTION OF $NiCl_2$ IN FISCHER RATS[a, b]

Constituent	Units	Excretion		
		Day before injection	Day 1 after injection	Day 2 after injection
Volume	ml/day	7.1 ± 1.0	7.8 ± 0.9	8.8 ± 1.8
Osmolality	mOsmol/g urine	2.7 ± 0.2	2.1 ± 0.4[c]	1.9 ± 0.3[c]
Sodium	mmol/day	1.6 ± 0.1	1.6 ± 0.2	1.3 ± 0.1[d]
Potassium	mmol/day	2.9 ± 0.2	2.3 ± 0.3[c]	2.5 ± 0.2[c]
Calcium	mmol/day	0.20 ± 0.05	0.09 ± 0.01[d]	0.08 ± 0.02[d]
Chloride	mmol/day	2.0 ± 0.2	2.0 ± 0.3	1.5 ± 0.2[d]
Inorganic phosphorus	mg P/day	13 ± 2	15 ± 2	15 ± 2

[a] $NiCl_2$, ip, in dosage of 68 μmol/kg.
[b] Each value is the mean ± SD in six rats.
[c] $p < 0.01$, paired-sample t test.
[d] $p < 0.001$, paired-sample t test.

TABLE 4

EFFECT OF IP INJECTION OF $NiCl_2$ UPON PLASMA AND URINE CONSTITUENTS AND ESTIMATED RENAL CLEARANCES IN FISCHER RATS[a, b]

Measurement	Units	Control rats [$N = 5$]	Ni(II)-treated rats [$N = 5$]
Plasma sodium	mmol/liter	142 ± 2[c]	142 ± 2
Plasma potassium	mmol/liter	4.0 ± 0.3	3.5 ± 0.3
Plasma calcium	mmol/liter	2.5 ± 0.1	2.6 ± 0.1
Plasma chloride	mmol/liter	100 ± 1	95 ± 2[d]
Plasma inorganic phosphorus	mg P/100 ml	6.8 ± 0.3	6.6 ± 1.0
Plasma creatinine	mg/100 ml	0.49 ± 0.08	0.67 ± 0.18
Urine creatinine	mg/day	88 ± 23	57 ± 21
Estimated creatinine clearance	ml/min	0.89 ± 0.19	0.60 ± 0.27
Plasma urea nitrogen	mg N/100 ml	21 ± 1	40 ± 12
Urine urea nitrogen	g N/day	3.8 ± 0.6	2.1 ± 0.6[d]
Estimated urea clearance	ml/min	0.90 ± 0.06	0.38 ± 0.17[e]

[a] $NiCl_2$, ip, in dosage of 68 μmol/kg. Plasma samples were obtained at 48 hr after injection and urine samples were collected from 24 to 48 hr after injection.
[b] Renal clearances estimated by the formula: Clearance = UV/P, where U = concentration of constituent in urine during 24 hr before sacrifice, V = volume of urine excreted during 24 hr before sacrifice, and P = concentration of constituent in plasma collected at time of sacrifice.
[c] Each value is the mean ± SD for five rats.
[d] $p < 0.01$, t test.
[e] $p < 0.001$, t test.

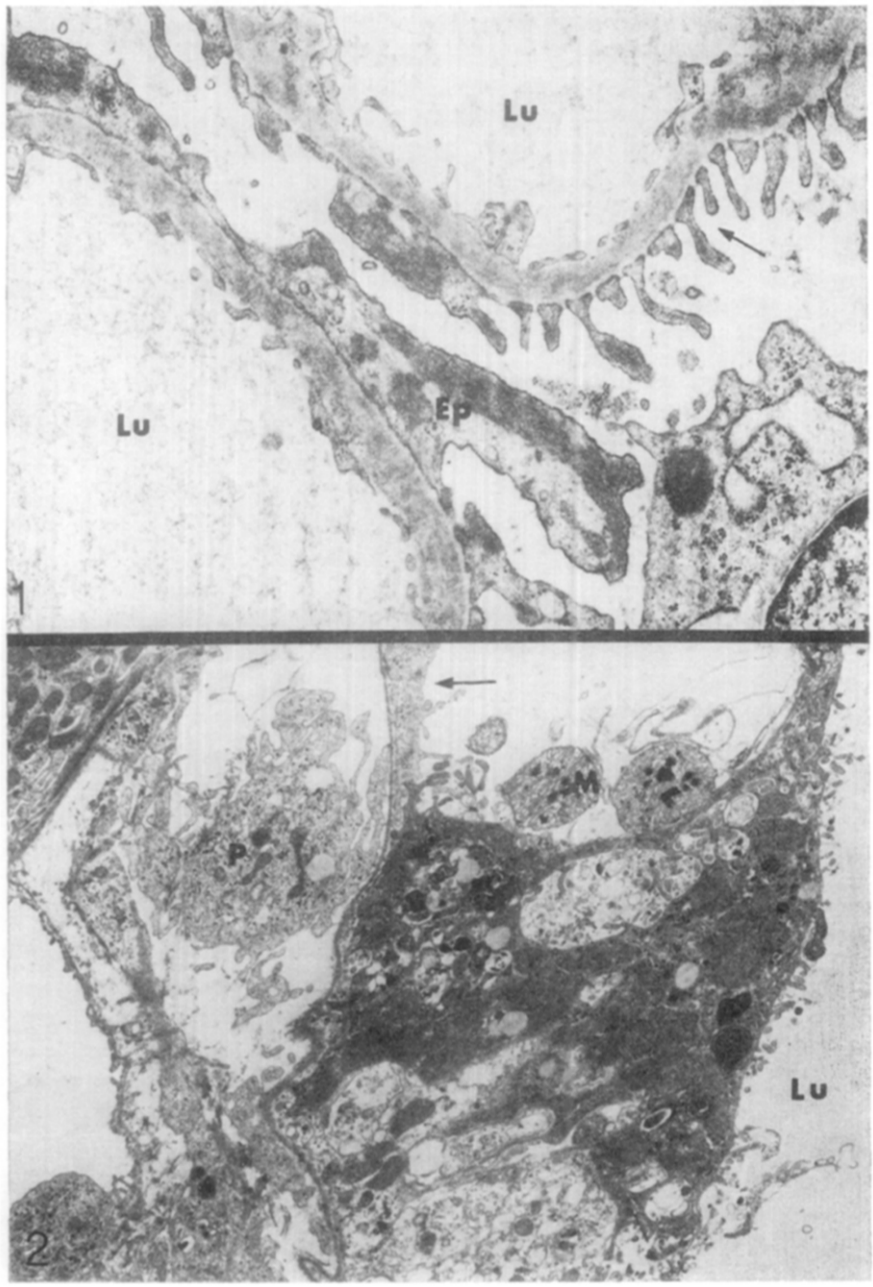

FIG. 1. Glomerular capillaries in kidney of a rat at 48 hr after ip $NiCl_2$ (68 μmol/kg). Two capillary lumens (Lu) are seen. The normal epithelial cell foot processes (arrow) of the capillary at the upper right are absent (fused) along the area of the epithelial cell process (Ep) to the lower left. Glutaraldehyde fixation; stained with uranyl acetate and lead citrate; ×18,000.

FIG. 2. Necrotic tubule in renal cortex of a rat at 48 hr after ip $NiCl_2$ (68 μmol/kg). The lumen (Lu) of the tubule is seen at lower right. Mitochondria (M) with dense matrix accumulations are seen in a vacuole in the necrotic cell at the top. There appears to be beginning regeneration (arrow). A platelet (P) is seen in the capillary at the left. Glutaraldehyde fixation; stained with uranyl acetate and lead citrate; ×7300.

urine osmolality and in urinary excretions of sodium, potassium, calcium, and chloride. Measurements of plasma electrolyte concentrations and estimations of renal clearances of creatinine and urea were performed upon samples from five sham-treated control rats and five Ni(II)-treated rats (68 μmol/kg). As specified in Table 4, the most significant finding was a marked reduction in the renal clearance of urea ($p < 0.001$).

Electron microscopy consistently revealed fusion of the foot processes of epithelial cells in renal glomeruli from all of the rats which were killed at 48 hr after ip injection of Ni(II) in dosage of 68 μmol/kg (Fig. 1). This focal lesion was not seen in glomeruli from any of the control rats or from the rats which were killed at 24 or 96 hr after an ip injection of Ni(II). Pathologic alterations of the renal tubules were found in one rat which was killed at 48 hr after ip injection of Ni(II). In this rat, focal necrosis of tubular epithelial cells was present in several blocks of cortical and medullary tissue (Fig. 2.) The necrotic segment of the renal tubule could not be identified, owing to the advanced degenerative changes.

DISCUSSION

The toxicology of nickel compounds has been reviewed recently (Sunderman and Coulston, 1975). Review of the literature has disclosed only a few previous reports of nephrotoxic effects of nickel compounds. Poisoning of dogs and cats by nickel nitrate was reported to produce acute renal injury with proteinuria and hyaline casts (Azary, 1879). Inhalation or iv injection of nickel carbonyl was found to cause transient azotemia, with pathological lesions of the renal glomeruli and tubules (Kincaid *et al.*, 1953; Sunderman *et al.*, 1961; Hackett and Sunderman, 1967). Proteinuria was noted in a few workmen who were accidently poisoned with nickel carbonyl (Brandes, 1934; Carmichael, 1953). In an unpublished study,[8] proteinuria was detected in persons who chronically drank water from a well which was contaminated with Ni(II).

In the present study, Ni(II) caused proteinuria after ip administration to rats at a dosage (34 μmol/kg) insufficient to produce aminoaciduria. The proteinuria probably was the result of glomerular injury and may be a biochemical correlate of fusion of foot processes of glomerular epithelial cells. At a higher dosage of Ni(II) (51 μmol/kg), significant histidinuria developed without any increase in the urinary excretions of other amino acids. This finding is compatible with the suggestion (van Soestbergen and Sunderman, 1972) that a Ni–histidine chelate may be one of several ultrafiltrable complexes which are involved in the renal excretion of Ni(II). At dosages of 68 and 85 μmol/kg, Ni(II) caused greatly increased excretions of most α-amino acids, presumably mediated by inhibition of amino acid transport systems located on luminal and/or peritubular membranes of the renal tubules (Foulkes and Gieske, 1973). No increase was found in the urinary excretion of aspartic acid, or of substituted α-amino acids [1-methylhistidine, 3-methylhistidine, and sarcosine (*N*-methylglycine)] and β- or γ-amino acids (β-alanine, γ-aminobutyric acid, and taurine). The consistent failure of Ni(II) to increase the urinary excretion of aspartic acid is particularly noteworthy and unexpected, since aspartic acid is believed to be reabsorbed by renal tubules of mammals via the same transport system as glutamic acid (Webber, 1963).

[8] Personal communication to F. William Sunderman, Jr., from Richard Gadsden, M.D., Charlestown, S.C.

Acute nephropathy has been produced in rodents after a single injection of uranyl or mercuric salts (Foulkes, 1971; Nomiyama *et al.*, 1974; Wada *et al.*, 1974; Wright and Plummer, 1974; Ganote *et al.*, 1975). The nephropathy which is produced in rodents by cadmium salts requires prolonged oral administration or multiple daily injections (Axelsson and Piscator, 1966; Berry, 1972; Itokawa *et al.*, 1974; Nomiyama *et al.*, 1975), unless the cadmium salt is injected in conjunction with mercaptoethanol (Gieske and Foulkes, 1974; Foulkes, 1974). Goyer *et al.* (1970) produced nephropathy with aminoaciduria in rats which received a diet containing 1% lead acetate for 10 weeks. The lead-induced aminoaciduria was only moderately severe and was manifested by a two- or threefold increase in urinary excretion of most α-amino acids. The pathogenesis of metal-induced nephropathies has been described by Oken (1972), Schreiner (1972), Hirsch (1973), Foulkes and Gieske (1973), Foulkes (1974), and Maher (1975).

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