THE RENAL CLEARANCE OF NICKEL IN MAN

Implications for Biological Monitoring

BY

WILLIAM EDWARD SANFORD

A THESIS
Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree
Doctor of Philosophy

University of Surrey
November, 1987
To Kathy

Who made all this possible!
DOCTOR OF PHILOSOPHY (1988)  
(Toxicology)  
University of Surrey  
Surrey, England

TITLE:  
THE RENAL CLEARANCE OF NICKEL IN MAN  
Implications for Biological Monitoring

AUTHOR:  
William Edward Sanford

SUPERVISORS:  
Dr. Evert Nieboer (McMaster University)  
and  
Dr. Brian Stace (University of Surrey)

NUMBER OF PAGES: xxvi, 378
ABSTRACT

The work described in this thesis examines the renal excretion of nickel in humans and the Wistar Albino male rat. The renal clearance of nickel is characterized in two groups of electrolytic nickel-refinery workers. In the animal work, the mechanisms of nickel uptake in kidney tissue and its pathological consequences are investigated.

Multi-void 24-h urine collections were obtained from 26 workers, as well as serum samples at the beginning and end of this sampling period. Nickel, β₂-microglobulin and creatinine concentrations were measured in both body fluids. In addition, specific gravity, protein and qualitative indices of kidney dysfunction (by Dip Stick) were assessed in urine. Examination of the functional dependence in individuals of urinary nickel, creatinine and specific gravity on urine flow-rate indicates that specific gravity adjustment of spot-nickel voids is more appropriate than employing creatinine. The systematic strategy devised in this study to overcome concentration-dilution effects for nickel has wide application in biological monitoring. It is demonstrated that if the specific gravity values of spot urine voids in a group of 20 individuals are between 1.010 and 1.039, then the uncompensated uncertainty in specific-gravity adjusted urinary-nickel concentrations does not exceed ± 10% (95% Confidence Level). Nickel clearance
studies and the determination of nickel in serum ultrafiltrates indicated that 24 ± 6% of serum nickel is available for renal filtration, of which 65% on average is reabsorbed in the human kidney. It is concluded for the nickel-refinery workers studied, that there was little evidence of kidney dysfunction.

Studies with rat renal slices and isolated proximal tubules showed that the uptake of Ni$^{2+}$ and its histidine complexes is probably passive. Ni(His)$_2$ reduced the uptake of L-histidine and proline, but not of thymidine. The renal accumulation of nickel from nontoxic i.v. doses of $^{63}$Ni(His)$_2$ (6 µg Ni kg$^{-1}$) was followed by rapid subcellular clearance during the 24-h period after injection. Autoradiography of the nephron illustrated that only the S3 segment of the proximal tubule accumulated and retained nickel. There was no evidence of nephrotoxicity in the histopathological examination of these tissues. By contrast, high i.p. doses (3 and 6 mg Ni kg$^{-1}$) induced a decrease in Bowman's space and minor changes along the entire length of affected nephrons. Compartmentalization of nickel within subcellular fractions of the rat kidney is interpreted in terms of an "Equilibrium" model for metal-ion uptake under steady state conditions (i.e., at fixed pH, redox potential, intracellular and extracellular ligand concentrations). Effectively, nickel distribution is determined by thermodynamic parameters such as complex stability.
The human and animal evidence support a passive mechanism of nickel reabsorption in the kidney. Simple pulmonary deposition and absorption considerations in man suggest that the dose of nickel acquired during an 8-h shift of work at a Threshold Limit Value (TLV) of 0.1 mg Ni m\(^{-3}\) is roughly equivalent to the 6 \(\mu g\) Ni kg\(^{-1}\) dose used in the rat study. Therefore, the absence of signs of significant nephrotoxicity in both the human and animal models appears to be consistent.
ACKNOWLEDGEMENTS

I would like to thank Dr. Nieboer and Dr. Stace for their guidance, understanding, help and patience. I am grateful for the help and encouragement from my friends in lab 3H43 at McMaster University. In particular, I would like to thank Mr. A. Jusys for his guidance in the analytical skills of electrothermal atomic absorption spectroscopy (EAAS) and assistance with the Port Colborne collections, and Mr. P. Stetsko for his assistance with the serum and urine collections made for both the Port Colborne and Thompson cohorts. I would also like to acknowledge the support of the Occupational Health Program at McMaster University; in particular Dr. D. C. F. Muir for creating my initial interest in Industrial Toxicology. I would also like to thank Dr. P. Bach, Dr. M. Dobrota, Dr. M. Taylor, N. Gregg and C. Ketley and all my friends at the Robens Institute of Industrial and Environmental Health and Safety at the University of Surrey for making my sabbatical work in England very interesting and introducing me to in vivo and in vitro methods of investigating nephrotoxicity.

The financial support of the Nickel Producers and Environmental Research Association (NiPERA) is gratefully acknowledged. The support and cooperation of the International Nickel Company (INCO) personnel at Port Colborne and Thompson is also gratefully appreciated; in particular the assistance of T. Fregren (Port Colborne) and T. Farrell (Thompson).
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APDC</td>
<td>Ammonium pyrrolidinedithiocarbamate</td>
</tr>
<tr>
<td>BHK</td>
<td>Baby hamster kidney cells</td>
</tr>
<tr>
<td>CD</td>
<td>Collecting duct</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary cells</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>cpm</td>
<td>Counts per minute</td>
</tr>
<tr>
<td>Cys</td>
<td>Cysteine</td>
</tr>
<tr>
<td>CYT</td>
<td>Cytosol</td>
</tr>
<tr>
<td>DD</td>
<td>Double distilled</td>
</tr>
<tr>
<td>DDC</td>
<td>Diethyldithiocarbamate</td>
</tr>
<tr>
<td>DDW</td>
<td>Ultra-pure water</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNP</td>
<td>2,4-Dinitrophenol</td>
</tr>
<tr>
<td>D-PEN</td>
<td>D-penicillamine</td>
</tr>
<tr>
<td>DT</td>
<td>Distal tubule</td>
</tr>
<tr>
<td>EAAS</td>
<td>Electrothermal atomic absorption spectrometry</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetra-acetic acid</td>
</tr>
<tr>
<td>G.I.</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GL</td>
<td>Glomerulus</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-2-Hydroxyethylpiperazine-N´-2-ethanesulfonic acid</td>
</tr>
<tr>
<td>HMM</td>
<td>High molecular mass</td>
</tr>
<tr>
<td>HSA</td>
<td>Human serum albumin</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>i.m.</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>INCO</td>
<td>International Nickel Company</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraparenteral</td>
</tr>
<tr>
<td>i.r.</td>
<td>Intrarenal</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
</tr>
<tr>
<td>LMM</td>
<td>Low molecular mass</td>
</tr>
<tr>
<td>MIBK</td>
<td>4-Methylpentane-2-one (Methylisobutyl ketone)</td>
</tr>
<tr>
<td>MIC</td>
<td>Microsome</td>
</tr>
<tr>
<td>ML</td>
<td>Mitochondria/lysosome</td>
</tr>
<tr>
<td>N</td>
<td>Nuclear</td>
</tr>
<tr>
<td>NAG</td>
<td>N-acetyl-D-glucosaminidase</td>
</tr>
<tr>
<td>P3</td>
<td>Luminal and basolateral membrane vesicle pellet</td>
</tr>
<tr>
<td>PAH</td>
<td>p-Aminohippuric acid</td>
</tr>
<tr>
<td>PE</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PMSF</td>
<td>Phenylmethylsulfonyl fluoride</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RPN</td>
<td>Renal papillary necrosis</td>
</tr>
<tr>
<td>s.c.</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SHE</td>
<td>Syrian hamster embryo cells</td>
</tr>
<tr>
<td>S1</td>
<td>S1 segment of the proximal tubule</td>
</tr>
<tr>
<td>S2</td>
<td>S2 segment of the proximal tubule</td>
</tr>
<tr>
<td>S3</td>
<td>S3 segment of the proximal tubule</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descriptive Note</td>
<td>ii</td>
</tr>
<tr>
<td>Abstract</td>
<td>iii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>vi</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xvii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xx</td>
</tr>
</tbody>
</table>

## CHAPTER 1 GENERAL INTRODUCTION

A Preface                                                            1

B Sources, Production and Uses of Nickel                            2
   (i) Sources                                                      2
   (ii) Production                                                   4
   (iii) Uses                                                       5

C Nickel Toxicology                                                 6
   (i) Nickel Essentiality                                          6
   (ii) Absorption Distribution and Excretion                      9
      (a) Absorption                                                9
      (b) Distribution                                              11
      (c) Excretion                                                  15
   (iii) Toxic Effects of Nickel Compounds                         18
      (a) Historical Aspects                                       18
      (b) Effects on the General Population                       20
         Nickel dermatitis                                          20
         Other Health Effects                                       23
      (c) Industrial Exposure                                      23
Nickel Carbonyl (Ni(CO)₄) 23
Cancer 30
Nickel Induced Asthma 38
(d) Biochemistry of Nickel
(Including Determinants of Reactivity) 40
Introduction 40
Absorption 40
Cellular Uptake 42
Biological Role of the Nickel(II) Ion 44
Reactivity of the Nickel(II) Ion 45
Complex Formation 48
Geometric Preferences 51
(e) Other Toxic Effects of Nickel 56
Reproductive and Developmental Effects of Nickel 56
(f) Nephrotoxic Effects of Nickel 56

CHAPTER 2 A SYSTEMATIC APPROACH TO ADJUSTMENT OF URINARY NICKEL CONCENTRATIONS FOR RATES OF URINE FLOW
A Introduction 59
B Materials and Methods 65
(i) Materials 65
(a) Chemical Reagents 65
(b) Laboratory Solutions
(Auto Analyzer) 66
Sodium Chloride (0.9% w/v) 66
Sodium Hydroxide (0.5 M) 66
Saturated Picric Acid 66
Stock Creatinine Standard (1 mg/mL) 66
(c) Laboratory Solutions
(Nickel Determination)

  Potassium Phosphate Buffer 66
  Bromothymol Blue Indicator Solution 69
  APDC (2% w/v) 69
  Stock Nickel Standard (1000 mg/L) 69
  Nickel Intermediate Standard (1 mg/mL) 69

(d) Decontamination of Plastic and Glasswares 70

(ii) Donor Populations 72
  (a) Nickel 72
  (b) Lead 73

(iii) Methods 73
  (a) Urine Collection Procedures 73
  (b) Serum Collection Procedure 76
  (c) Determination of Urinary Nickel 77
  (d) Determination of Urinary Lead 78
  (e) Determination of Serum Nickel 81
  (f) Determination of Serum and Urinary Creatinine 82
  (g) Determination of Specific Gravity 84
  (h) Determination of Osmolality 84

C Results 85

(i) Nickel Concentrations In Body Fluids 85
  (a) Urinary Nickel 85
  (b) Serum Nickel 85
  (c) Urinary Nickel 85

(ii) 24-h Collections 86
### D Discussion

(i) Current Practices of Urine Adjustments and their Limitations 124

(ii) Theoretical Considerations 128

(iii) Creatinine Normalization 132

(iv) Specific Gravity Normalization 138

(v) Biological Monitoring 141

(a) Definition, Uses and Practices 141

(b) Need for Urine-Flow Corrections 143

(c) Standardization to Unitary Flow Rate 146

---

### CHAPTER 3 RENAL CLEARANCE OF NICKEL AND BIOCHEMICAL INDICES OF KIDNEY FUNCTION IN ELECTROLYTIC REFINERY WORKERS

A Introduction 162

B Theoretical Considerations of Renal Clearance 165

(i) Introduction 165

(ii) Fractional Clearance 168

(iii) Serum/Urine Relationship 170

C Review of Laboratory Methods for Serum Creatinine Determination 170

D Materials and Methods 174

(i) Materials 174

(a) Chemical Reagents 174

(b) Laboratory Solutions (Auto Analyzer) 174

(c) Laboratory Solutions (Nickel Determination) 174

(d) Laboratory Solutions (Total Protein Determination) 174

Veronal Buffer (pH = 7.4) 174
Stock Total Protein Standard 174
(4.5 mg/mL)

(e) Decontamination of Plastic and glassware 175

(ii) Donor Populations 175

(iii) Methods 176

(a) Urine Collection Procedures 176
(b) Serum Collection Procedure 176
(c) Determination of Serum and Urinary Nickel 178
(d) Determination of Serum and Urinary Creatinine 178
(e) Determination of Specific Gravity 178
(f) Determination of Osmolality 178
(g) Determination of Urinary Parameters by Dip-Sticks 178
(h) Determination of Total Protein 179
(i) Determination of $\alpha_{Ni}$ 180
(j) Determination of $pH$ 181
(k) Determination of $\beta_2$-microglobulin 181

E Results 188

(i) Urinary $\beta_2$-microglobulin 188
(ii) Biochemical Indices 188
(iii) Nickel Clearance and $\alpha_{Ni}$ 195
(iv) Urinary Osmolality 198
(v) Effect of Exposure on $U_{Ni}^c$ 204

F Discussion 204

(i) Biochemical Indices of Kidney Function 204

-xiii-
CHAPTER 4 UPTAKE AND DISTRIBUTION OF NICKEL IN THE RAT KIDNEY

A Introduction  
(i) Renal Toxicity of Metals  
(ii) Experimental Approaches to Nephrotoxicity

B Materials and Methods  
(i) Materials  
(a) Chemical Reagents  
(b) Nickel-injection Solutions  
(c) Laboratory Solutions (Renal Slices)  
   HEPES Incubation Medium  
   (pH = 7.4 and 5.5)  
(d) Preparation of Labelled Solutions  
   $^{63}$Ni$^{2+}$ (100 μg Ni L$^{-1}$)  
   Histidine  
   $^{63}$Ni(His)$_2$ (100 μg Ni L$^{-1}$)  
   $^{63}$Ni(His)$_1$ (100 μg Ni L$^{-1}$)  
   $^{3}$H-PAH (0.25 mM)  
(e) Preparation of Unlabelled Solutions  
   Probenecid (0.04 M)
2,4-Dinitrophenol (DNP, 20 mM) 243
Na/K ATP-ase 243
Alkaline Phosphatase 244

(ii) Methods 245
(a) NiCl₂ Injection Study 245
Injection Protocol 245
Histology 245
(b) Uptake of Nickel in Renal Slices 245
(c) Isolation of Glomeruli and Tubules 250
(d) Simultaneous Isolation of Basolateral and Brush-Border Membranes 252
(e) Intravenous Injection Experiments 255
(f) Incubation with Subcellular Fractions 258
(g) Autoradiography 258
(h) Cation Column Chromatography 263

C Results 264
(i) NiCl₂ Injection Study 264
(a) Post Injection Status 264
(b) Solid and Liquid Balance 264
(c) Histology 268
(ii) Uptake of Nickel in Renal Slices 276
(iii) Studies with Isolated Proximal Tubules 285
(iv) Intravenous Injection Experiments 285
(v) Cation Column Chromatography 296

D Discussion 301
(i) NiCl₂ Injection Study 301
(a) Post-Injection Status 301
(b) Relationship between Physiological Function and Structural Changes Induced by Nickel

(ii) Uptake of Nickel in Renal Slices
(a) Comments on Experimental Procedures
(b) Interpretation of Results

(iii) Uptake of Nickel with Isolated Proximal Tubules

(iv) Renal Handling of iv Administered Ni(His)$_2$
(a) Association of Nickel with Soluble Intracellular Components
(b) Mechanism of Uptake
(c) Tissue Distribution of Nickel
(d) Distribution of Ni in Different Regions of the Nephron
(e) Serum and Intracellular Distribution of Ni
(f) Relationship Between Distribution and Toxicity

CHAPTER 5 GENERAL DISCUSSION AND CONCLUSIONS

A Renal Clearance of Nickel(II) and Implications for Biological Monitoring

B Absorption and Toxic Effects of Nickel in Rat Kidney

C Kidney Function Status in Electrolytic Refinery Workers

REFERENCES
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Replacement of essential metal ions by Ni(^{2+}).</td>
<td>49</td>
</tr>
<tr>
<td>2.1</td>
<td>Chemical reagents.</td>
<td>67</td>
</tr>
<tr>
<td>2.2</td>
<td>Plasticware.</td>
<td>71</td>
</tr>
<tr>
<td>2.3</td>
<td>24-h urine collection record.</td>
<td>74</td>
</tr>
<tr>
<td>2.4</td>
<td>Instructions for 24-h urine collection.</td>
<td>75</td>
</tr>
<tr>
<td>2.5</td>
<td>Instrumental parameters and settings for Ni analysis by EAAS.</td>
<td>79</td>
</tr>
<tr>
<td>2.6</td>
<td>Instrumental parameters and settings for Pb analysis by EASS.</td>
<td>80</td>
</tr>
<tr>
<td>2.7</td>
<td>Volume exponents ((b_1)) and urinary concentrations ((U_i^0)) at unit urine flow rates.</td>
<td>101</td>
</tr>
<tr>
<td>2.8</td>
<td>Observed flow rates.</td>
<td>111</td>
</tr>
<tr>
<td>2.9</td>
<td>Experimental relationships observed for creatinine normalized nickel in body fluids.</td>
<td>134</td>
</tr>
<tr>
<td>2.10</td>
<td>Assessment of the flow-rate power coefficient (b_1) for creatinine, (\rho)-1 and nickel, and the corresponding (\Delta b) values.</td>
<td>149</td>
</tr>
<tr>
<td>2.11</td>
<td>Boundary values of selected urinary parameters corresponding to (V^{\Delta b} = \pm 0.1) for selected (\Delta b) values.</td>
<td>152</td>
</tr>
<tr>
<td>2.12</td>
<td>Boundary values of selected urinary parameters corresponding to (V^{\Delta b} = \pm 0.2) for selected (\Delta b) values.</td>
<td>153</td>
</tr>
<tr>
<td>3.1</td>
<td>Job description of workers</td>
<td>177</td>
</tr>
<tr>
<td>3.2</td>
<td>(\beta_2)-Microglobulin radioimmunoassay reagents and assay protocols.</td>
<td>185</td>
</tr>
<tr>
<td>3.3</td>
<td>Laboratory test results (Port Colborne study group).</td>
<td>189</td>
</tr>
<tr>
<td>3.4</td>
<td>Dip-stick test results for urine samples (Port Colborne study group).</td>
<td>191</td>
</tr>
</tbody>
</table>
3.5 Laboratory test results
(Thompson study group).

3.6 Dip-stick test results for urine samples
(Thompson study group)

3.7 Clearance summary

3.8 Prediction of creatinine clearance

3.9 Biochemical indicies of kidney function
(Donors with 24-h flowrate less than
0.6 mL min\(^{-1}\) or unusually low creatinine
clearances)

3.10 Assessment of the urine flow-rate power
coefficient \(b_i\) for creatinine, and
nickel, and the corresponding
\(\Delta b\) values.

3.11 Estimate of current exposure \(\Delta U_{Ni,\max}\)
in the Thompson cohort in relation to job
classification.

4.1 Chemical reagents and diet.

4.2 Radiolabelled reagents and scintillation
liquid employed.

4.3 Equipment, plasticware and glassware
employed.

4.4 Slice/medium (s/m) ratio normalized
uptake data.

4.5 First-order kinetic analysis of PAH
and nickel \textit{in vitro} efflux from rat
renal slices.

4.6 Distribution of \(^{63}\)Ni determined by
scintillation spectrometry on subcellular
fractions after i.v. injection of \(^{63}\)Ni(His)\(_2\)
or after \textit{in vitro} incubation of subcellular
fractions with \(^{63}\)Ni\(^{2+}\) or \(^{63}\)Ni(His)\(_2\).

4.7 Cytosol profile.
4.8 Percent of injected dose in tissues and body fluids following a 6 μg Ni kg\(^{-1}\) iv injection of \(^{63}\)Ni(His)\(_2\).

4.9 Tissue concentration of \(^{63}\)Nickel in the rat 10 min and 24-h following a 6 μg Ni/kg i.v. injection of \(^{63}\)Ni(His)\(_2\).

4.10 Quantitative assessment of \(^{63}\)Ni in selected kidney sections by autoradiography after i.v. injection of \(^{63}\)Ni(his)\(_2\).

4.11 Effect of parenteral NiCl\(_2\) upon excretion of total protein and amino acids.

4.12 Distribution of \(^{63}\)Ni determined by scintillation spectrometry on subcellular fractions after i.v. injection or in vitro incubation of subcellular fractions.

4.13 Renal subcellular fractionation studies.
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>46</td>
</tr>
<tr>
<td>1.2</td>
<td>47</td>
</tr>
<tr>
<td>1.3</td>
<td>52</td>
</tr>
<tr>
<td>2.1</td>
<td>61</td>
</tr>
<tr>
<td>2.2</td>
<td>63</td>
</tr>
<tr>
<td>2.3</td>
<td>83</td>
</tr>
<tr>
<td>2.4</td>
<td>87</td>
</tr>
<tr>
<td>2.5</td>
<td>88</td>
</tr>
<tr>
<td>2.6</td>
<td>89</td>
</tr>
<tr>
<td>2.7</td>
<td>90</td>
</tr>
</tbody>
</table>

1.1 Major biological roles of metals.

1.2 Classification of metal ions according to binding preferences.

1.3 Proposed structure of the nickel-transport site of human albumin.

2.1 Nickel concentrations in urine samples from a worker accidentally exposed to nickel carbonyl.

2.2 General flowchart illustrating the principles of secondary refining by the electrolytic processes electrorefining and electrowinning.

2.3 Continuous-flow analyzer manifold employed in creatinine analysis.

2.4 Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the day work shift for the Port Colborne collection which started at 7:00 am and ended at 3:00 pm.

2.5 Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the day work shift for the Thompson collection which started at 7:00 am and ended at 3:00 pm.

2.6 Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the afternoon work shift for the Thompson collection which started at 3:00 pm and ended at 11:00 pm.

2.7 Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the midnight work shift for the Thompson collection which started at 11:00 pm and ended at 7:00 am.
2.8 A scatter diagram showing the linear relationship between log(U_Ni) and log(V) for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure (p<0.005).

2.9 A scatter diagram showing the linear relationship between log(U_{creat}) and log(V) for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure (p<0.001).

2.10 A scatter diagram showing the linear relationship between log(U_{ρ-1}) and log(V) for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure (p<0.001).

2.11 A scatter diagram showing the linear relationship between log(U_{pb}) and log(V) for one donor. The regression parameters are indicated in the figure (p<0.001).

2.12 A scatter diagram showing the linear relationship between log(U_{osmol}) and log(V) for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure (p<0.001).

2.13 Dependence of the magnitude of the volume power coefficient b_i on donor.

2.14 Linearization of the b_i values represented in Figure 2.13.

2.15 Linearization of b_{NI} values.

2.16 Linearization of b_{ρ-1} values

2.17 Distribution of urine flow rates for individual voids corresponding to the Port Colborne and Thompson collections (210 voids).

2.18 Distribution of urine flow rates for 24-h collections corresponding to the Port Colborne and Thompson collections (n = 32).
2.19 A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\text{creat}}$ for selected donors in the Thompson cohort.

2.20 A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\text{creat}}$ for selected donors in the Thompson $(C_T, K_T, F_T)$ and Port Colborne $(E_P)$ cohorts.

2.21 A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\text{creat}}$ for selected donors in the Thompson cohort.

2.22 A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\rho -1}$ for selected donors in the Thompson cohort.

2.23 A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\rho -1}$ for selected donors in the Thompson $(C_T, K_T, F_T)$ and Port Colborne $(E_P)$ cohorts.

2.24 A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\rho -1}$ for selected donors in the Thompson cohort.

2.25 A scatter diagram showing the relationship between $U_{Ni}/U_{\text{creat}}$ and $V$ for selected donors in the Thompson cohort.

2.26 A scatter diagram showing the relationship between $U_{Ni}/U_{\text{creat}}$ and $V$ for selected donors in the Thompson $(C_T, K_T, F_T)$ and Port Colborne $(E_P)$ cohorts.

2.27 A scatter diagram showing the relationship between $U_{Ni}/U_{\rho -1}$ and $V$ for selected donors in the Thompson cohort.

2.28 A scatter diagram showing the relationship between $U_{Ni}/U_{\rho -1}$ and $V$ for selected donors in the Thompson cohort.
2.29 A scatter diagram showing the relationship between $U_{Ni}/U_{\text{creat}}$ and $V$ for selected donors in the Thompson ($C_T$, $K_T$, $F_T$) and Port Colborne ($E_p$) cohorts.

2.30 A scatter diagram showing the relationship between $U_{Ni}/U_{\rho_1}$ and $V$ for selected donors in the Thompson cohort.

2.31 A scatter diagram showing the linear relationships between $U_{Ni}/U_{\text{creat}}$ and $U_{Ni}$ corresponding to 28 of the 32 donors.

2.32 Classification of $U_{Ni}/U_{\text{creat}}$ according to the urinary concentration factor ($U_{\text{creat}}/S_{\text{creat}}$). Data corresponds to 24-h urine collections in Figure 3.31.

2.33 A scatter diagram showing the relationship between $U_{Ni}/U_{\text{creat}}$ and nickel excretion ($E_{Ni}$). Data corresponds to the 24-h collections in Figures 2.31 and 2.32.

2.34 A scatter diagram showing the relationship between $U_{Ni}/U_{\rho_1}$ and nickel $U_{Ni}$ excretion ($E_{Ni}$). Data corresponds to the 24-h collections in Figures 2.31 and 2.32.

2.35 A scatter diagram showing the relationship between $U_{Ni}/U_{\rho_1}$ and nickel excretion ($E_{Ni}$). Data corresponds to the 24-h collections in Figure 2.31.

2.36 Correlation graphs of nickel concentrations in urine specimens from five non-exposed, healthy, adult men, versus urine creatinine concentrations and urine specific gravity measurements.

2.37 A composite diagram showing three possible linear relationships between $U_{Ni}/U_{\text{creat}}$ and $U_{Ni}$.
2.38 The family of curves for the uncompensated residual flow factor ($V^{\Delta b}$) at various flow rates ($V$) and selected values of the power coefficient difference $\Delta b$.

2.39 The boundary flow rates for various $\Delta b$ values at which the uncompensated residual flow factor ($V^{\Delta b}$) deviates $\pm$ 10% or $\pm$ 20% from unity.

3.1 Urinary nickel concentrations (adjusted to a specific gravity of 1.018) for subject A during a work week.

3.2 Fractional clearance of nickel(%) as a function of urinary flow rate.

3.3 Fractional clearance of nickel(%) as a function of urinary flow rate.

3.4 Relationship between serum nickel concentrations and nickel excretion ($E_{Ni}$).

3.5 Observed relationship between specific gravity of spot urine samples and corresponding osmolality for three donors with a total of 31 voids for the Port Colborne study group.

3.6 Fluctuations in standardized urine nickel concentrations ($U^o_{Ni}$) for a nickel electrolytic refinery work.

3.7 Fluctuations in standardized urine nickel concentrations ($U^o_{Ni}$) for a nickel electrolytic refinery worker (donor $A_{PC}$) during two 24-h multi-void collections (Port Colborne cohort).

3.8 Fluctuations in standardized urine nickel concentrations for a nickel electrolytic refinery worker (donor $A_{T}$) during a 24-h multi-void collection.

3.9 Fluctuations in standardized urine nickel concentrations for a nickel electrolytic refinery worker (donor $E_T$) during a 24-h multi-void collection.
3.10 Fluctuations in standardized urine nickel concentrations for a nickel electrolytic refinery worker (donor N_f) during a 24-h multi-void collection.

4.1 Diagram illustrating the lines along which surgical cuts were made on the excised kidney.

4.2 Dietary and excretion parameters of male Wistar Albino rats, housed in metabolic cages measured before and after i.p. injections of NiCl_2.

4.3 Photomicrographs of kidney tissue from rats 48-h after i.p. injections of NiCl_2.

4.4 The accumulation of PAH in renal tissue slices, measured by the slice/medium (s/m) ratio over a 4-h incubation period.

4.5 The accumulation of 100 \( \mu g \ L^{-1} \) Ni^{2+} in renal tissue slices, measured by the slice/medium (s/m) ratio over a 4-h incubation period.

4.6 The accumulation of \( ^{63}\text{Ni}^{2+} \), \( ^{63}\text{Ni}(\text{His})_{2} \) and \(^{3}\text{H}-\text{PAH} \) in renal tissue slices, measured by the slice/medium (s/m) ratio over a 2-h incubation period.

4.7 The total efflux of PAH and \(^{63}\text{Ni}^{2+} \) from tissue expressed as a percent of that accumulated during a 2-h incubation.

4.8 (A) Time course of \( ^{63}\text{Ni} \) in subcellular fractions after i.v. injection of \( ^{63}\text{Ni}(\text{His})_{2} \) (6 \( \mu g \) Ni kg\(^{-1} \)).
(B) Distribution of \(^{63}\text{Ni} \) in subcellular fractions incubated for 2-h (25\(^{\circ}\)C) with \( ^{63}\text{Ni}^{2+} \) or \( ^{63}\text{Ni}(\text{His})_{2} \) (200 \( \mu g \) Ni L\(^{-1} \)).

4.9 G-75 Molecular-mass profiles of kidney-cell cytosol and authentic \(^{63}\text{Ni}(\text{His})_{2} \).
4.10 Autoradiography of kidney sections, after $^{63}\text{Ni(His)}_2$ i.v. injection, illustrating structure and grains developed. The regions outlined represent examples of the masking of specific nephron segments identified and selected for counting on the Quantimet 920 image analyzer.

4.11 Time-course distribution of nickel along the nephron identified by $^{63}\text{Ni}$ autoradiography and quantified by image analysis of grains using the Quantimet 920 analyzer.

4.12 "Equilibrium" model for the transport of nickel across a biological membrane.
CHAPTER 1 GENERAL INTRODUCTION

A PREFACE

In this thesis, experimental evidence is presented that describes the manner of nickel excretion by the human kidney. A rationale for volume correction of spot urine samples for use in biological monitoring of nickel workers is also addressed. These human studies are extended to an investigation of the uptake of nickel(II) salts and nickel (II)-histidine complexes in the rat kidney.

The health effects of exposure to nickel compounds may be divided into three categories: (i) nickel dermatitis; (ii) human cancers; and (iii) nickel carbonyl poisoning. These areas have been well-studied. In contrast, very little work has been done on the renal excretion of nickel in animals and humans. The objective of this thesis is to attempt to fill this void.

This thesis is divided into five chapters. The first chapter constitutes a general introduction to the sources, production, uses, toxicology and biochemistry of nickel. The second and third chapters report the results for the renal clearance and toxicity of nickel among electrolytic nickel refinery workers; empirical and theoretical treatments of adjusting spot urine nickel concentrations for urine flow rates are also presented. In Chapter 4 the uptake and distribution of nickel(II) salts and nickel(II)-histidine complexes in the rat kidney are investigated. Finally in
Chapter 5, the conclusions of Chapters 2 to 4 are used to help unify the work in this thesis, namely: the excretion of nickel(II) salts, the distribution of nickel(II)-histidine complexes within intracellular kidney compartments, and the biological residence time of nickel in animals and man.

**B SOURCES, PRODUCTION AND USES OF NICKEL**

(i) Sources.

Nickel (Ni), atomic number 28, is the 24th element in the order of abundance in the earth's crust. It is a high melting (1453°C) metal with excellent corrosion-resistance and the ability to form many alloys (Tien and Howson, 1978). It is a widely known element because of its use in coinage, but has become more important for its many domestic, industrial and military applications. Although the pure metal itself can be used in many kinds of mechanical equipment, it is more commonly employed in the form of alloys such as nickel/chromium/iron known as stainless steel (Mackenzie, 1968). Nickel is known to occur in trace quantities in many foods such as fresh oysters, 1.5 ppm; wheat, 0.0 - 6.5 ppm; rye, 2.7 ppm; black pepper, 3.9 ppm; cocoa, 5 ppm; tea, 7.6 ppm; baking powder, 13 ppm; and gelatin, 4.5 ppm; all of these values are based on fresh-weight (Schroeder et al., 1961).

The use of nickel dates from prehistoric times, when early man fashioned some implements from meteoric iron, which normally contains between 5 and 15% nickel. Nickel was
first isolated by Cronstedt in 1751 from an ore containing niccolite (NiAs). An ore of this type had earlier caused copper and silver miners in Saxony considerable trouble because, although it resembled copper in colour, it yielded a brittle unfamiliar product. They referred to it as "kupfernickel", after "Old Nick" and his mischievous gnomes. Thus Cronstedt named this new element after this historical name. It was not until 1775 when Bergman first produced nickel in a fairly pure state that Cronstedt's work was widely accepted (Mackenzie, 1968; Mastromatteo, 1986).

The most important sources of nickel in Canada, and to a lesser extent in Finland, are the mixed sulfide ores (pentlandite, (Fe,Ni)S; nickel-bearing pyrrhotite, Fe₅S₆ to Fe₁₆S₁₇; and nickel-bearing chalcopyrite, CuFeS₂). Minor deposits of these sulfide ores can also be found in Norway, China, India and the United States. The oxide ores, e.g. hydrous magnesium silicates, garnierite, (Ni,Mg)SiO₃·nH₂O and nickel-bearing iron oxide (laterite), are becoming more important sources of this metal. The oxide ores are found in New Caledonia, United States, Cuba, Phillipines, Indonesia, Brazil and Venezuela (Boldt, 1967). However, with the depressed market for nickel in the last few years, many of the projects in these countries have been severely restricted or temporarily terminated.
(ii) Production.

The refining processes of the nickel-bearing sulfide ores found in Canada have the following main stages (Morgan, 1979):

(a) Beneficiation. The ore is crushed and the sulfide content is separated by mechanical, magnetic and flotation processes.

(b) Pyrometallurgy. The ore concentrates from the previous step are passed to a furnace and allowed to burn with the release of \( \text{SO}_2 \) (roasting). Various grades of nickel-containing material can be placed into a furnace to melt the roasted concentrates (smelting). The molten material separates into an iron-rich silicate slag and a heavier sulfur-rich liquid called "matte". The liquid matte can be further treated with oxygen. Silicious material is added to remove the iron and much of the remaining sulfur. The separation of nickel and copper sulfides is carried out mainly by controlling slow cooling, or leaching principles (matte separation). After separation, the sulfides are then roasted to oxides. The nickel oxide may be sold directly or be reduced to metal and refined by the carbonyl or electrolytic process (Morgan, 1979). Details of these secondary refining
processes are discussed later.

(c) Hydrometallurgy/Leaching. This process may be used as an alternative to roasting methods. The ore (sulfide or laterite) or the metal concentrate is leached to take nickel and other metals into aqueous solution. The metals are then selectively precipitated (Duke, 1980). Ammonia is used in a pressure leaching method. Iron forms insoluble ferric hydroxide, whereas nickel, copper and cobalt are taken into solution as amine complexes. The so-called "pregnant" leach solution is heated to drive off the ammonia and the copper is precipitated as the sulfide. The remaining copper-free solution is then reduced with molecular hydrogen resulting in the precipitation of pure nickel powder (Duke, 1980).

(iii) Uses.

Nickel is extensively used in electroplating, in the manufacture of steel and other alloys and in the manufacture of electronic devices. Very finely ground nickel is also used as a catalyst in the hydrogenation of oils. Nickel is also employed in coloured glass and for colour development in the ceramic industry. Many nickel compounds are released into the atmosphere during mining, smelting and refining operations. Coal-fired power plants also release nickel
compounds into the air. Because it is not profitable to recover nickel from this type of process, such releases will result in an increase in exposure to the general population as economic and energy considerations demand more coal to be consumed (Swaine, 1980).

Da Costa, in 1883, reported on the therapeutic effects of nickel salts. For example, nickel(II) sulfate was used in the treatment of rheumatism and nickel(II) bromide in reducing the frequency of epileptic attacks.

C NICKEL TOXICOLOGY

(i) NICKEL ESSENTIALITY

The data and rationale implicating nickel as an essential trace metal in man has been summarized by Cecutti and Nieboer (1981), and is reproduced below:

(a) the occurrence of nickel in human fetal tissues;
(b) the well-defined narrow range of nickel concentration in human body fluids (e.g. whole blood and urine) imply physiological or homeostatic control;
(c) the pathophysiological fluctuations in serum concentrations of nickel that occur in certain trauma states (e.g. myocardial infarction, acute stroke and accompanying severe burns);
(d) nickel deficiency symptoms in certain vertebrate species including rats, chicks,
swine, goats and sheep (among the more common were ultrastructural changes in the liver, reduced oxidative ability along with diminished activity of other key liver enzymes and impaired intestinal absorption of iron with concurrent indications of anaemia and of changes in iron metabolism and growth retardation);

(e) a number of eukaryotic and prokaryotic organisms have nickel-dependent enzymes (e.g. urease obtained from jack bean plants has been shown to be a nickel metalloenzyme and a number of enzymes requiring nickel as a cofactor have been isolated from bacteria which have been shown to involve the Ni(III)/Ni(II) redox couple) (Thauer et al., 1980; Thomson, 1982; Xavier, 1986).

The very low dietary concentrations of nickel required for the demonstration of nickel deficiency in animals suggests that nickel deficiency in man is an unlikely problem (Cecutti and Nieboer, 1981). Thus, as pointed out by Nielsen (1984), its importance in human nutrition can only be inferred from animal studies. Extrapolation of experimental findings from animals to humans is difficult. Quite often it is necessary to link animal studies to observations that can be made in humans before the scientific community will accept a metal like nickel as essential in human nutrition. For
example, Spoerl and Kirchgessner (1977) detected an enhanced nickel retention during pregnancy of rats. The one paper that reports this trend in humans (Rubanyi et al., 1982) reports serum nickel levels in control subjects that are approximately 100 times the currently accepted concentrations for occupationally-unexposed individuals. Thus, proof that this phenomenon occurs in humans remains to be demonstrated. If further investigations are able to confirm this, or other specific biological functions of nickel, then its acceptance as an essential metal in humans would be enhanced.

Nielsen (1971) was able to show that chicks on low-nickel diets had a much higher uptake of $^{63}\text{Ni}$ into their liver, spleen, kidneys and aorta when compared to control animals. This has been interpreted as evidence for the existence of a mechanism that regulates nickel absorption according to need. For nickel to be kept in homeostasis, it is quite probable that there is a mechanism that will conserve nickel when it is excreted via the kidney. The work in this thesis may help to clarify this aspect (see Chapter 3). Absorption from food may also be expected to be regulated.

Although Nielsen (1984) considered only animal and plant studies, he concluded that: "it is reasonable to postulate that nickel is required by humans".
(ii) ABSORPTION, DISTRIBUTION AND EXCRETION

(a) Absorption.

Inhalation and ingestion via mouth and gastrointestinal tract are the major routes of nickel intake for man. Although absorption through the skin (percutaneous) does not appear to be a major source of nickel in man, it is of great importance in such disorders as nickel dermatitis.

A typical Canadian consumes about 350-580 μg/day of nickel. This wide range illustrates regional dependence (Kirkpatrick and Coffin, 1974, 1977). Despite this large intake of nickel, only 1-10% is absorbed from the gastrointestinal tract in man and animals (EPA, 1978).

The most important route of nickel uptake, especially in an industrial setting, is respiratory absorption. This has been documented for workers in nickel refineries (including nickel carbonyl plants) and electroplating shops. The average person dwelling in an urban centre is exposed to approximately 0.025 μg Ni/m³ in air. The amount of nickel inhaled annually would be about 0.2 mg, assuming that 20 m³ of air is breathed per day (Cecutti and Nieboer, 1981; Barrie, 1981).

Nickel has been found in most human tissues. However, it has been suggested that the highest concentrations of nickel occur in tissues exposed to exogeneous sources of nickel, like the lung, large and small intestines, and skin (Sumino et al., 1975). Large amounts of nickel have also
been found in the kidney, muscle and rib bone. An "average" 70 kg person appears to have a total nickel content of 0.5 mg (Bennett, 1984).

Reliable methods are now available for the detection of nickel in body fluids and tissues. Until recently the accepted normal values were: whole blood (3-7 µg/L), serum (1-5 µg/L), urine (0.7-5.2 µg/L), saliva (2.2 ± 10.2 µg/L), sweat (52 ± 36 µg/L) and feces (14.2 ± 2.2 µg/g or 258 ± 126 µg/day) in healthy, industrially-unexposed persons (Cecutti and Nieboer, 1981; Sunderman, 1983; Zober et al., 1984). Recently, much lower values have been reported for nickel in serum of 0.46 ± 0.26 µg L⁻¹ (Sunderman et al., 1984) and 0.28 ± 0.24 µg L⁻¹ (Linden et al., 1985; Sunderman et al., 1986a), while in whole blood levels of 1.26 ± 0.33 µg L⁻¹ (Sunderman et al., 1984) and 0.34 ± 0.28 µg L⁻¹ (Linden et al., 1985; Sunderman et al., 1986a) were found. Although lower serum and plasma nickel values are now commonly reported, urine concentrations appear to hold near 2 µg/L. For example, Sunderman et al. (1986b), by direct quantitation of urine diluted with dilute nitric acid, found urinary nickel levels to be 2.0 ± 1.5 µg L⁻¹ (range 0.5 to 6.0 µg L⁻¹) for four non-exposed healthy adults. The consensus is that part of this reduction in serum levels is due to better contamination prevention and the availability of atomic absorption spectrometers with modern background correction accessories (mostly by Zeeman technique). For workers occupationally exposed to nickel and its compounds,
values of plasma or serum concentrations up to 35 \( \mu g/L \) and urine levels up to 400 \( \mu g/L \) have been reported (Nieboer et al., 1984a).

(b) **Distribution.**

The uptake, transport and excretion of nickel depends not only on the mode of absorption but also on its chemical form while being absorbed (see next section for a further discussion of this). Blood is the main vehicle for the transport of absorbed nickel. Although the exact division between erythrocytes and the plasma or serum has not been determined, serum and plasma reliably reflect blood burdens and exposure (National Academy of Sciences, NAS, 1975; Nieboer et al., 1984a). Nickel is transported in the body via plasma where it is bound to both high and low molecular mass carriers. The high molecular mass ligand is human albumin, and possibly a nickel metalloprotein which is believed to be an alpha-2-macroglobulin, also called nickeloplasmin (Sunderman, 1977; Nomoto, 1980; Scott and Bradwell, 1983). The low molecular mass component has been shown to be a nickel(II)-amino acid complex by in \textit{vitro} \(^{63}\text{Ni}\)-addition experiments. Of the 22 amino acids tested, the main Ni\(^{2+}\)-binding amino acid was found to be L-histidine (L-His) (Lucassen and Sarkar, 1979). The exact distribution between these fractions has not been firmly established. The metalloprotein nickeloplasmin has not been well characterized, and there is some doubt about its importance.
in the distribution of Ni$^{2+}$ in serum. Work by Nomoto et al. (1971) and Sunderman et al. (1972) determined nickel associated with albumin, nickeloplasmin and ultrafiltrable components of rabbit and human serum. This early work involved atomic absorption spectrometry measurements on the fractions of serum that had been separated by ultrafiltration and column chromatography. They found that approximately 40% of nickel(II) present in rabbit serum was associated with albumin, 44% with the nickeloplasmin fraction and 16% with the ultrafiltrable fraction; whereas approximately 34% of the nickel in human serum was associated with albumin, 26% with nickeloplasmin and 40% was ultrafiltrable. Asato et al. (1975) found the amount of $^{63}$Ni$^{2+}$ (introduced by intravenous (i.v.) injection) associated with the ultrafiltrable fraction of rabbit serum to be 15%. Nomoto (1980) separated alpha-2-macroglobulin by affinity column chromatography from the serum of healthy humans who had no industrial exposure to nickel. Using atomic absorption spectrometry he found that 43% of the total nickel content was bound to alpha-2-macroglobulin. By comparison, Lucassen and Sarkar (1979) found that only about 0.1% of the total Ni$^{2+}$ in human serum was bound to the nickeloplasmin, with 95.7% bound to albumin and 4.2% to L-histidine in the ultrafiltrable fraction. They added $^{63}$NiCl$_2$ to 2.0 mL of serum and diluted to a constant volume of 2.5 mL before ultracentrifugation. The values reported are thus for nickel(II) added to serum and do not necessarily represent the endogenous nickel distribution.
Caution in interpretation is thus warranted since the added nickel resulted in concentrations about 35,000-fold higher than those observed naturally in serum. The in vitro distribution studies involving 22 amino acids were also carried out under these artificial conditions. There appears to be no agreement on the exact size of the ultrafiltrable or high molecular mass fractions of nickel(II) in human serum. The work described in Chapter 3 measures the actual endogenous low and high molecular mass fractions by ultracentrifugation and atomic absorption spectrometry in serum of electrolytic nickel-refinery workers.

The binding of Ni$^{2+}$ to these protein and amino acid ligands allows its rapid transport throughout the body. Although the distribution of nickel in the human body is of great importance, relatively little is known in comparison to animal studies. Usually the nickel content in human tissue has been studied employing autopsy specimens. Most of the early data appears to be of questionable analytical reliability. Also many studies (e.g. Schroeder et al. 1961; Tipton and Cook, 1963; Tipton et al., 1965) found that the nickel contents of some of the autopsy tissues were below the detection limits available at that time. However, these workers were able to illustrate the presence of nickel in lung, kidney, liver, heart, trachea, aorta, spleen, skin and intestine. Generally, the levels were found to be less than 0.05 µg/g wet tissue. The skin, intestine and lung had higher levels with the highest concentrations accumulating in
the lung. Similarly, Sunderman et al. (1971) found that lung tissue from accidental death victims had the highest levels of nickel (0.016 μg/g wet tissue) of the tissues sampled. In more recent studies of post-mortem specimens from adults without known occupational or iatrogenic exposure to nickel compounds, the highest concentrations of nickel were found in bone, followed by lung, kidney, liver and heart (Nomoto, 1974; Sunderman, 1984d; Zober et al., 1984; Kollmeier et al., 1985).

Nickel concentrations in lung tissue obtained from random autopsy tissue of industrially non-exposed individuals are positively correlated with age (Sunderman, 1984d) with a correlation coefficient of 0.82. In another study, Kollmeier et al. (1985) found this trend with a correlation coefficient of 0.71. Recent and the earlier data has been summarized by the relationship: Lung Nickel (μg kg⁻¹ dry wt) = 3.25 Age (years) - 38.4, with r = 0.77 and P< 0.001 (Rezuke et al., 1987). No age-dependent increases of nickel were observed in bone, kidney, liver or heart (Sunderman, 1984d). In a preliminary study Morgan and Adams (1980) found elevated levels of nickel in post-mortem lung tissue of nickel refinery workers. Andersen and Hogetveit (1984) found that autopsied lung samples from former nickel refinery workers in Norway had nickel values ranging from 2 to 1360 ppm, depending on worksite classification. Therefore, it appears that lung nickel levels are related to industrial exposure. The discussion on cancer will illustrate the importance of
nickel accumulation in the lung.

The estimated body burden of nickel in healthy adults averages 7.4 μg/kg body weight, with the highest contribution (7.3 μg/kg) coming from ingestion (including drinking water) and the remainder from inhalation (Bennett, 1984).

In comparison to the limited amount of human work, a large amount of information is available for acute and chronic animal exposure studies. Nickel(II) salts administered parenterally to a variety of animals show the largest accumulation in lung, kidney, liver and endocrine gland, with relatively little nickel found in neural tissue (Mushak, 1980). Thus both animal and human data illustrate high levels of nickel in lung and kidney.

(c) Excretion.

Many studies have been completed on the kinetics of distribution and clearance of injected nickel compounds in experimental animals. All of these studies show a relatively short half-life for water-soluble Ni(II)-salts of 24 h (or less). In rats and rabbits, Onkelinx et al. (1973) determined a two-compartment clearance model with fast and slow components. One day after injection, 78% of the dose is excreted in the rabbit, and in the rat it takes about 72 hours. In both species, $^{63}\text{Ni}^{2+}$ was rapidly cleared from the plasma or serum in the first two days after injection and disappeared at a much lower rate from three to seven days. This work also showed that most of the nickel is cleared by
the kidney (during the three days after injection of rats, 78% of the dose was excreted in the urine and 15% was excreted in faeces presumably via bile). In contrast to the large amount of absorbed nickel excreted by the kidney, the excretion of total ingested nickel is mainly faecal, with only about 10% appearing in the urine, for both dogs (Tedeschi and Sunderman, 1957) and humans (Horak and Sunderman, 1973).

Asato et al. (1975) have studied the ultrafiltrates from rabbits given i.v. injections of $^{63}$NiCl$_2$. They found that the Ni(II)-ultrafiltrable complexes (i.e. low molecular mass complexes) were rapidly cleared from the serum via urine. Although the identities of all the ultrafiltrable Ni(II)-ligands were not totally established, preliminary studies indicated that Ni$^{2+}$ was preferentially complexed to the amino acids L-His, L-Cysteine (L-Cys) and L-Aspartic (L-Asp), both singly or as mixed-ligand species.

Biliary excretion of nickel has been known to occur in calves with a nickel-supplemented diet (O'Dell et al., 1971), and in rabbits (9.2% of i.v. $^{63}$Ni$^{2+}$ injected dose) (Onkelinx et al., 1973). Recently, Marzouk and Sunderman (1985) have found that biliary excretion of nickel in rats injected with single subcutaneous doses only amounted to approximately 0.3% of the total dose over a 24-h period. By contrast, bile may be an important excretion route of nickel for humans. Bile specimens obtained during the postmortem examination of gallbladders from five non-occupationally exposed subjects
averaged $2.3 \pm 0.8 \, \mu g \, Ni \, L^{-1}$ (Rezuke et al., 1987). This level appears to be comparable to normal urinary nickel levels of $2.2 \pm 1.2 \, \mu g \, L^{-1}$ (or $2.6 \pm 1.4 \, \mu g \, d^{-1}$) (Sunderman et al., 1986a). If there is no significant intestinal reabsorption of the bile-nickel in the gastrointestinal tract, Rezuke et al. (1987) estimate that the net biliary excretion of nickel in non-exposed persons averages $2-5 \, \mu g \, d^{-1}$. This work suggests a net excretion of $\approx 6 \, \mu g \, Ni \, d^{-1}$ for the combined biliary and urinary routes.

One study by Hohnadel et al. (1973) indicates that sweat may be a major route of nickel excretion in humans. They determined nickel levels in the sweat collected from healthy subjects during sauna bathing for brief periods at $93^\circ C$ to be $52 \pm 36 \, \mu g/L$ for men and $131 \pm 65 \, \mu g/L$ for women. The importance of this route of excretion for electrolytic nickel refinery workers is unknown.

The role of hair as an excretory mechanism in man has inspired a large number of studies. Most of this interest has evolved from the possibility that nickel levels in hair may be used to assess overall nickel body burdens. This approach has not been successful, although hair may be an index to external environment exposure. In addition, there is controversy as to the correct collection and analytical procedures. Most of this debate is concerned with the type of sample to be collected, namely bulk hair sample (Sunderman, 1983) \textit{versus} proximal (first centimetre) scalp hair (Nieboer et al., 1984a). Washing protocols are also
controversial; whether three consecutive washings with a non-ionic detergent (Sunderman, 1983) or with distilled water (Chittleborough, 1980) should be adopted. In spite of these problems, Nieboer et al. (1984a) came to the conclusion that human hair shows considerable promise as an environmental indicator of exposure, providing more research is done on the complexities of hair-nickel interactions.

It may be concluded from the above discussion that the major route of excretion of absorbed nickel in man and many animals appears to be renal. This is most likely the reason why urinary nickel is a reliable indicator of environmental exposure. Urine is also the most convenient body fluid for biological monitoring because its collection is non-invasive and its nickel concentration is considerably higher than in plasma, serum or whole blood, which facilitates analysis (Nieboer et al., 1984a).

(iii) **TOXIC EFFECTS OF NICKEL COMPOUNDS**

(a) **Historical Aspects.**

As early as 1889, dermatitis resulting from exposure to chemicals used in nickel plating has been reported by Blaschko (NIOSH, 1977). Eczema was more severe in those who had worked longest and the use of rubber gloves and protective creams was recommended to reduce such outbreaks. Originally considered to be an occupational problem, recent clinical and epidemiological reports suggest that non-occupational exposures to nickel can present significant
problems to the general population. In contrast, there has been a decline of incidents related to industrial exposure (Nieboer et al., 1984a).

In 1891 McKendrick and Snodgrass recognized the extreme toxicity of nickel carbonyl. They concluded that air concentrations of less than 0.05% v/v are dangerous. In 1902 the first reports of nickel carbonyl poisoning due to industrial exposure occurred soon after the construction of the Mond nickel refinery in Clydach, Wales (Sunderman, 1977). From 1902 to the present there have been many case study reports of nickel carbonyl poisoning. Recently, Zhicheng (1986) has reviewed 179 cases that have occurred in China since 1961. All of the patients recovered including the six classified as severe. In North America and Europe there were 19 deaths reported from 1903 to 1971 (Sunderman et al., 1975). Lately, there has been a large decrease in accidental gassings, for example from 17 in 1971 to 2 in 1981 at the International Nickel Company (INCO) Clydach refinery (Nieboer et al., 1984a).

In Great Britain, the first indications of lung and nasal cancer of nickel workers were listed in the Annual Reports of the Chief Inspector of Factories for 1932 and 1949 (NIOSH, 1977; Mastromatteo, 1986). In the 1932 report there were 10 cases of cancer of the nasal cavities and paranasal sinuses. However, in the 1949 report there were 47 cases of cancer of the nose and 82 cases of lung cancer. In a report to the Ontario Department of Health in 1959, Sutherland
estimated that during 1930-1957 workers at INCO's Port Colborne refinery had a 37-fold increase in risk of dying from cancer of the nasal cavities (Sunderman et al., 1975). In the 1960's, cancer of the respiratory tract was also recognized among workers at a sintering plant in Copper Cliff, near Sudbury (Sunderman et al., 1975).

(b) Effects on the General Population.

The population at large (i.e. those not exposed to nickel in their work place) are exposed to nickel in their everyday life due to the widespread use of this metal and its compounds. Nickel is found in food, water, stainless steel products (e.g. jewelry, cutlery, etc.), permanent magnets, radios, generators, turbochargers, high stress and high temperature copper-nickel alloys and Ni-Cd batteries. These lead to exposure by skin contact and gastrointestinal tract absorption. As mentioned, nickel is also found in the air mainly due to the combustion and incineration sources (e.g. coal and oil burning units such as utilities, commercial and residential dwellings and municipal and sewage sludge incinerators). These anthropogenic sources account for the observation that the respiratory tract constitutes a significant route of intake besides the diet.

Nickel dermatitis. Nickel sensitivity is found in approximately 5% of the general population, with a tenfold higher incidence in women than men (Kieffer, 1979; Sun, 1980; Menne and Hjorth, 1982; Nieboer et al., 1984a). This type of
eczema is characterized by inflammation of the skin upon continued contact with nickel-containing items (Sunderman et al., 1975). Calnan (1956) described two patterns of nickel dermatitis: a primary outburst at the site of direct metal contact, and a secondary eruption or area of "spread" remote from the metal-contact site. Secondary distant eruptions are found in 75% of patients (Menne and Hjorth, 1982). The usual method for diagnosing individuals for nickel sensitivity is the "patch test". It involves placing a few drops of a water-soluble nickel compound (e.g. 2.5% NiSO₄ in either water or petrolatum; Wilkinson et al., 1970) on a cotton patch and taping it to an individual's skin. A positive test is considered to occur when inflammation develops under this patch after 24 to 48 h. This test is not very sensitive for persons who produce only mild reactions to nickel, or who display a very delayed reaction. Also the patch test itself has the potential of inducing nickel sensitivity in an individual. It can also cause local irritation or non-specific (false) positives (e.g. due to the adhesive tape) and exacerbation of the dermatitis (Svengaard et al., 1978; Veien et al., 1979). The patch test diagnosis of nickel dermatitis can now be complemented with the lymphocyte transformation test. The lymphocyte transformation induced by NiSO₄ in vitro has been shown to be beneficial in the diagnosis of nickel sensitivity in dermatitis patients (Veien et al., 1979; Al-Tawil et al., 1981). This test is based on the fact that lymphocytes isolated from the nickel-sensitive
person undergo a lymphocyte to lymphoblast transformation after 7 to 8 days incubation in the presence of a Ni(II) salt. This transformation is monitored by an increased uptake of radiolabelled $^3$H-thymidine when cells proliferate during blastogenesis. Although this is a more difficult test to perform than the patch test, it may prove to be more specific and sensitive (Al-Tawil et al., 1981). It is also less invasive than the patch test, in that it does not cause a primary eruption of the skin.

The mechanisms of sensitization are not completely understood. However, it is generally accepted to include the following phases: (1) diffusion of nickel through the skin; (2) subsequent binding (conjugation) of the nickel(II) ion (the hapten) with protein(s) and/or other skin components to become the allergen; and (3) a cellular response to the nickel-macromolecular complex (Sunderman et al., 1975; Spruit et al., 1980; Adams, 1983). The allergen is believed to initiate a cascade of biological reactions in regional lymph nodes, spleen and bone marrow, ultimately including stimulation and proliferation of effector T-lymphocytes (thymus-dependent lymphocytes) that recognize and react with the hapten. After a secondary challenge, effector cells upon contact with the allergen release chemotactic substances that cause the inflammatory skin reaction by inducing vascular permeability, attraction of mononuclear cells, and retention and proliferation of cells within the area penetrated by the hapten. It is clear that nickel dermatitis is a delayed,
cell-mediated hypersensitivity response.

Despite the suggestion by some investigators that there is an association between nickel dermatitis and atopy (or the hereditary predisposition to mount a humoral immunological reaction which is characterized by high plasma IgE titres), the expected correlation with serum IgE levels has not been generally observed (Nieboer et al., 1984b). In fact, atopic people are known to have a reduced predisposition to contact sensitization. However, very recently, Belda et al. (1985) found that the IgE levels in a group of nickel-sensitive individuals when compared to those in a non-allergic control population failed to provide evidence of a reduced tendency to elevated IgE levels in people with contact dermatitis. This suggests that IgE and IgG antibodies are most likely indirectly involved in the cellular response.

**Other Health Effects.** Although the general population is exposed to nickel by way of the lung, there have been no reports of respiratory hypersensitivity (i.e. asthma) nor respiratory cancer. Neither are there reports of nickel carbonyl poisoning in people not industrially exposed.

**(c) Industrial Exposure.**

**Nickel Carbonyl (Ni(CO)₄).** This is a very volatile colourless liquid (b.p. 42.6°C) that decomposes rapidly above room temperature unless held under a pressurized atmosphere of carbon monoxide (Antonsen, 1978). Exposure may arise from a variety of operations in refineries, manufacturing plants
and research laboratories. The Mond or Carbonyl process of nickel refining, discovered by Ludwig Mond and Carl Langer in 1889, uses these properties to isolate pure nickel (Boldt, 1967). At Clydach, Wales, refinery feed material (usually nickel(II) oxide from Canada) is passed into rotating kilns where it is treated with molecular hydrogen to reduce the nickel. It is then exposed to CO at room temperature to form nickel carbonyl gas. Subsequently, the gas is passed over nickel seed pellets, which have been preheated to 200-250°C to decompose the nickel carbonyl. The pure nickel metal deposits on the pellets and the CO is then recirculated.

\[ \text{Ni} + 4\text{CO} \rightarrow \text{Ni} (\text{CO})_4 \quad (1.1) \]

Nickel powder can also be manufactured by this process if the nickel carbonyl vapour is shock heated in a special decomposer (Morgan, 1979).

In addition to gassing in nickel refineries, nickel carbonyl exposure may also result from its inadvertent formation (i.e. when carbon monoxide, especially under high partial pressure, comes in contact with an active form of nickel such as nickel catalysts used in hydrogenation) (Kincaid et al., 1953). Exposure in these circumstances is likely to be particularly hazardous because the presence of nickel carbonyl may not be expected or recognized as a hazard.

Nickel carbonyl is a very lipophilic compound. Hence,
when its vapour is inhaled, it passes through the lung's membranes very easily and causes nickel to be deposited in the lungs and other tissues. The clinical symptoms of nickel carbonyl poisoning have been divided into two phases. The first or immediate phase has symptoms similar to influenza: headache, vertigo, nausea, vomiting, fatigue and sometimes sternal pain and epigastric discomfort (Carmichael, 1953; Sunderman and Kincaid, 1954; Vuopala et al., 1970; Sunderman Sr., 1970; NAS, 1975; Sunderman Jr., 1977). These early symptoms of the initial phase are usually mild and disappear within 24 hours. The second or delayed phase symptoms usually develop gradually 2-10 days following exposure. The second phase symptoms are similar to chemical pneumonia, as is apparent from the following quote from NAS (1975): "Constrictive pain in the chest is usually the first symptom. This is followed by cough, hypernea, cyanosis, occasionally gastrointestinal symptoms and a profound weakness". In the most severe cases, death occurs 4 to 13 days after exposure (Armit, 1907; Carmichael, 1953; Sunderman and Kincaid, 1954; Sunderman, 1970; Sunderman, 1977). In a recent study, Zhicheng (1986) found the general symptoms to be very similar to those reported previously, and he was the first to document the occurrence of cardiotoxicity. Three of six severe cases had inflammation of the myocardium (myocarditis).

The lesions induced by nickel carbonyl exposure in lung tissue of animals and man are very similar. Autopsy
examination has also documented the occurrence of hepatic and adrenal cortical degeneration, as well as cardiac irregularities and liver enlargement. Brain and renal congestion are often observed (Sunderman Sr., 1981), although the loss of life is usually attributed to respiratory failure.

Convalescence in patients recovering from acute nickel carbonyl poisoning is usually quite long and is customarily characterized by fatigue on slight exertion. Commonly two to three months elapse before it is possible for a patient to return to work.

Studies of nickel carbonyl in mice and rats after inhalation or intraperitoneal administration have shown that nickel is deposited primarily in the lungs, and to a lesser extent in the heart, diaphragm, central nervous system, kidney, liver, adipose tissue and blood (Sunderman and Selin, 1968; Oskarsson and Tjalve, 1979a; Tjalve et al., 1984). The pulmonary parenchyma is the principal target tissue regardless of the route of administration. It is known that Ni(CO)$_4$ can cross the alveolar membrane in either direction without undergoing a metabolic change. After a review of the literature, Nieboer et al. (1984a) postulate the following mechanism of action in nickel carbonyl poisoning. The first step is the decomposition of Ni(CO)$_4$ to produce metallic Ni.

\[
\text{Ni(CO)}_4 \rightarrow \text{Ni}^0 + 4\text{CO} \quad (1.2)
\]
This reaction may be promoted by haemoglobin which serves as a receptor for the released CO. The second step is the oxidation of the metallic Ni⁰ to Ni²⁺ by a suitable oxidizing agent (Ox), perhaps aided by enzyme catalysts.

\[ \text{Ni}^0 + \text{Ox} \rightarrow \text{Ni}^{2+} + \text{Ox}^{2-} \quad (1.3) \]

It is not clear whether the direct interaction of Ni(CO)₄ with tissues or the resulting Ni⁰ or Ni²⁺ is responsible for the severe tissue lesions.

It is currently thought that the toxic action of nickel carbonyl is not due to carbon monoxide (Armit, 1907; Kincaid et al., 1953). These studies have evaluated the relative amounts of CO present as carboxyhaemoglobin. It was concluded there was not a high enough level of CO in the blood to account for the observed toxic action. Oskarsson and Tjalve (1979a) found from the tissue distribution of ⁶³Ni and ¹⁴C-labelled nickel carbonyl that the toxic effect of Ni(CO)₄ appeared to correlate with the distribution of nickel. Thus the lung was the main depository.

Zhicheng (1986) has reported that individuals subjected to mild exposure were successfully treated with bed rest and symptomatic drugs like bronchodilators. Moderate cases also received oxygen, glucose, vitamin C and corticosteroids. The severe cases were treated with a series of drugs and other methods, such as limitation of water intake, administration
of oxygen, hibernation therapy, and parenteral administration of large doses of corticosteroids, dithiocarb, antibiotics and other drugs to help resolve pulmonary oedema, pneumonia and toxic myocarditis. This work confirmed that corticosteroids are useful in the treatment of Ni(CO)$_4$ gassing, and it was found that prompt help (first aid) and comprehensive treatment are important for the recovery of the patients.

Currently accepted clinical practice in North America and Europe is based upon the hypothesis that administration of chelating agents, especially oral administration of diethyldithiocarbamate (DDC), improves the prognosis (Sunderman Sr., 1981). It is thought that the role of DDC is to lower the body burden of Ni$^{2+}$. Unfortunately, the available data does not demonstrate an unequivocal increase in urinary nickel levels due to DDC chelation therapy. The clinical worth of DDC therapy is also clouded by the fact that in clinical cases it was given 3 - 4 days after exposure and that no controlled therapeutic trials have been conducted to evaluate the effectiveness of this chelating agent (Nieboer et al., 1984a).

A novel mechanistic point of view arises from reports that DDC acts as an effective scavenger of free radicals, such as the superoxide anion (Nieboer, unpublished results). In the animal model, it protects tissue against radiation damage (Evans et al., 1982, 1983). The injury to the lung induced by Ni(CO)$_4$ resembles that caused by other chemicals
like phosgene and paraquat that are known to have a toxic mechanism involving redox reactions (Menzel and McClellan, 1980; Minchin and Boyd, 1983). A similar mechanism can be postulated for Ni(CO)$_4$ because it decomposes rapidly, releasing Ni$^0$ which is assumed to be the source of electrons when oxidized to Ni$^{2+}$. If this mechanism proves to be operative, then it follows that it is crucial for DDC to be administered quickly after the exposure to nickel carbonyl.

This radical mechanism of nickel carbonyl toxicity may also explain differences found for the chelating agents DDC and D-penicillamine (D-PEN) in studies of Ni(CO)$_4$ administered to animals. Both are known to act as free radical scavengers in animal models (Evans et al., 1982, 1983). However, DDC is lipophilic, and can act as an intracellular radical scavenger, whereas the D-PEN is hydrophilic and presumably cannot act as an intracellular free radical scavenger. Animal studies have indicated that D-PEN is more effective than DDC in NiCl$_2$ poisoning, while the reverse has been observed for Ni(CO)$_4$ (Horak et al., 1976; Baselt et al., 1977). In fact, $^{63}$Ni-autoradiography has demonstrated that DDC enhances the deposition of nickel administered as NiCl$_2$ in lipophilic tissues (Oskarsson and Tjalve, 1980). In vitro cellular nickel(II) uptake studies with a number of cell types have also confirmed such intracellular compartmentalization effects in the presence of DDC (Nieboer et al., 1984c).

After a review of the literature, Nieboer et al. (1984a)
concluded that high urinary levels of nickel generally reflect not only the extent of \( \text{Ni(CO)}_4 \) exposure but also the clinical severity that may be expected. For example, a urinary nickel level of more than 100 \( \mu \text{g/L} \) on the day of exposure suggests that careful monitoring of the patient would be judicious even in the absence of initial symptoms. The urinary nickel action levels reported in the literature are based on an 8-h urine collection. However, as pointed out above and by Nieboer et al. (1984a) and Zhicheng (1986), more immediate treatment seems advisable. Therefore waiting for an 8-h urine collection before treatment is initiated may not be appropriate. This demonstrates the need to use spot urine samples that can be accurately and confidently corrected for concentration/dilution effects. This aspect is addressed in Chapter 2.

**Cancer.** The incidence of increased cancer risk associated with industrial exposure to nickel compounds shows nasal and lung cancers to be predominant. The incidence of these two cancers has been most strongly correlated with exposure to pyrometallurgical refinery intermediates (Cecutti and Nieboer, 1981; Nieboer et al., 1984a; Sunderman, 1984a).

It appears that inhalation of newly generated insoluble dusts and/or fumes composed mostly of nickel-sulphide and/or nickel-oxide mixtures produced in the pyrometallurgical refining processes are carcinogenic. Nickel and nickel oxide dusts have also been associated with welding, grinding and
polishing and the casting of alloys, although no excess cancer risk is known for the associated occupations. There has been no increased risk observed in the electrolytic department at INCO's Port Colborne plant, while there have been reports of cancer risk in the electrolytic refining processes employed at other locations, especially in Norway and the USSR. These apparently conflicting findings for electrolytic refining have been attributed to the possibility of mixed exposures of water-soluble and relatively water-insoluble nickel compounds, or to engineering and operational differences which generate airborne nickel of different composition (Nieboer et al., 1984a). The carcinogenicity of water-soluble nickel(II) salts is still controversial. There have also been isolated reports indicating slight elevations in kidney cancer, stomach cancer and cancer of the prostate. However these were not consistent, nor statistically significant, and their importance or the role of nickel exposure in these instances has to be clarified (Nieboer et al., 1984a; Roberts et al., 1984).

Water-insoluble compounds (e.g. Ni_3S_2, Ni powder and NiO) have all been demonstrated to be carcinogenic in animal studies, but water-soluble salts (e.g. NiCl_2, NiSO_4) have no carcinogenic potential (Leonard et al., 1981; Sunderman, 1984c). Nasal tissues biopsies of nickel workers have demonstrated that nasal epithelial dysplasia may precede nasal cancer (Boysen et al., 1980a,b; Boysen and Reith,
1982). Recently, Boysen et al. (1984) have studied the histological changes in nasal biopsy specimens and urinary levels of nickel in retired workers. They found that the presence of hyperplastic/polypoid nasal lesions was inversely related to the duration of retirement, possibly reflecting gradual regression of the mucosal swelling. Lower nickel body burden, assessed by plasma and urinary nickel levels, showed a similar dependence. The nickel concentrations in the plasma and urine were significantly higher among former roasting/smelting workers than in former electrolysis and non-process (control) workers. This is probably due to the slow release and excretion of nickel(II) from deposits of particulate nickel compounds that have accumulated in the first group prior to retirement. This slow release of insoluble nickel compounds from lung tissue has also been demonstrated in intratracheal injections of rats with insoluble NiO when compared to soluble NiCl₂ (English et al., 1981). In part, the carcinogenic potential of the different nickel compounds appears to correlate with the degree and speed with which the lung can remove these materials (Sunderman, 1984b; Nieboer et al., 1986). Clearance will, of course, depend on the solubility of the compounds in body fluids. The more soluble compounds will be released from the body more quickly than the insoluble compounds. This illustrates the importance of biological residence time and bioavailability in nickel carcinogenesis.

Animal inhalation studies support the carcinogenic
effects found in man (Ottolenghi et al., 1975; Wehner et al., 1984). Ottolenghi et al. (1975) exposed Fischer 344 rats to airborne nickel subsulfide in concentrations of 0.97 mg nickel m\(^{-3}\) (70% < 1μm diameter) 6 hours/day, 5 days/week for 78 - 84 weeks and found that the lungs were most affected. Within two years after starting the experiment, 14 malignant lung tumors were found in the exposed group, compared to 1 malignant tumor in the control group.

Among studies employing injection protocols are many reports that nickel subsulfide (Ni\(_3\)S\(_2\)) is a potent carcinogen. Sunderman and Hopfer (1983) have shown in animal studies that crystalline alpha-Ni\(_3\)S\(_2\) and crystalline beta-NiS can cause 100% induction of sarcomas at the injection site, whereas Ni dust and amorphous NiS caused 55% and only 4% respectively. These results show that nickel compounds differ substantially in their carcinogenic potencies (Sunderman, 1984b). This work also suggests that compounds that have well defined crystal structures are the most potent carcinogens. Kasprzak et al. (1983) were able to show that colloidal Ni(OH)\(_2\) is non-carcinogenic in rats, whereas "dried" and more crystalline forms appear to be carcinogenic. Thus crystal structure and surface properties of nickel compounds appear important to nickel carcinogenesis. More recent studies have suggested that surface passivity of solid nickel compounds (i.e. "smooth exterior, crystallinity, low surface charge, low surface activity with respect to protein adsorption and cell lysis, and moderate solubility") appears
to be predisposing to carcinogenicity" (Nieboer et al., 1984d; Nieboer et al., 1986). Surface inactivity was interpreted to reduce biological clearance and to promote intracellular delivery of Ni$^{2+}$.

A number of studies have been reported on the intrarenal (i.r.) injection of nickel subsulfide in rats. After i.r. administration of 5 mg of alpha-Ni$_3$S$_2$, the blood haematocrit increased within one week concomitant with intense stimulation of erythropoiesis (Hopfer et al., 1980). Sunderman (1984b) has pointed out a significant rank correlation ($p < 0.0001$) between the sarcoma incidences and the capacities of the Ni-compounds to induce erythrocytosis after i.r. injection to rats. This has been interpreted that somehow regulation of renal production of erythropoietin can serve as an index to carcinogenic activity of nickel compounds in rats. After i.r. injection of Ni$_3$S$_2$, renal tumors developed only at doses of 5 and 10 mg/rat with 28 and 75% incidences, respectively. It is thus possible to induce renal tumors by direct injection of the solid Ni$_3$S$_2$ into the target organ. However, these experiments do not demonstrate that the kidney is a target organ of Ni$_3$S$_2$ when the exposure route is by the respiratory system. Presumably only the Ni(II) ion reaches the kidney.

From this work we see that in an animal model not only is it possible to induce lung cancer, as found in humans, but it is also possible to generate injection site tumors. The significance of the latter to the human situation is
difficult to assess (Environmental Protection Agency, (EPA), 1986).

Manganese dust has been demonstrated to inhibit the carcinogenicity of alpha-Ni$_3$S$_2$ when both were given as intramuscular (i.m.) injections in the same thigh (Sunderman and McCully, 1983). It is interesting to note that in rats that received injections of alpha-Ni$_3$S$_2$ in one thigh and Mn dust in the other, no reduction in the incidence of sarcoma was evident. This illustrates that interaction with other metal ions may alter the potency of nickel compounds.

In the somatic mutation model of nickel carcinogenesis it is often assumed that the Ni$^{2+}$ ion interferes with the fundamental molecular processes of replication, transcription and translation (Cecutti and Nieboer, 1981), perhaps by interaction with the DNA of cells and causing irreversible damage. Friedberg (1985) has stated that "if the cellular response(s) to this damage involves the misrepair of lesions, or if DNA repair does not occur and the dominant response is a mechanism that is error-prone, then mutations may arise in the descendants of the affected cells. These mutations, affecting specific genes (or sets of genes) regulate the critical aspects of cellular growth and may express themselves phenotypically as neoplastic transformations". Waksvik et al. (1984) have shown increased incidence of chromosomal aberrations (breaks and gaps) but no incidence of sister chromatid exchanges in peripheral lymphocytes of retired nickel refinery workers who were exposed to furnace
dust of Ni$_3$S$_2$ and NiO or aerosols of NiCl$_2$ and NiSO$_4$ for more than 25 years. The relation of these observations to cancer is unknown. Ni$^{2+}$ like other divalent metal ions has been shown to be mutagenic in cultured Chinese hamster ovary cells (Sunderman, 1984b). With respect to these \textit{in vitro} assays, the response of nickel(II) appears to be non-specific in that both essential and toxic metals have been shown to be mutagenic in these systems. Recently, the EPA (1986) reviewed the testing of various inorganic nickel compounds for mutagenicity and other genotoxic effects in a number of test systems. Although it is suggested that the data reported must be considered preliminary, it appears that nickel may induce gene mutations in bacteria and cultured mammalian cells. Nickel(II) also appears to be able to induce chromosomal aberrations in cultured mammalian cells and sister chromatid exchange in both cultured mammalian cells and human lymphocytes. It also exhibits the ability to induce morphological cell transformations \textit{in vitro} and to interact with DNA resulting in cross-links and strand breaks. The EPA (1986) document concludes that it has been demonstrated that nickel compounds can induce genotoxic effects. However, the "translation of these effects into actual mutations is not clearly understood".

The major postulate of nickel carcinogenesis at the present time is that the Ni$^{2+}$ ion is the ultimate carcinogen (Sunderman, 1984b and e). Work by Hansen and Stern (1983) is frequently cited to support this "Ni$^{2+}$ ion hypothesis". They
examined the in vitro transformation potency of nickel compounds employing BHK-21 cells (baby hamster kidney cell line), and established a correlation between the cytotoxicity and morphological cell transformation induced by nickel compounds. The results are suggestive that bioavailability and the intracellular level of Ni$^{2+}$ may determine carcinogenicity. However, the Hansen and Stern study (1983) does not firmly establish the "nickel ion hypothesis" because of large inter-experiment variability, lack of statistical treatment of the results, and since no actual measurement of the intracellular nickel content was made. Although it has been demonstrated that Ni$^{2+}$ can interact with the genetic machinery (see Section iii d), its exact role in mutagenicity and cancer development has not been clarified.

In a recent study by Nieboer et al. (1986), it was pointed out that consideration must be given to a number of factors that may mediate or modify the nickel ion hypothesis. Among these are: the surface and other physicochemical properties of relatively insoluble nickel compounds; the concepts of the bioavailability of Ni$^{2+}$, mode of entry and delivery of Ni$^{2+}$ or Ni(II)-complexes to cells and the nucleus of the cell; the biological residence time of nickel and its compounds; and the balance between the intracellular compartmentalization of Ni$^{2+}$ and its extracellular transport/excretion.

Farber (1981, 1984) has pointed out that cancer is a multistage process. A number of steps have been recognized:
(1) initiation (a single or brief exposure to a carcinogen induces a permanent change in tissues recognized after promotion as focal proliferations, such as papillomas, nodules or polyps); (2) promotion (the process involving cell proliferation whereby tumor formation is accelerated in a tissue that has been initiated); and (3) progression (focal proliferation lesions resulting from a promoting environment become precancerous lesions before malignant behaviour is expressed). Chemical promoters of cancer appear to share an ability to produce radicals such as the superoxide anion (O$_2^-$), the hydroxyl radical (-OH) and the peroxy radical (RO$_2^-$) from molecular oxygen (O$_2$) (Marx, 1983). Since studies indicate that with the enzyme system xanthine/xanthine oxidase and with stimulated human polymorphonuclear leukocytes (PMNs) the Ni(III)/Ni(II) redox couple participates in dioxygen radical biochemistry (Nieboer et al., 1986), it is tempting to postulate that Ni(II) may also be involved in the promotion stage of carcinogenesis. However, the direct participation of Ni$^{2+}$ in any of the steps outlined by Farber has not been demonstrated.

It is obvious from this brief review that nickel carcinogenesis is a complicated process.

**Nickel Induced Asthma.** Very few well-documented cases have been reported of workers who have experienced asthma due to exposure to nickel. The first case of asthma that was clearly attributable to inhalation of nickel salts was
reported by McConnell et al. (1973) for a metal plater. They concluded from skin and haemoglutionation tests that circulating antibodies to nickel were involved. The second case was reported by Malo et al. (1982) for a young worker in an electroplating factory. They showed the presence of nickel-specific IgE antibodies. Block and Young (1982) described a third case of a metal polisher. All three workers had nickel dermatitis. They also had positive reactions to prick testing with nickel sulphate solution (in concentrations of 1 mg/mL or 10 mg/mL). Sunderman and Sunderman (1961) have reported an incident of nickel-related asthma due to exposure to nickel carbonyl in a patient with Loeffler's syndrome (excess cellular infiltration of the lung). Recently, Davies (1986) found evidence of three cases of occupational asthma (out of 53 studied) due to exposure to nickel salts in the manufacture of a nickel catalyst. However, these cases were identified by clinical observation with only strong circumstantial evidence to support the diagnosis.

Novey et al. (1983) found a metal plating worker who upon inhalation challenge developed acute asthma to chromium sulfate and a biphasic asthma-like response to nickel sulfate. They were able to show specific IgE antibodies to the challenge materials (industrial solutions of chromium sulfate and nickel sulfate) but not to another metal, gold, which the patient could tolerate. They concluded that this work supports the postulate that bronchial reactivity can be
specifically induced by fumes of metallic salts, even in a previously nonallergic individual, and that an IgE type I immunopathogenic mechanism is involved. An antibody with nickel specificity has been characterized in the serum of a patient (Dolovich et al., 1984; Nieboer et al., 1984b). This antibody recognized Ni\(^{2+}\) bound to the natural Cu\(^{2+}\)-binding site of human serum albumin (HSA) as illustrated by the high titres of radiolabelled nickel (\(^{63}\)Ni) that coprecipitated with IgE and IgG antibodies. By contrast, Cu\(^{2+}\) bound to this site was not recognized by the antibodies.

As pointed out by Davies (1986), it is surprising that so few cases of nickel induced asthma have been described. He makes the comment that since serum antibodies with nickel specificity can now be identified, it should be easier to investigate and positively diagnose nickel asthma.

(d) **Biochemistry of Nickel**  
*(Including Determinants of Reactivity)*

**Introduction.** Nieboer and Sanford (1985) have illustrated the great variety of determinants of metal-ions that facilitate the understanding of their biochemistry, toxicity and therapeutic uses. This review of nickel biochemistry will use many examples from cancer studies and other areas such as enzyme reactions to provide interesting information on metal-ion parameters that have widespread implications for all endogenous and exogenous metal ions.

**Absorption.** The mode of entry into the body that a chemical has may determine its toxic (or beneficial) effect. The
relatively small amount of nickel that is absorbed via the gastrointestinal (G.I.) tract has been interpreted in two ways: (1) as evidence that nickel is an essential element with uptake as part of its homeostatic control; or (2) as evidence that this is in effect a detoxification mechanism that serves to exclude, at least in part, the Ni$^{2+}$-ion from the human body. Indeed, it appears that Ni$^{2+}$ interacts with constituents of foods and beverages in a differential manner causing a variable uptake in humans because of diet (Solomons et al., 1982). A recent study by Foulkes and McMullen (1986) found a two-step mechanism for the absorption of $^{63}$Ni$^{2+}$ from the lumen of perfused rat jejunum. The first step was found to be linearly dependent on concentrations up to 20 µM Ni$^{2+}$ with saturation being approached between 20 and 100 µM. They also found that this step was inhibited by a foodstuff namely dried skim milk. The absorption of Ni$^{2+}$ from the G.I. tract probably results from an equilibrium of Ni$^{2+}$ with various food components which limit the amount of free Ni$^{2+}$ that can be absorbed. The absorption of low molecular mass Ni(II)-complexes in the G.I. tract was not investigated. The second step, the movement from the jejunum mucosa into the body was shown to be different for Cd$^{2+}$ and Ni$^{2+}$. For Cd$^{2+}$, high retention in the mucosa occurred with subsequent slow movement to the body. Ni$^{2+}$ (Foulkes and McMullen, 1986) and Zn$^{2+}$ (Bonewitz et al., 1983) show little tendency to be retained in the mucosa. This indicates that Ni$^{2+}$ acts more like the essential ion Zn$^{2+}$ than the non-essential and toxic
Cd$^{2+}$ ion. Thus the uptake of Ni$^{2+}$ appears to resemble that of an essential metal with a means of limiting uptake to partially regulate body levels. It is well known that Ni$^{2+}$ can penetrate the skin and produce allergic skin reaction. However the incidence of any G.I. tract irritation is very low, with no reports of cancer due to nickel. This is in contrast to the high prevalence of respiratory cancers in nickel workers and dermatitis in the general population.

Cellular Uptake. Because of the high incidence of lung cancer for workers exposed to insoluble nickel compounds, most of the work on cellular uptake of nickel has centered around the phagocytosis and solubilization of such compounds. Costa and Mollenhauer (1980) have shown that crystalline nickel subsulfide is actively phagocytized by cultured Chinese hamster ovary (CHO) and Syrian hamster embryo (SHE) cells. In contrast, no active phagocytosis was observed in cells exposed to amorphous nickel monosulfide nor to water-soluble nickel(II) salts. Phagocytized particles were solubilized to a form capable of entering the nucleus and interacting with nuclear macromolecules (Costa et al., 1981). The kinetics of solubilization may be expected to play some role in the toxic action of nickel compounds. For example, in a study with Ni$^{2+}$-hydroxides and NiSO$_4$, Kasprzak et al. (1983) found an inverse relationship between carcinogenic activity and dissolution kinetics in human serum, artificial lung fluid and ammonium acetate buffer. By contrast, Sunderman (1984c) found that the sarcoma incidence at two
years after i.m. injection of various nickel compounds (14 mg Ni/rat) was correlated (p = 0.02) with their nickel mass fractions, but not with dissolution half-times in serum or renal cytosol, nor with in vitro phagocytic indices assessed for rat peritoneal macrophages. He also found a significant rank-correlation (p < 0.0001) between the carcinogenic activities and the potencies of the compounds to induce erythrocytosis of rats two months after i.r. injection of nickel compounds (7 mg Ni/rat). By comparison to the slow solubilization of crystalline nickel subsulfide, the highly lipid-soluble nickel carbonyl and aerosols of soluble nickel salts are absorbed by the body very quickly through the lungs. As already indicated, nickel absorbed or ingested as soluble nickel compounds has a much shorter half-life than tissue deposits of insoluble compounds (such as in the lungs).

Cellular uptake of soluble Ni(II) salts may be mediated by the presence of extracellular and intracellular ligands. The uptake of Ni$^{2+}$ by cultured cells (human B-lymphoblasts, human erythrocytes and rabbit alveolar macrophages) has been shown to be promoted by DDC presumably because of the lipophilicity of the resulting Ni(DDC)$_2$ complex. L-His and HSA at physiological concentrations, like the exogenous ligands D-PEN and EDTA, inhibited the uptake of physiological levels of Ni$^{2+}$ (Nieboer et al., 1984c). Preincubation with DDC has also been shown to increase the uptake of Ni$^{2+}$ by human peripheral mononuclear leukocytes (mostly lymphocytes
with approximately 15% monocytes) (Menon and Nieboer, 1986). They interpreted the predominant incorporation and subsequent cytosolic accumulation rather than in the cell pellet (consisting of the plasma membrane, nuclei, mitochondria, lysosomes and cell debris) in terms of the "Equilibrium" model of metal ion uptake by cells (Williams, 1981). This model proposes that the distribution of metal ions among the various compartments of a cell under steady state conditions (i.e. at fixed pH, redox potential, intracellular and extracellular ligand concentrations) is determined by thermodynamic parameters such as the pKₐ values of ligands, the binding constants of the metal-ligand complexes, and their solubilities in aqueous and lipid phases. This "Equilibrium" model may be envisioned to mediate cellular uptake, distribution and clearance of Ni²⁺.

**Biological Role of the Ni(II) Ion.** Ingested nickel is presumably taken up as the Ni(II) ion. Intracellular dissolution of particulates of a nickel compound in phagocytic cells is also believed to result in an increased availability of nickel ions. Nickel(II) released from, for example, alveolar macrophages, is likely available to the surrounding tissue. Presumably once inside the cell, Ni²⁺ then has the potential of interacting with a large selection of intracellular ligands (from amino acids to proteins to DNA etc.). Subsequently it is most likely transported to various parts of the cell (e.g. to the nucleus) and, in part,
transported out into the bloodstream, from which it can be delivered to other tissues or be excreted via the kidney. Thus absorbed Ni\(^{2+}\) now has the potential of participating in some of the many biological roles of metal ions (Fig. 1.1).

**Reactivity of the Nickel(II) Ion.** A system of classification has been proposed by Nieboer and Richardson (1980) that separates the binding preferences of metal ions into class A (oxygen-seeking), class B (nitrogen/sulfur-seeking) and borderline (or ambivalent). A survey of crystallographic data for metalloproteins and metalloenzymes is consistent with these designations (Blundell and Johnson, 1976; Nieboer and Richardson, 1980). The charge-to-size ratio (Z\(^2/r\)) is a measure of the ability of metal ions to participate in ionic or class A interactions, while the product of electronegativity and size (X\(\mu\)r) reflects covalent-bonding or class B tendency. These covalent and ionic indices plotted in Figure 1.2 allow a graphical display of the metals in each of these three categories (Nieboer and Richardson, 1980; Nieboer and Sanford, 1985).

The Ni\(^{2+}\) ion is borderline in its binding preference. Thus in addition to its affinity for class B (nitrogen and sulfur) ligands, it tends to bind to class A (oxygen) sites. Consequently, Ni\(^{2+}\) can compete effectively with class A ions for their binding sites and at the same time seek out other non-oxygen centres. A prime example is the tendency of Ni\(^{2+}\) to bind with oxygen sites even better than Ca\(^{2+}\) and Mg\(^{2+}\). Ni\(^{2+}\) behaves as a partial antagonist in the response observed
Figure 1.1  Major biological roles of metals.
Figure 1.2  Classification of metal ions according to binding preferences: Class A, oxygen-seeking; Class B, nitrogen/sulfur seeking; Borderline, intermediate or ambivalent.

\[ Z = \text{formal charge}; \ r = \text{ionic radius}; \ x_m = \text{metal ion electronegativity} \]

For fixed covalent index, stability increases with ionic index; and conversely for fixed ionic index, stability increases with covalent index.

Consequently, endogenous ions such as \( \text{Mg}^{2+} \) and \( \text{Ca}^{2+} \) are readily displaced by ions above them in the figure (e.g. \( \text{Pb}^{2+} \) and \( \text{Cd}^{2+} \)) and to their right (e.g. \( \text{Al}^{3+} \), \( \text{Be}^{2+} \) and \( \text{La}^{3+} \)).

Reproduced from Nieboer and Richardson, 1980.
for Ca$^{2+}$-dependent processes in excitable tissues such as muscle and nerve cells. On the other hand, Ni$^{2+}$ closely mimics Ca$^{2+}$ in generating action potentials, but cannot replace Ca$^{2+}$ in generating the myosin-actin interaction (see Table 1.1). Ni$^{2+}$ has also been shown to have ambivalent action on the contractile activity of isolated rat uterus (Rubanyi and Balogh, 1982). In low concentrations (10^{-7} to 10^{-5} M), NiCl$_2$ increased the basal tone (sustained contracture) probably by enhancing the influx of Ca$^{2+}$ and/or inhibiting mitochondrial sequestration of Ca$^{2+}$. In high doses (10^{-4} to 10^{-3} M), NiCl$_2$ depressed spontaneous contractions by antagonizing the influx of Ca$^{2+}$ into uterine smooth muscle cells.

Complex Formation is very rapid for Ca$^{2+}$ (forward rate constant, $k_f = 10^8 - 10^9$ s$^{-1}$), which is faster than for Mg$^{2+}$ ($k_f = 10^5$ s$^{-1}$) and for Ni$^{2+}$ ($k_f = 10^4$ s$^{-1}$) (Nieboer, 1981). Ni$^{2+}$ complexes also tend to be more stable than those of Ca$^{2+}$ and Mg$^{2+}$. Substitution of Ni$^{2+}$ for Ca$^{2+}$ and Mg$^{2+}$ can lead to activation or inactivation of enzymes (Nielsen, 1974, 1980; Nieboer et al., 1984a). Other examples, in addition to those mentioned earlier, of the replacement of essential metal ions by Ni$^{2+}$ are given in Table 1.1. Recently King et al. (1985) found that Ni$^{2+}$ dramatically activates the calmodulin-dependent phosphoprotein phosphatase in a time-dependent and irreversible manner. The Ni$^{2+}$ ion, like the endogenous divalent metal ions Mn$^{2+}$ and Mg$^{2+}$ maintains
Table 1.1

REPLACEMENT OF ESSENTIAL METAL IONS BY Ni\(^{2+}\)

<table>
<thead>
<tr>
<th>Biological system*</th>
<th>Effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Ni(^{2+})/Ca(^{2+}) antagonism</strong></td>
<td></td>
</tr>
<tr>
<td>1. Enzymes</td>
<td></td>
</tr>
<tr>
<td>Human’salivary α-amylase</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcal nuclease</td>
<td>-</td>
</tr>
<tr>
<td>2. Excitable tissues</td>
<td></td>
</tr>
<tr>
<td>Frog skeletal and cat heart muscles:</td>
<td></td>
</tr>
<tr>
<td>Uncoupling of:</td>
<td></td>
</tr>
<tr>
<td>Excitation</td>
<td>+</td>
</tr>
<tr>
<td>Contraction</td>
<td>-</td>
</tr>
<tr>
<td>Uterine contraction in rat:</td>
<td></td>
</tr>
<tr>
<td>Low levels</td>
<td>+</td>
</tr>
<tr>
<td>High levels</td>
<td>-</td>
</tr>
<tr>
<td>3. Exocytosis</td>
<td></td>
</tr>
<tr>
<td>Epinephrine in frog adrenal</td>
<td>+</td>
</tr>
<tr>
<td>Prolongs transmitter release in frog</td>
<td>+</td>
</tr>
<tr>
<td>neuromuscular junction</td>
<td></td>
</tr>
<tr>
<td>Amylase (rat parotid)</td>
<td>-</td>
</tr>
<tr>
<td>Growth hormone (bovine pituitary)</td>
<td>-</td>
</tr>
<tr>
<td><strong>B. Ni(^{2+})/Mg(^{2+}) antagonism</strong></td>
<td></td>
</tr>
<tr>
<td>Enzymes</td>
<td></td>
</tr>
<tr>
<td>Phosphoglucomutase (rabbit muscle)</td>
<td>+</td>
</tr>
<tr>
<td>Ribulose diphosphate carboxylase</td>
<td>+</td>
</tr>
<tr>
<td>Yeast enolase</td>
<td>-</td>
</tr>
<tr>
<td>Pyruvate kinase (rabbit muscle)</td>
<td>-</td>
</tr>
<tr>
<td>Avion myeloblastosis virus and <em>E. coli</em></td>
<td>-</td>
</tr>
<tr>
<td>DNA polymerases</td>
<td></td>
</tr>
<tr>
<td><strong>C. Ni(^{2+})/Zn(^{2+}) antagonism</strong></td>
<td></td>
</tr>
<tr>
<td>Enzymes</td>
<td></td>
</tr>
<tr>
<td>Carboxypeptidase A (bovine)</td>
<td>+</td>
</tr>
<tr>
<td>Aspartate transcarbamoylase (<em>E. coli</em>)</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatases (<em>E. coli</em>)</td>
<td>-</td>
</tr>
<tr>
<td>Carbonic anhydrase B (human)</td>
<td>-</td>
</tr>
</tbody>
</table>

* A decrease in enzyme activity of >70% relative to the native enzyme was considered inhibitory.
* Activation or stimulation +; inhibition -.

Reproduced from Nieboer *et al.* 1984d.
the phosphatase's structural stability. They speculate that the irreversible activation of the phosphatase by Ni\(^{2+}\) in the presence and absence of calmodulin indicates that the effect may be toxic by interfering with normal cellular regulation.

Ni\(^{2+}\) has been implicated in the inhibition of Ca\(^{2+}\) requiring hormone release. For example, Dormer et al. (1973) studied three different Ca\(^{2+}\)-requiring hormone systems in detail: the release of the salivary enzyme amylase from the parotid gland; the release of insulin from mouse pancreatic islets; and the release of growth hormone from bovine pituitary slices. In all of these cases the presence of Ni\(^{2+}\) inhibited the release of hormone or enzyme under conditions which would have been expected to cause stimulation.

As pointed out in the Cancer section of this chapter, Mn dust inhibits the carcinogenic response of alpha-Ni\(_{3}\)S\(_2\) when both were given as i.m. injections in the same thigh (Sunderman and McCully, 1983). This can be explained by the fact that Mn\(^{2+}\) might be expected to be a good replacement for Ni\(^{2+}\) (Mn\(^{2+}\) has borderline character; is slightly larger than Ni\(^{2+}\); and both prefer octahedral complexes) providing that the redox properties of Mn\(^{2+}\) do not become critical. In vitro and in vivo studies indicate that the presence of Mn dust retards the dissolution of of Ni\(_{3}\)S\(_2\) (Sunderman et al., 1976a; Hopfer and Sunderman, 1978). Presumably, the simultaneous dissolution of both Ni\(_{3}\)S\(_2\) and Mn dust (at the same injection point) sets up a competition of Mn\(^{2+}\) and Ni\(^{2+}\) for the same binding sites. In normal tissue, manganese is
generally present at higher concentrations than nickel (up to 15X) (Sumino et al., 1975). Mn$^{2+}$ deficiency occurs in cattle grazing on peat pasture that lacks this element, but human deficiency is not known (Passmore, 1976). Manganese compounds do not appear to be carcinogenic.

**Geometric preferences** of metal ions may play important roles in biochemical reactions. This is illustrated by the differences between Zn$^{2+}$ and Ni$^{2+}$. They are both borderline ions and form complexes of comparable stabilities. Most Zn$^{2+}$ metalloenzymes require a tetrahedral arrangement of ligands which is common for Zn$^{2+}$ stereochemistry, while the majority of four-coordinate complexes with Ni$^{2+}$ are square planar (Nieboer et al., 1984d). In Table 1.1, some Zn$^{2+}$-enzymes are inhibited, as expected, by Ni$^{2+}$ substitution. The enzymes that are activated by Ni$^{2+}$ substitution presumably do not strictly require the tetrahedral geometry favoured by Zn$^{2+}$.

As discussed in the absorption, distribution and excretion section of this chapter, there is a Cu$^{2+}$/Ni$^{2+}$ binding site on HSA. This primary and natural complex of Ni$^{2+}$ is characterized by a square pyramid with both nitrogen and oxygen binding sites (see Fig. 1.3) (Glennon and Sarkar, 1982; Sarkar, 1984; Dolovich et al., 1984). This illustrates the borderline character of both Cu$^{2+}$ and Ni$^{2+}$. Both Cu$^{2+}$ and Ni$^{2+}$ have almost the same ionic radii and are capable of 5-coordinate square pyramid geometry (Cotton and Wilkinson, 1980). Cu$^{2+}$ exhibits stronger binding to this site than does
Ni\(^{2+}\), which could have been predicted by the larger class B character possessed by Cu\(^{2+}\); the log (association constant) for Cu\(^{2+}\)-HSA is 16.1 and 9.5 for Ni\(^{2+}\)-HSA (Glennon and Sarkar, 1982). Not surprisingly, Cu\(^{2+}\) can replace Ni\(^{2+}\) bound to HSA. However, the human body is capable of generating specific antibodies that only recognize Ni\(^{2+}\) bound at this natural Cu\(^{2+}\) binding site (see Nickel Induced Asthma section of this chapter).

Figure 1.3. Proposed structure of the nickel-transport site of human albumin. Reproduced from Sarkar, 1981.

The binding of nickel to proteins after intraperitoneal (i.p.) injection, and in vitro incubation of \(^{63}\text{NiCl}_2\) with isolated fractions obtained from the lung and liver of
untreated mice, was investigated by Herlant-Peers et al. (1983). They found, in both in vivo and in vitro studies, that all cellular fractions (except the nuclear fraction) contained several nickel-binding proteins. In vivo incorporation of $^{63}$Ni$^{2+}$ into lung fractions (especially the mitochondrial and microsomal) showed consistently higher concentrations of label than did the liver fractions. This investigation not only illustrates that the lung is a target organ for nickel-retention (and possible toxicity), but also demonstrates that Ni$^{2+}$ is preferentially bound to the mitochondrial and microsomal fractions. Several proteins are implicated in the transport and metabolism of nickel in the cell. Thus we see that cells are able to compartmentalize nickel within them. A number of other studies have illustrated a similar ability of Ni$^{2+}$ to bind to low and high molecular mass components of rat, cow and human renal cytosol (Sunderman et al., 1981; Sunderman et al., 1983; Abdulwajid and Sarkar, 1983; Templeton and Sarkar, 1985; Templeton and Sarkar, 1986). The significance of renal intracellular compartmentalization will be discussed in Chapters 4 and 5.

Ni-transport proteins may be expected to mediate delivery of nickel to the nucleus of a cell. Once Ni$^{2+}$ is in the nucleus it can cause potentially detrimental effects. Mg$^{2+}$ is the counter ion to negatively charged phosphate groups and thus stabilizes genetic material (Eichhorn, 1981). It is also needed to regulate the incorporation of the proper nucleotides in both DNA and RNA synthesis (Spiro, 1980).
Ni$^{2+}$ has the ability to displace Mg$^{2+}$ (both have approximately the same radii and Ni$^{2+}$ will bind to an oxygen ligand better than Mg$^{2+}$). Replacement of Mg$^{2+}$ by Ni$^{2+}$ is known to decrease the fidelity of DNA synthesis (Sirover and Loeb, 1976). Ni$^{2+}$ has also been shown to bind to DNA and RNA polymerases and appears to deactivate them (Sirover and Loeb, 1976), probably by displacing Zn$^{2+}$. Thus it is not surprising that DNA lesions have been observed in nuclei isolated from rat tissues (kidney, liver and lungs) following i.p. injection of nickel carbonate (Ciccarelli and Wetterhahn, 1982). Recently Ciccarelli and Wetterhahn (1985) have found that $^{63}$Ni$^{2+}$ became bound to chromatin, polynucleosomes (DNA + histone octamer protein complex), and to deproteinized DNA, both in isolated intact nuclei and in vitro incubation of chromatin fractions. The amount of Ni$^{2+}$ bound depended on the concentration of Ni$^{2+}$, the presence of chromosomal proteins and the binding sites on DNA which provide a stable coordination environment for Ni$^{2+}$. As DNA containing Ni$^{2+}$ was isolated from chromatin, Ni$^{2+}$ appears to interact directly with the stable binding sites of the DNA molecule in this molecular moiety (Ciccarelli and Wetterhahn, 1985). The binding of Ni$^{2+}$ to chromatin and to DNA in isolated whole nuclei was much slower than in vitro incubation of isolated chromatin, indicating that accessibility of the DNA binding sites was influenced by the presence of the nuclear membrane. This illustrates the possibility of Ni$^{2+}$ being genotoxic and DNA its molecular target. Additional details on the
genotoxic and mutagenic potential of nickel compounds are provided in the Cancer section of this chapter.

It has been speculated that conformational changes in oligodeoxynucleotides from the normal right-handed B-helix to the left-handed Z-helix may have a role in the nickel(II) carcinogenic process (Boutayre et al., 1984; Rich et al., 1984). In a recent study, Rossetto and Nieboer (1987) have shown that conformational changes with poly d(G-C)·poly d(G-C) support the theory that interactions of Ni\(^{2+}\) with DNA are not specific. They found that all 20 metal ions tested, including several whose compounds are non-carcinogenic, promote the conversion of the B-helix to a Z-helix. Thus, although Ni\(^{2+}\) can interact with DNA, it may not be specific and hence may not be the ultimate carcinogen postulated in the somatic mutation model of cancer induction, unless preferential intranuclear compartmentalization occurs. This is in agreement with the non-specific mutagenic potential of nickel(II) that was pointed out earlier in the Cancer section.

Studies with the enzyme system xanthine/xanthine oxidase and with human PMNs indicate that the Ni\(^{3+}/Ni^{2+}\) redox couple can participate in dioxygen radical biochemistry (Nieboer et al., 1986; 1987a and b). This redox property of nickel suggests that it may have the ability to act as a chemical promoter of cancer (see Cancer section).

A large number of parameters and processes have been shown to be of importance in nickel biochemistry/toxicology:
namely, ion size, preferred geometry, binding preferences, isomorphous and non-isomorphous replacement of endogenous metal ions, complex stability, redox properties, lipid solubility, insolubility; and mode of entry, cellular uptake, phagocytosis, solubilization, membranes, compartmentalization, target organ, and molecular target. Surface passivity of solid nickel compounds, biological clearance, biological residence time and bioavailability were identified as possible determinants of reactivity in the discussion of nickel carcinogenesis (see Cancer section).

From this brief review of nickel biochemistry/toxicology it is obvious that the subleties of the inorganic chemistry and solution chemistry of nickel(II) provide it with agonistic and antagonistic roles.

(e) Other Toxic Effects of Nickel

Reproductive and Developmental Effects of Nickel. Since nickel has been demonstrated to cross the placenta, there has been concern over its reproductive and developmental effects. Because these effects of nickel have not been demonstrated in humans (EPA, 1986), they will not be reviewed in this thesis.

(f) Nephrotoxic Effects of Nickel

There are some reports of nephrotoxicity in humans attributed to nickel exposure. Such observations are very rare in comparison to the well-documented nephropathies induced by uranium, cadmium and lead (Nieboer et al., 1984a).
Gitlitz et al. (1975) reported proteinuria in persons who chronically drank water from a well contaminated with nickel, and Sunderman and Horak (1981) observed a significant relationship between increased urinary concentration of beta-2-microglobulin and hypernickeluria in nickel workers. However, Wall and Calnan (1980) found that there was no evidence of proteinuria in 17 nickel process workers in an electroforming plant during an outbreak of occupational dermatitis. It is important to note that other than in two cases of nickel carbonyl poisoning (Sunderman, 1977), no other cases of severe proteinuria, nor any other markers of significant renal disease have been reported despite years of collecting urine specimens (Nieboer et al., 1984a).

Gitlitz et al. (1975) have studied the biochemical and ultrastructural changes induced in the rat kidney after a single i.p. injection of a water-soluble nickel salt. They concluded that an acute reversible nephropathy is associated with nickel. Their work indicates that NiCl₂-induced nephropathy results mainly in glomerular damage, with renal tubular damage being less prominent and with focal tubular necrosis occasionally seen. Sunderman and Horak (1981) found that two days after injection of NiCl₂ in rats (6mg/kg b.w.), excretion of protein in the urine was increased four-fold, urine N-acetyl-D-glucosaminidase (NAG) activity was doubled, and the excretion of selected amino acids was increased ten-fold. A dose-response relationship was demonstrated for these three indices, with urinary protein being the most
sensitive index of NiCl$_2$-induced nephrotoxicity.

Because there is relatively little known about the renal excretion of nickel in man and animals, the present study was initiated in an attempt to fill part of this void. This work will assess the renal function of electrolytic refinery workers for clearance of nickel, and urinary markers of nephrotoxicity. The uptake and distribution of nickel in the kidney of an animal model will also be investigated.
CHAPTER 2 A SYSTEMATIC APPROACH TO ADJUSTMENT OF URINARY NICKEL CONCENTRATIONS FOR RATES OF URINE FLOW

INTRODUCTION

It is widely recognized that the volume of urine excreted varies widely with collection time. Even during short time periods, urinary flow rates are variable. Yet urine analysis is commonly used in all branches of medicine and particularly in the biological monitoring of workers. A number of strategies have been used to overcome these concentration-dilution effects on the analyte concentration of interest. Practices currently used include: (i) the 24-h collection; (ii) collection over a shorter but timed period (e.g., an 8-h collection); and (iii) the standardized, untimed specimen (the spot sample), such as the first void in the morning, and preshift or postshift specimens. The spot samples are often adjusted (normalized) to a constant specific gravity or are expressed per mole or gram of creatinine. However, results are also frequently reported without any adjustment (Pryde 1982). Thus practices for adjusting urinary concentrations in spot samples for different flow rates are inconsistent.

For urinary nickel spot samples, Tola et al. (1979) found that specific gravity normalization was more effective than creatinine adjustment in reducing the variability in nickel concentrations. Bernacki et al. (1978) chose to reject urine specimens with a specific gravity ($\rho$) below 1.012. However, Hagedorn-Gotz et al. (1977) and Adams (1980)
were able to reduce considerably the variation in individuals exposed to nickel carbonyl by creatinine normalization, as illustrated in Figure 2.1. Here, the uncorrected urinary-nickel values have a large variation with a probable but not systematic decrease over a four day period. When these nickel values were adjusted to a creatinine concentration of 1.6 g L\(^{-1}\), a reasonably smooth decay curve with less variation was the result. With reference to Figure 2.1, subsequent to the initial time point of exposure there appears to be a gradual increase in nickel levels. After a maximum was achieved, a slow and relatively smooth decrease in nickel levels occurred. Thus, from this information it appears that nickel reaches a maximum in the urine approximately 24-h after exposure. It is evident that volume standardization increased the toxicological information about this Ni(CO)\(_4\) exposure incident. Most researchers report unadjusted nickel levels, perhaps because specific guidelines are not available and because data for other urinary analytes often indicate that neither specific gravity nor creatinine normalization reduces the standard deviation from that found in unadjusted results (Graul and Stanley, 1982).

It is widely held that the 24-h urine collection obviates the concentration-dilution problems since amounts of analyte per unit time are measured (Pryde, 1982). However, there is evidence that concentrations in 24-h collections adjusted for specific gravity are a better index of exposure
than 24-h excretion rates (Elkins and Pagnotto, 1965). In the occupational setting, the single spot sample has been accepted as the most practical and convenient. Although 24-h collections are preferable in a clinical setting, a single spot or grab sample is commonly used for practical reasons. When 24-h collections are taken, it is common to express the analyte of interest in the amount excreted per day. With untimed spot samples, the common practice is to normalize the analyte concentration with creatinine rather than correct to a fixed specific gravity.

In this chapter a systematic approach to urinary nickel adjustments is outlined to provide more rigorous treatment to
this problem. The two cohorts examined involved International Nickel Company of Canada (INCO) employees working in electrolytic refining, one at Port Colborne (Ontario, Canada) and the second at Thompson (Manitoba, Canada). The nature of the electrolytic processes are now briefly summarised.

The secondary refining of nickel by electrolytic processes takes impure nickel (see Chapter 1B ii) to produce very pure nickel (>99.9%), while recovering impurities such as Fe, Co, Cu and precious metals (e.g., Au, Ag, and Pt). The following description is based on Boldt (1967), Mackenzie (1968) and Morgan (1979). The generalized flowchart for the two common electrolytic refining processes (electrorefining and electrowinning) is illustrated in Figure 2.2. The major difference depends on the type of anode utilized. At Port Colborne the majority of the anodes were made by the reduction of nickel oxide (≈74% Ni) to impure metal with petroleum coke as the reducing agent and then cast into anodes. These anodes were then placed into tanks containing electrolyte at ≈60°C as depicted in Figure 2.2. This yields an electrolytic process (electrorefining) that does not have a net cell reaction as illustrated below in Equations 2.1 and 2.2.

\[
\begin{align*}
\text{Ni}^{2+} \text{(in anolyte)} &+ 2e^- \text{(anode)} \rightarrow \text{Ni} \text{(anode)} \\
\text{Ni}^{2+} \text{(catholyte)} &+ 2e^- \text{(in cathode)} \rightarrow \text{Ni} \text{(cathode)}
\end{align*}
\]
Concrete electrolytic tank

Power source

Hydrostatic head

Impure electrolyte

Anode

Anode slimes

Canvas container

Cathode

Pure electrolyte

Purified electrolyte

Nia*(Catholyte)

Precipitation of Fe

Precipitation of Co

Precipitation of Cu

Recovery of sulphur

Precious metal recovery

Sulphide anode slimes

For removal of soluble impurities

Impure electrolyte

Cu, Co, Fe, Ni, As, Pb ions (Anolyte)

Anode slimes

Figure 2.2 General flowchart illustrating the principles of secondary refining by the electrolytic processes electrorefining and electrowinning. Adapted from Mackenzie, 1968.
However, at Thompson the anodes are cast from nickel matte (≈20% S) directly from the converter (smelter). This results in an electrolytic process that has a net cell reaction (electrowinning). These anodes are then placed into tanks containing electrolyte as depicted in Figure 2.2. The principal anode reaction is:

$$\text{Ni}_3\text{S}_2 \rightarrow 3\text{Ni}^{2+} + 2\text{S} + 6e^-$$  \hspace{1cm} (2.3)

while at the cathode:

$$3\text{Ni}^{2+} + 6e^- \rightarrow 3\text{Ni}$$  \hspace{1cm} (2.4)

resulting in a net cell reaction of

$$\text{Ni}_3\text{S}_2 \rightarrow 3\text{Ni} + 2\text{S}$$  \hspace{1cm} (2.5)

Around each cathode is a canvas-covered compartment that is used to divide the cell so that elements like Cu and Pb (lower in the electromotive series than Ni) in the anolyte will not plate out on the cathode. The anolyte passes from the anode part of the cell containing such elements as Co, Cu, Fe, Pb, and As while the Ag, Au, and Pt group of metals remain undissolved and are recovered from the slime that deposits at the bottom and sides of the tank. Iron is usually removed by first aerating the anolyte to obtain the ferric ion which is precipitated as the ferric hydroxide. Coprecipitation of Pb and As also occurs. Cobalt is precipitated as the cobalt(III) hydroxide after oxidizing with (e.g., Cl₂). Copper is removed by the addition of metallic nickel causing the precipitation of metallic Cu or
addition of H$_2$S to precipitate the copper as CuS. After removal of impurities, the electrolyte is pumped into the compartment around the cathode. The higher level of the catholyte creates a hydrostatic pressure so that there is continual flow of electrolyte out of this compartment into the anolyte, not allowing any mixing of the anolyte in the catholyte compartment. The pure electrolyte around the cathode that now contains "pure" Ni$^{2+}$ is plated out on the cathode with a purity of ≈99.93%.

From this description of electrolytic refining processes it is obvious that both groups of workers in this study are exposed mainly to soluble nickel. However, anode casting and the purification steps do result in exposure to particulates containing nickel and/or other elements.

B MATERIALS AND METHODS

For convenience and to avoid duplication, both the determination of nickel in urine and sera are described in this section, although the reporting of the serum nickel levels is delayed until Chapter 3.

(i) MATERIALS
(a) Chemical reagents

Pertinent information about the routine chemical reagents used are summarized in Table 2.1.
(b) Laboratory Solutions (Auto Analyzer)

Sodium Chloride (0.9% w/v). To prepare 1 L of sodium chloride solution, 9.0 g of NaCl was placed in a 1 L volumetric. Approximately 500 mL of ultra-pure water (DDW; Table 2.1) were added and mixed until completely dissolved. Prior to dilution to volume, 0.5 mL of the detergent Brij-35 were added.

Sodium Hydroxide (0.5 M). Approximately 800 mL of DDW were added to a 1 L volumetric flask containing 20.0 g of NaOH and allowed to dissolve. It was then brought to volume.

Saturated Picric Acid. Approximately 14 g of picric acid were added to a 1 L volumetric flask and DDW added to the mark. The mixture was periodically agitated during 4-h equilibration period. The solution was then filtered into a polyethylene (PE) bottle and stored in the dark.

Stock Creatinine Standard (1 mg/mL). Exactly 1.0 g of creatinine was transferred to a 1 L volumetric flask. This was dissolved and brought to volume with 0.1 M HCl.

(c) Laboratory Solutions (Nickel Determination)

Potassium Phosphate Buffer. To prepare 250 mL of buffer, 17.0 g of anhydrous KH₂PO₄ and 21.8 g of K₂HPO₄ were added to ~150 mL of DDW. The beaker was covered with Parafilm and allowed to sit overnight. The resulting solution was transferred to a 250-mL volumetric flask and brought to the mark with DDW. To this 0.1 g of APDC was added. The resulting solution was extracted a number of times with CHCl₃.
<table>
<thead>
<tr>
<th>Chemical Reagent</th>
<th>Source</th>
<th>Grade</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium pyrolidine dithiocarbamate</td>
<td>BDH, Toronto, Canada</td>
<td>Spectrosol</td>
<td>For atomic absorption spectrometry.</td>
</tr>
<tr>
<td>(APDC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\beta_2)-microglobulin Test Kits</td>
<td>Pharmacia Montreal</td>
<td>RIA-1</td>
<td>Determination of (\beta_2)-microglobulin in serum and urine by radioimmunoassay (RIA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\beta_2)-micro and RIA-2</td>
<td></td>
</tr>
<tr>
<td>Barbital IV (Sodium diethylbarbiturate)</td>
<td>Fisher</td>
<td>Purified</td>
<td></td>
</tr>
<tr>
<td>Barbital (5,5-diethylbarbituric acid)</td>
<td>Fisher</td>
<td>Purified</td>
<td></td>
</tr>
<tr>
<td>Brij-35</td>
<td>Technicon</td>
<td></td>
<td>Used to obtain optimum bubble pattern and maintain low noise in creatinine determination (0.5 mL per litre of saline solution).</td>
</tr>
<tr>
<td>Bromothymol Blue</td>
<td>BDH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{CHCl}_3)</td>
<td>BDH</td>
<td>Analara</td>
<td></td>
</tr>
<tr>
<td>Coomassie Brilliant Blue</td>
<td>Bio-Rad</td>
<td>G-250</td>
<td>5-fold concentrate</td>
</tr>
<tr>
<td>Creatinine</td>
<td>BDH</td>
<td>Analara</td>
<td></td>
</tr>
<tr>
<td>DD HCl</td>
<td>Double Distilled HCl</td>
<td></td>
<td>Double distilled constant boiling mixture (6 M HCl) of reagent grade HCl.</td>
</tr>
<tr>
<td>HCl</td>
<td>J.T. Baker</td>
<td>Analytical Reagent</td>
<td></td>
</tr>
<tr>
<td>(\text{HNO}_3)</td>
<td>J.T. Baker</td>
<td>Ultrex</td>
<td>60% (w/v)-solution.</td>
</tr>
</tbody>
</table>
Table 2.1a continued

<table>
<thead>
<tr>
<th>Chemical Reagent</th>
<th>Source</th>
<th>Grade</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Serum Albumin</td>
<td>Sigma</td>
<td>Albumin</td>
<td>Used for total protein standards in Bio-Rad determination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human Fraction V</td>
<td></td>
</tr>
<tr>
<td>K_2PO_4</td>
<td>BDH</td>
<td>ACS Assured</td>
<td></td>
</tr>
<tr>
<td>KH_2PO_4</td>
<td>BDH</td>
<td>ACS Assured</td>
<td></td>
</tr>
<tr>
<td>NH_4OH</td>
<td>Merck, Toronto, Canada</td>
<td>Ultrapure</td>
<td>13.4 M solution</td>
</tr>
<tr>
<td>Nickel Powder</td>
<td>Alpha cat. no. 0063</td>
<td>Ultrapure</td>
<td>Used for nickel standards Spherical 5 μm diameter.</td>
</tr>
<tr>
<td>NaCl</td>
<td>BDH</td>
<td>AnalaR</td>
<td></td>
</tr>
<tr>
<td>NaOH</td>
<td>BDH</td>
<td>Analytical Reagent</td>
<td></td>
</tr>
<tr>
<td>4-Methylpentane-2-one (MIBK)</td>
<td>BDH</td>
<td>Laboratory Reagent</td>
<td>For atomic absorption spectroscopy.</td>
</tr>
<tr>
<td>Picric Acid</td>
<td>BDH</td>
<td>AnalaR</td>
<td></td>
</tr>
<tr>
<td>Pb(NO_3)_2</td>
<td>BDH</td>
<td>AnalaR</td>
<td>Used for lead standards.</td>
</tr>
<tr>
<td>Ultra-pure water</td>
<td></td>
<td></td>
<td>Prepared by demineralizing laboratory distilled water (Corning LD-2a demineralizer) and distilling it in a Corning Mega-Pure still.</td>
</tr>
<tr>
<td>(DDW)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Some of the items listed in this Table are employed for the work described in Chapter 3.
until there was no trace of yellow in the CHCl₃ layer. The purified buffer solution and the last CHCl₃ extract were tested for nickel content. The buffer was transferred to a polyethylene bottle the cap sealed with Parafilm; storage was in the dark. The final pH of the solution was 6.7.

Bromothymol Blue Indicator Solution. Into a 60 mL screw-cap polyethylene drop-dispenser bottle, 20 mg of bromothymol blue and 1 mL of dilute NH₄OH solution were added. The contents were diluted to 50 mL with DDW and stored in a wash bottle. The delivery tube was capped and the bottle was wrapped with Parafilm. It was stored in the dark.

APDC (2% w/v). 0.5 g of APDC was added to a 50 mL Falcon Tube (not acid washed). This was dissolved in 25 mL of DDW and extracted with MIBK until the last two extractions were colourless (usually three extractions were required).

Stock Nickel Standard (1000 mg/L). Into an acid washed 150-mL beaker, exactly 0.50 g of nickel powder (spherical 5 μm diameter) was added. Approximately 5 mL of HNO₃ (Ultrex) were added and the mixture was stirred under low heat until dissolution was complete. The cooled solution was transferred to a 500 mL volumetric flask and brought to the mark with DDW. The solution was stored in a Parafilm-wrapped screw-capped polyethylene bottle. This solution was stable for ~1 year.

Nickel Intermediate Standard (1 mg/mL). With a fixed volume 50-μL Eppendorf pipettor (calibrated for delivery with water), 50 μL of the nickel stock standard solution was
transferred to a 50 mL (acid-washed) glass volumetric flask and diluted to volume with 1% DD HCl (0.12 M HCl). This solution was kept for up to six weeks.

(d) Decontamination of Plastic and Glasswares

In Table 2.2, details are provided about the glassware and plasticware employed. The protocols to prevent extraneous additions during collection, handling and analytical procedures were patterned after Nieboer and Jusys (1983). Throughout the cleaning of wares, plastic gloves were worn and special effort was taken to avoid contact with stainless steel items (e.g. fume-hood linings and sinks). All wares used in the procedures for nickel were first washed with a hot non-ionic detergent solution, rinsed with DDW and then subjected to acid-washing. Items were first placed in acid-washed snap-capped polyethylene canisters and soaked in 6 M HCl in a batch fashion for 2 to 7 days on a plastic tray in a fume hood cupboard. In the case of large items (e.g., 500-mL PE bottles) the 6 M HCl was poured directly into the containers, and the tops screwed on tightly so as to ensure direct contact with the screw cap. After decantation of the HCl, the wares were rinsed at least three times with DDW. This was followed by a soaking for 2-7 days in dilute double-distilled (DD) HCl (≈ 1M HCl) until use. At that time, they were then rinsed several times with DDW and dried in an aluminium oven at 110°C on plastic coated test-tube
Table 2.2a

<table>
<thead>
<tr>
<th>Description</th>
<th>Source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide mouth bottles</td>
<td>Nalgene, Fisher</td>
<td>Polyethylene (PE) screw cap bottles (500 mL ); collection bottles for urine voids.</td>
</tr>
<tr>
<td>Centrifuge tubes</td>
<td>Corning 25319-15, Fisher</td>
<td>Polypropylene (PP) screw cap centrifuge tubes (15 mL); for storage of serum.</td>
</tr>
<tr>
<td>Centrifuge tubes</td>
<td>Falcon 2098, Becton-Dickinson</td>
<td>Polypropylene (PP) screw cap centrifuge tubes (50 mL); for storage of urine.</td>
</tr>
<tr>
<td>Centrifuge tubes</td>
<td>Falcon 2059, Becton-Dickinson</td>
<td>Polypropylene (PP) snap-cap round bottom tubes 17x100 mm; for determination of nickel in serum.</td>
</tr>
<tr>
<td>Syringes</td>
<td>Sarstedt</td>
<td>Serum Monovette syringes (polypropylene 10 mL) with separation aid; for collection of blood.</td>
</tr>
<tr>
<td>Needles</td>
<td>Intravenous Cannulae 6745, Becton-Dickinson</td>
<td>I. V. catheter (polyethylene cannulae with teflon needle hubs) 20G 5 cm length with 23G inner needle; for collection of blood.</td>
</tr>
<tr>
<td>Ultracentrifuge tubes</td>
<td>Beckman 339573</td>
<td>Threaded-top polycarbonate tubes with a plug, O-ring and noral cap (maximum speed 650000 RPM).</td>
</tr>
</tbody>
</table>

a. Some of the items listed in this Table are employed for the work described in Chapter 3.
racks. The 500 mL PE urine specimen bottles, the 50-mL Falcon tubes and the 15-mL Corning centrifuge tubes were allowed to air-dry after vigorous shaking to remove excess water by inverting on a clean lab bench covered with absorbent bench paper (Canlab) placed on paper towels. After drying, all wares were wrapped with Parafilm, placed in sealed plastic bags and stored in boxes until use.

The syringes used in blood collection required special treatment. They were subjected to the same acid washes described above except that this was done in smaller batches and special care was taken to ensure that they were completely filled with the wash or rinse solutions. After removal from the last DDW rinse, they were dried by lyophilization while being held in an acid-washed glass jar. Subsequently, the tops and bottoms of the syringes were wrapped with Parafilm and stored in sealed plastic bags until use.

(ii) DONOR POPULATIONS
(a) Nickel.

This study was carried out on 26 subjects occupationally exposed to nickel at INCO's electrolytic refining operations in Port Colborne (Ontario, Canada) and Thompson (Manitoba, Canada). A single multi-void 24-h collection involving twenty workers was conducted at Thompson. The study group consisted of workers from each of three shifts (day shift, 7am - 3pm; afternoon shift, 3pm - 11pm; midnight shift, 11pm
- 7am). The Port Colborne collection had two components. The first 24-h multi-void collection (day shift, 7am - 3pm) was made just prior to a one-month layoff and the second multi-void 24-h collection was made on their return to work. The mean ± SD age of the Port Colborne donors was 48 ± 12 years while for Thompson it was 45 ± 8 years.

(b) Lead.

This study was carried out on one subject who was occupationally exposed to lead when cutting lead-chromate painted surfaces. The subject was 50 years old.

(iii) METHODS

(a) Urine Collection Procedures

Each individual urine void during the 24-h collections was made directly into a separate acid-washed 500 mL PE bottle. The time of each void was recorded on the form provided (Table 2.3) so that the flow rate in mL min⁻¹ could be calculated from the urine volume and the elapsed time between voids. Particular care was taken to inform the donors as to the importance of chance contamination from hands and clothing, and recording the correct time of each void. In Table 2.4, the instructions given each donor for specimen collection are reproduced. All samples were weighed in the 500 mL preweighed polyethylene collection bottles and wrapped with Parafilm before transport to the laboratory. The urine was not acidified, but was transported by car to the analytical laboratory as soon as possible (Port Colborne).
Table 2.3

24-HOUR URINE COLLECTION RECORD

Company/Location ____________________________________
Donors Code ______________
Employee Identification Number ________________
Dates from _____________ to ________________

<table>
<thead>
<tr>
<th>Sample Code Number</th>
<th>Time of Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TO BE COMPLETED AT END OF 24-HOUR COLLECTION;
Numbers of urine samples returned: ________________
Numbers of empty sample bottles returned: __________
COMMENTS; __________________________________________
Table 2.4

INSTRUCTIONS FOR 24-HOUR URINE COLLECTION

These instructions must be followed to avoid contamination of the specimen, to assure proper collection and to avoid the mixing up of samples.

1. Use the urine collection bottles in sequence (for example: A1, A2, A3, etc.)

2. A new bottle must be used for each urination.

3. All urine must be collected for each urination (even if a second bottle is required). Make sure your bladder is completely empty.

4. Please record the time of each urination on the collection record.

5. Never rinse out any of the collection bottles with soap or water (each bottle has been specially treated).

6. Never add any tap water to the urine.

7. Your hands must be washed before giving the urine sample.

8. Males are asked to lower pants before urinating.

9. Collection bottle must not come in contact with any clothing.

10. After urine has been collected, screw the cap on tightly.

11. At work, all urine specimen collection should be done in the Nurses' Station.

12. All bottles (used and empty) must be returned.
or frozen for transport by air to Hamilton (Thompson). Each void was analyzed for nickel, creatinine and specific gravity ($\rho$).

(b) **Serum Collection Procedure**

Blood samples were collected using the IUPAC reference procedure (Brown et al., 1981). The shallow depression at the bend of the elbow (antecubital fossa) was cleansed with an ethanol swab and allowed to dry by evaporation. A tourniquet was applied while a polyethylene intravenous catheter was inserted into an antecubical vein. The stylus of the catheter was removed, and blood (>3 mL) was allowed to flow through the plastic catheter to remove any nickel contamination in the catheter. This blood was discarded. A polypropylene syringe (with separation beads) was used to collect 10 mL of blood. After collection, the handle of the syringe was removed and the open tip of the syringe was wrapped in Parafilm. The blood was allowed to clot for 50 to 90 min at room temperature in the syringe held in an upright position by a plastic coated testube rack. The syringe was then centrifuged at 900 g for 20 min. The serum was transferred with an acid-washed Pasteur pipet to an acid-washed 15 mL acid-washed PP centrifuge tube, wrapped with Parafilm, and transported as soon as possible by car (Port Colborne) or was frozen for air transport to Hamilton (Thompson). Samples were kept frozen until they were analyzed for nickel.
(c) Determination of Urinary Nickel

On arrival at the laboratory, urine samples were mixed to resuspend any solids and a representative aliquot was transferred to a 50-mL Falcon tube and stored in a cold room at 4°C until analysis. Urinary nickel was determined by direct extraction (without digestion) of nickel into a MIBK layer for detection by electrothermal atomic absorption spectrometry (EAAS). Verification of this method relative to the IUPAC nickel reference method (Brown et al., 1981) indicated comparable accuracy and precision (Jusys et al., 1982). Care was taken to avoid contaminating the samples and measures to assure low blank values as recommended by Nieboer and Jusys (1983) are incorporated in the analytical procedures described below.

The nickel working standards were prepared fresh daily by pipeting accurately with a 2-mL graduated Mohr pipet (500 μL, 1000 μL, 1500 μL, 2000 μL) of the nickel intermediate standard solution into 50-mL glass volumetric flasks. This constituted standards of 10, 20, 30 or 40 μg L⁻¹ used for urine with high nickel concentrations. For samples with low urinary nickel values the working standards were prepared by pipeting accurately with a 1 mL Mohr pipet 200, 400, 800, or 1200 μL of the intermediate nickel standard into a 100-mL glass volumetric flasks. This resulted in standards of 2, 4, 8 or 12 μg L⁻¹. All of the working standards were brought to volume with DDW.

Exactly 2 mL (calibrated Gilson pipettor) of standards
or blanks (DDW) were transferred to acid-washed test tubes. For urine samples with low amounts of nickel exactly 2 mL was transferred to acid-washed glass centrifuge tubes. When high urinary nickel was anticipated, the urine samples were diluted with DDW and 2 mL transferred to glass centrifuge tubes.

Prior to extraction, bromothymol blue indicator solution (3 drops) was added to each tube. Then 50 μL (from a fixed volume 50-μL Eppendorf pipettor) of phosphate buffer was added. The solutions were adjusted to a pH 7 by dropwise addition of dilute NH₃ (1:3 v/v). Following this, 0.5 mL (Gilson pipettor) of 2% (w/v) APDC solution was added. The resulting mixture was vortexed for 10s and allowed to stand for 5 min. From a calibrated automatic dispenser, 1.0 mL of MIBK was then added and vortexing for 35s followed. When the aqueous and MIBK phases had separated on standing (standards and blanks) or after centrifugation (15 min at 1000 g) of the urine samples, the MIBK layer was transferred to sample cups and analyzed for Ni by EAAS. Instrumental settings and the temperature-control program employed are summarized in Table 2-5.

(d) Determination of Urinary Lead

The approach adopted was identical to that of the direct extraction of urinary nickel except that the graphite furnace temperature control program was altered because of the
Table 2.5

INSTRUMENTAL PARAMETERS AND SETTINGS FOR Ni ANALYSIS BY EAAS

A  AA Spectrometer Parameters
(Perkin-Elmer Model 703)

1. Lamp Current 25 mA (Hollow cathode)
2. Wavelength 232.0 nm
3. Slit Width 3 (0.2 nm)
4. D₂ Background Corrector OFF
5. Peak Integration Time 5s
6. Signal Absorbance
7. Mode Peak Height
8. Recorder Int

B  AUTOMATIC SAMPLER
(Perkin-Elmer Model AS-1)

1. Sample Size 20 μL

C  GRAPHITE FURNACE PARAMETERS
(Perkin Elmer Model HGA-500)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp(°C)</th>
<th>Ramp(s)</th>
<th>Hold(s)</th>
<th>Read</th>
<th>Base Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Drying</td>
<td>150</td>
<td>20</td>
<td>10</td>
<td>off</td>
</tr>
<tr>
<td>2</td>
<td>Ashing</td>
<td>900</td>
<td>40</td>
<td>20</td>
<td>off</td>
</tr>
<tr>
<td>3</td>
<td>Atomization</td>
<td>2600</td>
<td>0</td>
<td>5</td>
<td>on</td>
</tr>
<tr>
<td>4</td>
<td>Conditioning</td>
<td>2700</td>
<td>0</td>
<td>2</td>
<td>off</td>
</tr>
<tr>
<td>5</td>
<td>Conditioning</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>at 18s</td>
</tr>
</tbody>
</table>

a. Internal argon flow was held at 300 mL min⁻¹ except in Step 3, where it was 50 mL min⁻¹.
Table 2.6

INSTRUMENTAL PARAMETERS AND SETTINGS FOR Pb ANALYSIS BY EAAS

A  **AA Spectrometer Parameters**  
(Perkin-Elmer Model 703)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lamp power</td>
<td>10 watts</td>
</tr>
<tr>
<td>(Continuous discharge)</td>
<td></td>
</tr>
<tr>
<td>2. Wavelength</td>
<td>283.3nm</td>
</tr>
<tr>
<td>3. Slit width</td>
<td>4</td>
</tr>
<tr>
<td>4. D₂ Background Corrector</td>
<td>On</td>
</tr>
<tr>
<td>5. Peak Integration Time</td>
<td>3s</td>
</tr>
<tr>
<td>6. Signal</td>
<td>Absorbance</td>
</tr>
<tr>
<td>7. Mode</td>
<td>Peak Height</td>
</tr>
<tr>
<td>8. Recorder</td>
<td>Int</td>
</tr>
</tbody>
</table>

B  **AUTOMATIC SAMPLER**  
(Perkin-Elmer Model AS-1)

| Sample size          | 20 µL |

C  **GRAPHITE FURNACE PARAMETERS**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp(°C)</th>
<th>Ramp(s)</th>
<th>Hold(s)</th>
<th>Read</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Drying</td>
<td>110</td>
<td>10</td>
<td>30</td>
<td>off</td>
</tr>
<tr>
<td>2</td>
<td>Ashing</td>
<td>650</td>
<td>10</td>
<td>30</td>
<td>at 20s</td>
</tr>
<tr>
<td>3</td>
<td>Atomization</td>
<td>1800</td>
<td>1</td>
<td>6</td>
<td>on</td>
</tr>
<tr>
<td>4</td>
<td>Conditioning</td>
<td>2500</td>
<td>1</td>
<td>2</td>
<td>off</td>
</tr>
<tr>
<td>5</td>
<td>Conditioning</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>at 32s</td>
</tr>
</tbody>
</table>

a. Internal argon flow was held at 300 mL min⁻¹ except in Step 3, where it was 40 mL min⁻¹.
relatively high volatility of lead. The instrumental settings and the temperature-control program employed are summarized in Table 2.6. The standards were made up with Pb(NO₃)₂ (Analar). Both samples and standards were treated in the same manner as in the determination of urinary nickel.

(e) Determination of Serum Nickel

The analytical procedure was adopted from Sunderman et al. (1984) and details are described below. The working standards and blanks were made up in an identical manner to the low standards (2, 4, 8 and 12 µg L⁻¹) used in the urinary nickel determination.

The serum was thawed at room temperature and 2.0 mL (1-mL Gilson pipettor) were added to 10-mL Falcon centrifuge tubes (model 2059). While the serum was being vortexed, 100 µL (100 µL fixed-volume Eppendorf) of concentrated HNO₃ (Ultrex) was added directly to the tube. The vortexing was continued for one min. Then each Falcon tube was stoppered, wrapped with Parafilm and placed in a water bath for 10 min at 70°C. After this incubation, the samples were then centrifuged for 30-40 min at 2300 rpm. One mL of protein-free supernatant was transferred to a 15-mL glass centrifuge tube and two drops of bromothymol blue indicator were added to the supernatant. Phosphate buffer (25 µL) was then added, followed by ~3 drops of concentrated (Ultrex, 13.4M) NH₃ and ~4 to 7 drops of dilute (1:3 v/v) NH₃ until the colour changed from a light yellow to a light blue-green. If there
were any signs of haemolysis, no phosphate buffer was added but instead 50 μL of concentrated NH₃ (13.4 M) was added after neutralization to bring the pH above 9.0. Then 0.25 mL of 2% APDC solution was added, and the sample was vortexed for 10s. The mixture was allowed to stand for 5 min and then 0.5 mL of MIBK was added prior to vortexing for 35s. When the aqueous and MIBK phases had separated (no centrifuging was necessary), the MIBK layer was transferred to acid-washed polyethylene sample cups and analyzed for Ni by EAAS. Instrumental settings and the temperature-control program employed are summarized in Table 2.5.

Blanks (DDW) and standards were treated the same as serum samples, except that they were not centrifuged and were extracted into 1 mL of MIBK after the addition of 0.5 mL of 2% APDC solution.

**f) Determination of Serum and Urinary Creatinine**

These were measured by the Jaffé reaction employing a single-channel Technicon Auto Analyzer. This method was compared to an established continuous-flow Jaffé micromethod used in the Clinical Chemistry Laboratory at the Chedoke-McMaster Medical Centre (Ryan and Walker, 1980) and against the manual Jaffé method according to Adams (1980).

The sera were run directly, while the urines were diluted by a factor of 33.33 with 0.02 M HCl. The working standards were prepared by adding 1, 3, 5, 7 and 10 mL of the stock standard (1 mg mL⁻¹) to a 100-mL glass volumetric flask
and bringing to volume with 0.02 M HCl to give a final concentration of 1, 3, 5, 7 and 10 mg L\(^{-1}\).

The samples and standards were placed in a series of sample cups on the rotating tray of the Technicon Autoanalyzer Sampler II. A sample of DDW was inserted between each sample to reduce "carry over". The sample is removed by a probe which is activated by a pump (Fig. 2.3). The sample is separated by air bubbles ("slugs") and passed through a dialyzer at 37°C which allows creatinine to pass from the sample stream to the DDW stream on the other side of the dialyzing membrane but impervious to high-molecular-mass compounds. Saturated picric acid is mixed with 0.5 M NaOH.

Figure 2.3. Continuous-flow analyzer manifold employed in creatinine analysis. The tubes with numbers eight and nine were switched in order to attain a good bubble pattern in tube nine. Reproduced from Technicon Auto Analyzer Methods Manual.
and then comes into contact with the sample from the dialyzer (see tubes 10 and 12, Fig. 2.3). Next the stream is passed through a 12m time-delay coil for colour development. From where it passes through a debubbler, to a 15-mm flowthrough UV/VIS cell where the absorbance is measured continuously at 505 nm.

(g) Determination of Specific Gravity

Specific gravity was measured on a hand-held, temperature-compensated, refractometer (American Optical Corporation). A small drop of urine was placed on the lower glass surface of the meter. The upper glass surface (hinged) was lowered on the drop bringing the two surfaces firmly in contact. The meter was held toward a light source allowing light to pass through the sample and prisms. The specific gravity was read directly from a scale at the point where a sharp line of contrasting light and dark area formed.

(h) Determination of Osmolality

This parameter was measured by the Clinical Chemistry Laboratory of the Chedoke-McMaster Hospital by freezing-point depression of centrifuged urine samples using an Advanced Instruments Osmometer Model No. 68-L.
C RESULTS

(i) NICKEL CONCENTRATIONS IN BODY FLUIDS

(a) Urinary Nickel.

The smallest concentration of nickel that could be detected with 95% probability [i.e., detection limit = \( \bar{x}(\text{blanks}) + 2.3\sigma(\text{blanks}) \); Nieboer and Jusys, (1983)] was 1.1 \( \mu \text{g L}^{-1} \) corresponding to four blanks per experiment. The blank was assessed to contain (mean \( \pm \) SD) 0.4 \( \pm \) 0.3 \( \mu \text{g L}^{-1} \) of Ni (4 experiments, 4 blanks per experiment). The run to run precision had a coefficient of variation (CV = 100 SD/\( \bar{X} \)) of 8.9 percent based upon 11 experiments employing the same urine sample with a concentration of 52 \( \pm \) 5 \( \mu \text{g L}^{-1} \).

(b) Serum Nickel.

Recovery tests were performed by adding 5 \( \mu \text{g L}^{-1} \) to each sample. The recovery of nickel averaged 101 \( \pm \) 11 percent (range 86 to 116; n=6). The smallest concentration of nickel that could be detected with 95 percent probability was 0.39 \( \mu \text{g L}^{-1} \) corresponding to four blanks per experiment. The blank was assessed to contain 0.14 \( \pm \) 0.11 \( \mu \text{g L}^{-1} \) of Ni. The run to run precision had a CV of 12.8 percent based on seven experiments employing the same serum sample. The serum nickel concentration from an unexposed donor was 0.38 \( \pm \) 0.17 \( \mu \text{g L}^{-1} \) (n=14, range 0.19 to 0.73).

(c) Urinary Lead.

The recovery tests were performed by adding 10 \( \mu \text{g L}^{-1} \) to
each sample. The recovery of lead averaged 105 ± 9 percent
(range 93 to 120; n=4).

(ii) 24-h COLLECTIONS

As illustrated in Figures 2.4 to 2.7, there are wide
variations in urine output during the day, and this must
obviously result in fluctuating analyte concentrations. In
general, the urinary analyte levels (nickel, creatinine and
specific gravity) have an inverse relationship with urine
flow rate, \( V \), (in mL min\(^{-1}\) and calculated from the urine
volume and the elapsed time between voids). As the urine
flow rate changes (increases or decreases), the analyte
concentration varies in the opposite direction. There
appears to be no relationship between the variation of these
analytes and the particular work shift that an individual is
on, nor on the time of collection. Because of the variations
observed, it is mandatory that for untimed urine samples some
manner of adjusting to a standard flow rate needs to be
sought.

The relationships developed by Araki (1980) for lead,
\( \delta \)-aminolaevulinic acid, coproporphyrin, creatinine, total
solute and urinary volume open up a more systematic approach
to volume correction. Urinary nickel, creatinine and total
solute were found to conform to the relationship in Equation
2.6 by Araki (1980)

\[
U^0_i = U_i V^{bi}
\]  

(2.6)
Figure 2.4. Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the day work shift for the Port Colborne collection which started at 7:00 am and ended at 3:00 pm.
Figure 2.5. Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the day work shift for the Thompson collection which started at 7:00 am and ended at 3:00 pm.
Figure 2.6. Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the afternoon work shift for the Thompson collection which started at 3:00 pm and ended at 11:00 pm.
Figure 2.7. Fluctuations in specific gravity, urine flow and urinary nickel and creatinine concentrations for a nickel electrolytic refinery worker during a 24-h multi-void collection. The diagonal lines indicate the midnight work shift for the Thompson collection which started at 11:00 pm and ended at 7:00 am.
where $U_i$ is the concentration of "i" in the urine, $V$ is the urine flow (mL min$^{-1}$), $U_0^i$ is the urinary concentration of i at a flow rate of 1 mL min$^{-1}$, and $b_i$ is the volume exponent or power coefficient. Typical regression analysis plots of $\log(U_i)$ versus $\log(V)$ for the individual voids from a single donor participating in the 24-h collections at Port Colborne and Thompson are illustrated in Figures 2.8 to 2.12. Estimates of $b_i$ and $U_0^i$ are summarized in Table 2.7. The averages of the power coefficients $b_{Ni}$, $b_{\rho-1}$ and $b_{\text{osmol}}$ are comparable, whereas that for $b_{\text{creat}}$ is substantially different. Thus it appears that creatinine has a different flow-dependence than nickel, total solids and osmolality.

The dependence of the magnitude of the power coefficient $b_i$ on donor is illustrated in Figures 2.13 to 2.16. In Figure 2.13, the magnitude of the volume coefficient $b_i$ versus donor is plotted. The $b_{\text{creat}}$ values are arranged in decreasing values from left to right. From this diagram it is evident that the $b_{Ni}$ and $b_{\rho-1}$ values are consistently smaller than those for creatinine, although it is not clear how close the values of $b_{Ni}$ and $b_{\rho-1}$ are to each other. In Figure 2.14, the $b_{\text{creat}}$ values are plotted against an arbitrary scale for individual donors so that a linear fit of this data is obtained. It is clear from this plot that $b_{Ni}$ and $b_{\rho-1}$ values are roughly arranged in a linear manner below that of the $b_{\text{creat}}$ line. Figures 2.15 and 2.16 depict the linear fit of $b_{Ni}$ and $b_{\rho-1}$ coefficients with respect to this arbitrary donor scale. A comparison of the regression
Figure 2.8. A scatter diagram showing the linear relationship between $\log(U_{Ni})$ and $\log(V)$ for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure ($p<0.005$).
Figure 2.9. A scatter diagram showing the linear relationship between \( \log(U_{\text{creat}}) \) and \( \log(V) \) for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure (\( p<0.001 \)).

\[ b_{\text{creat}} = 1.04 \]
\[ U_{\text{creat}}^0 = 1.33 \text{ g/L} \]
\[ r = 0.99 \]
Figure 2.10. A scatter diagram showing the linear relationship between $\log(U_{\text{solute}})$ and $\log(V)$ for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure ($p<0.001$).
Figure 2.11. A scatter diagram showing the linear relationship between log($U_{P_b}$) and log(V) for one donor. The regression parameters are indicated in the figure ($p<0.001$).
Figure 2.12. A scatter diagram showing the linear relationship between log($U_{Osmol}$) and log($V$) for one donor from the first Port Colborne collection. The regression parameters are indicated in the figure ($p<0.001$).
Figure 2.13. Dependence of the magnitude of the volume power coefficient $b_i$ on donor. The $b_{\text{creat}}$ values (−O−O−) are arbitrarily arranged from left to right according to decreasing value for 11 individuals. The corresponding $b_{N_i}$ (−A−A−) and $b_{P-1}$ (−■■■−) values for each donor are also plotted. As explained in the footnotes to Table 2.7, $b_i$ values were obtained by regression analysis plots of $\log(U_i)$ versus $\log(V)$. The subscripts $T$ and $P$ represents the Thompson and Port Colborne collections respectively.
Figure 2.14. Linearization of $b_i$ values represented in Figure 2.13. Instead of ranking the donors on a unitary scale donor $P_T$ was taken as 1 on an arbitrary $x$-scale and $K_T$ as 26. The remainder of the donors were plotted linearly according to $b_{\text{creat}}$ (○○○). The $b_{Ni}$ (▲▲▲) and $b_{\rho-1}$ (■■■) were plotted similarly.
Figure 2.15. Linearization of $b_{Ni}$ values. See legend to Figure 2.14 for explanation. ($r = 0.67$ and $p < 0.05$)
Figure 2.16. Linearization of $b_{\rho-1}$ values. See legend to Figure 2.14 for explanation. ($r = 0.78$ and $p < 0.005$)
Table 2.7  
VOLUME EXPONENTS ($b_i$) AND URINARY CONCENTRATIONS ($U_i^0$) AT UNIT URINE FLOW RATES$^a$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Port Colborne (First Collection)$^c$</th>
<th>Thompson Group$^d$</th>
<th>Overall$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_Ni$</td>
<td>—</td>
<td>—</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>$b_{\rho-1}$</td>
<td>—</td>
<td>—</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>$b_{\text{crea}}$</td>
<td>0.7 ± 0.1</td>
<td>—</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>$b_{\text{osmol}}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$U_Ni^0$</td>
<td>28 ± 6 $\mu$g L$^{-1}$</td>
<td>60 ± 30 $\mu$g L$^{-1}$</td>
<td>—</td>
</tr>
<tr>
<td>(range)</td>
<td>(29-33)</td>
<td>(19-123)</td>
<td>—</td>
</tr>
<tr>
<td>$U_{\text{crea}}^0$</td>
<td>1.26 ± 0.06 g L$^{-1}$</td>
<td>1.27 ± 0.18 g L$^{-1}$</td>
<td>—</td>
</tr>
<tr>
<td>$U_{\rho-1}^0$</td>
<td>0.0217 ± 0.001</td>
<td>0.018 ± 0.003</td>
<td>—</td>
</tr>
<tr>
<td>$U_{\text{osmol}}^0$</td>
<td>743 ± 4 m mol kg$^{-1}$</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$The parameters found for one 24-h multi-void collection from a worker exposed to lead are: $b_{\text{Pb}} = 0.9$; $U_{\text{Pb}}^0 = 40$ $\mu$g L$^{-1}$.

$^b$These parameters are defined in Equation 2.6, namely:
$U_i^0 = U_i V^{b_i}$. Here, $V$ is the urine flow (mL min$^{-1}$; calculated from the urine volume and the elapsed time between voids), $U_i$ is the concentration of "i" in the urine, $U_i^0$ is the urinary concentration of "i" at a flow rate of 1 mL min$^{-1}$, and $b_i$ is the volume exponent or power coefficient.

$^c$Only the data for three workers had a wide enough range of $V$ values for linear regression analysis.

$^d$Data for eight workers had a wide enough range of $V$ to allow linear regression analysis.

$^e$Average for both groups; like $U_i^0$ values, $b_i$ values were obtained from plots of log $U_i$ versus log $V$. 
parameters describing the fitted lines clearly illustrates that \( b_{\text{creat}} > b_{\rho 1} \approx b_{\text{Ni}} \).

As expected, there is a significant variation in the flow rates of the individual voids of each donor throughout the 24-h collection period (Fig. 2.17). By comparison and as illustrated in Figure 2.18 there is a smaller but still substantial variation in flow rate in the 24-h collections. The most frequently observed flow rate for both types of collections is centered around 1.0 mL min\(^{-1}\). The average values of the flow rate for individual voids and the 24-h collections are summarized in Table 2.8: overall averages of 1.6 ± 1.5 mL min\(^{-1}\) and 1.2 ± 0.5 mL min\(^{-1}\) for untimed spot samples and 24-h collections, respectively. In both cases, the Thompson collections have a lower flow rate than the Port Colborne collections. It is also noticeable that the variance as judged by the magnitude of the standard deviation is 2.5 to 3 times smaller for the 24-h collections in all instances.

In Figures 2.19 to 2.21, urinary nickel \( (U_{\text{Ni}}) \) and creatinine \( (U_{\text{creat}}) \) levels are compared. For arbitrary groupings of donors, a rough correlation exists between these two parameters, although the absence of a single relationship implies the variation of an underlying independent variable(s). A similar conclusion is reached from an examination of plots of urinary nickel versus total solutes \( (U_{\rho 1}) \) as illustrated in Figures 2.22 to 2.24. The data in Figures 2.23 and 2.24 do suggest an improvement for the
Figure 2.17. Distribution of urine flow rates for individual voids corresponding to the Port Colborne and Thompson collections (210 voids).
Figure 2.18. Distribution of urine flow rates for 24-h collections corresponding to the Port Colborne and Thompson collections (n = 32).
Figure 2.19. A scatter diagram showing the relationship between $U_{Ni}$ and $U_{creat}$ for selected donors in the Thompson cohort.
Figure 2.20. A scatter diagram showing the relationship between $U_{Ni}$ and $U_{creat}$ for selected donors in the Thompson ($C_T$, $K_T$, $F_T$) and Port Colborne ($E_P$) cohorts.
Figure 2.21. A scatter diagram showing the relationship between $U_{Ni}$ and $U_{crea^t}$ for selected donors in the Thompson ($I_T, K_T, P_T$) and Port Colborne ($A_P$) cohorts.
Figure 2.22. A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\rho-1}$ for selected donors in the Thompson cohort.
Figure 2.23. A scatter diagram showing the relationship between $U_{Ni}$ and $10^2 U_{\rho-1}$ for selected donors in the Thompson($C_T$, $F_T$, $K_T$) and Port Colborne ($E_P$) cohorts.
Figure 2.24. A scatter diagram showing the relationship between $U_{Ni}$ and $U_{\rho-1}$ for selected donors in the Thompson ($I_T$, $K_T$, $P_T$) and Port Colborne ($A_p$) cohorts.
Table 2.8

**OBSERVED FLOW RATES**

<table>
<thead>
<tr>
<th>Collection</th>
<th>Flow Rate (mL min⁻¹) MEAN ± SD</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thompson</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual Voids</td>
<td>1.3 ± 1.1</td>
<td>123</td>
</tr>
<tr>
<td>24-h Collections</td>
<td>1.1 ± 0.4</td>
<td>20</td>
</tr>
<tr>
<td><strong>Port Colborne</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual Voids</td>
<td>1.9 ± 1.8</td>
<td>87</td>
</tr>
<tr>
<td>24-h Collections</td>
<td>1.3 ± 0.6</td>
<td>12</td>
</tr>
<tr>
<td><strong>Thompson and</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Port Colborne</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual Voids</td>
<td>1.6 ± 1.5</td>
<td>210</td>
</tr>
<tr>
<td>24-h Collections</td>
<td>1.2 ± 0.5</td>
<td>32</td>
</tr>
</tbody>
</table>

<sup>a</sup> the number of individual voids or 24-h collections.
correlation $U_{Ni} \text{ versus } U_{\rho^{-1}}$ compared to $U_{Ni} \text{ versus } U_{\text{creat}}$ for individual donors, as indicated by the splitting of the data into separate donor curves.

The plots in Figures 2.25, 2.27, 2.28 and 2.30 illustrate that the normalized ratio $U_{Ni}/U_{\text{creat}}$ has considerably more uncompensated contribution from urine flow rate than $U_{Ni}/U_{\rho^{-1}}$. This is more difficult to assess for the data in Figures 2.26 and 2.29, although this generalization appears to hold (compare data for donors Ep and Ct in these two graphs). Certainly, there is less random scatter in the $U_{\rho^{-1}}$-normalized $U_{Ni}$, and for $V > 1 \text{ mL min}^{-1}$ greater urine flow independence is also achieved.

When $U_{Ni}/U_{\text{creat}}$ is plotted against $U_{Ni}$ for the 24-h collections in this study (Fig. 2.31), two apparently linear responses result. Such data separation has also been observed in other studies for both spot and 24-h collections (see Table 2.9). The placement of data points on lines A and B is not determined by collection site. However, this grouping appears to reflect different mean urine flow rates: line A data has a mean flow rate of $V = 1.7 \pm 0.4 \text{ mL min}^{-1}$, while line B data has a lower flow rate, namely $V = 0.8 \pm 0.2 \text{ mL min}^{-1}$. This dependence is further illustrated in Figure 2.32 where $U_{Ni}/U_{\text{creat}}$ is plotted versus $U_{\text{creat}}/S_{\text{creat}}$. Here the data corresponding to lines A and B of Figure 2.32 respectively occur below and above $U_{\text{creat}}/S_{\text{creat}} = 100$. When the $U_{Ni}/U_{\text{creat}}$ data are plotted against the amount of nickel excreted in 24-h ($E_{Ni}$), the clear separation into two groups
Figure 2.25. A scatter diagram showing the relationship between $U_{Ni}/U_{creat}$ and $V$ for selected donors in the Thompson cohort.
Figure 2.26. A scatter diagram showing the relationship between $U_{\text{Ni}}/U_{\text{creat}}$ and $V$ for selected donors in the Thompson ($C_T$, $K_T$, $F_T$) and Port Colborne ($E_P$) cohorts.
Figure 2.27. A scatter diagram showing the relationship between $U_{Ni}/U_{crea^t}$ and $V$ for selected donors in the Thompson ($I_T$, $K_T$, $P_T$) and Port Colborne ($A_P$) cohorts.
Figure 2.28. A scatter diagram showing the relationship between $\frac{U_{Ni}}{U_{p-1}}$ and $V$ for selected donors in the Thompson cohort.
Figure 2.29. A scatter diagram showing the relationship between $U_{NI}/U_{P-1}$ and $V$ for selected donors in the Thompson ($C_T$, $F_T$, $K_T$) and Port Colborne ($E_P$) cohorts.
Figure 2.30. A scatter diagram showing the relationship between $U_{Ni}/U_{p-1}$ and $V$ for selected donors in the Thompson ($I_T$, $K_T$, $P_T$) and Port Colborne ($A_p$) cohorts.
Figure 2.31. A scatter diagram showing the linear relationships between $U_{Ni}/U_{Creat}$ and $U_{Ni}$ corresponding to 28 of the 32 donors.

The regression equations are:

Curve A: \[
\frac{U_{Ni}}{U_{Creat}} = 1.7U_{Ni} - 3.0 \quad r = 0.99 \quad (p<0.001) \quad 13 \text{ donors}
\]

Curve B: \[
\frac{U_{Ni}}{U_{Creat}} = 0.67U_{Ni} + 0.52 \quad r = 0.99; \quad (p<0.001) \quad 15 \text{ donors}
\]
Figure 2.32. Classification of $U_{Ni}/U_{Creat}$ according to the urinary concentration factor ($U_{Creat}/S_{Creat}$). Data corresponds to 24-h urine collections in Figure 2.31.
Figure 2.33. A scatter diagram showing the relationship between $U_{Ni}/U_{Creat}$ and nickel excretion ($E_{Ni}$). Data corresponds to the 24-h collections in Figures 2.31 and 2.32.

$$\frac{U_{Ni}}{U_{Creat}} = \frac{1}{U^o_{Creat}} (U_{Ni} V)$$

$U^o_{Creat} = 1.2 \text{ mg min}^{-1}$

Slope = 0.86 min mg$^{-1}$

$$\frac{U_{Ni}}{U_{Creat}} = 0.86 E_{Ni} + 2.4; \quad r = 0.96, n = 28 \quad (p < 0.001)$$
Figure 2.34. A scatter diagram showing the relationship between $\frac{U_{Ni}}{U_{\rho-1}}$ and $U_{Ni}$. Data corresponds to the 24-h collections in Figure 2.31. The regression parameters are included in the figure ($p < 0.001$).
Figure 2.35. A scatter diagram showing the relationship between $U_{Ni}/U_{p-1}$ and nickel excretion ($E_{Ni}$). Data corresponds to the 24-h collections in Figure 2.31. The regression parameters are included in the figure ($p<0.001$).
vanishes as illustrated in Figure 2.33. By contrast to creatinine normalization of the 24-h collections (Fig. 2.31), when the same data is adjusted employing the total solids ($\rho_{-1}$) and plotted against $U_{Ni}$ as before, no grouping is evident (Fig. 2.34). Furthermore, when $U_{Ni}/U_{\rho_{-1}}$ is plotted against $E_{Ni}$ for the 24-h collections (Fig. 2.35), a plot similar to that observed for creatinine adjustment is obtained (compare Figs. 2.33 and 2.35).

D DISCUSSION

(i) CURRENT PRACTICES OF URINE ADJUSTMENTS AND THEIR LIMITATIONS

The focus in this section will be on the urine flow dependence of urinary nickel, creatinine and specific gravity. Recommendations for flow adjustments of urinary nickel concentrations will also be made. The interpretation of these data in relation to measures of kidney function will be presented in Chapter 3.

Observed urinary nickel levels for non-occupationally exposed individuals agree with recent literature data: namely, serum $= 0.46 \pm 0.26 \, \mu g \, L^{-1}$; urine $= 2.0 \pm 1.5 \, \mu g \, L^{-1}$ (Sunderman et al., 1984, 1986b). For exposed individuals, the observed concentrations fall in the lower end of the ranges reported by reputable laboratories (serum $\leq 35 \, \mu g \, L^{-1}$; urine $\leq 400 \, \mu g \, L^{-1}$; Nieboer et al. (1984a)).

As illustrated in Figures 2.4 to 2.7, the wide variation in flow rate has an inverse relationship to the analyte
concentration (nickel, creatinine and specific gravity). Although all these analyte concentrations vary in a similar manner, the question that needs to be answered is: which analyte (creatinine or specific gravity) most closely resembles nickel in its excretion pattern? As mentioned in the introduction, 24-h collections of urine are thought to eliminate the concentration/dilution effects associated with spot samples. The expected large deviation in flow rates is evident in the spot urine collections (see Fig. 2.17). However, the data in Figure 2.18 and Table 2.9 illustrate that for 24-h collections there is still a significant variation in urine flow. Thus, it is reasonable to ask if 24-h collections (without any further adjustments) are the best sampling strategy.

Figures 2.19 to 2.24 illustrate one possible approach to deciding what type of normalization is most appropriate for a particular analyte. In these plots of $U_Ni$ versus $U_{crea}$ or $U_{si}$, the objective is to determine which of urinary creatinine or urinary total solids most closely resembles urinary nickel. This is the strategy that was taken in a recent paper by Sunderman et. al. (1986b). The plots from this paper are reproduced in Figure 2.36. Sunderman et al. (1986b) concluded that normalization by either creatinine or specific gravity have approximately equal validity for nickel elimination in urine, based upon the successful correlations between nickel concentrations and creatinine concentration or
Figure 2.36. Correlation graphs of nickel concentrations in urine specimens from five non-exposed, healthy, adult men, (A to E, respectively), versus urine creatinine concentrations and urine specific gravity measurements. Reproduced from Sunderman et al. (1986b).
specific gravity of urine specimens from healthy individuals. They recommend that "the results of nickel analyses of random spot urine specimens should be reported as unadjusted nickel concentrations, with optional provision of supplemental values factored on the basis of creatinine and/or specific gravity, according to the preference of the responsible physician". This study was done on subjects who were not industrially exposed to nickel. Hence the maximum nickel values were below $6 \mu g \, L^{-1}$, possibly making this study invalid for any recommendations made for biological monitoring of nickel workers. Lauwerys (1983) recommends that analyses of dilute spot urine specimens (creatinine concentration $< 0.3 \, g \, L^{-1}$ or specific gravity $< 1.010$) should, whenever possible, be repeated on a different specimen.

From the data presented in the present study (Figs. 2.19 to 2.24), it can be seen that the relationships between $U_{Ni}$ and $U_{\text{creat}}$ or $U_{\rho^{-1}}$ are not necessarily linear for a single donor when $U_{Ni}$ varies over a much wider range than studied by Sunderman et al. (1986b). This leads to the speculation as to whether this approach is valid and whether a more satisfactory approach is feasible. The systematic, and internally rigorous, mathematical approach described in the remainder of this chapter is to some extent empirical, but will be shown in Chapter 3 to be rooted in the mathematical relationships of renal clearance.

For convenience, the remainder of this discussion will
be divided into four parts. The first three sections will develop some theoretical aspects of urine normalization and apply them to adjustments by creatinine and specific gravity of urinary nickel levels. The final part will discuss the implications of the new approach for biological monitoring.

(ii) THEORETICAL CONSIDERATIONS

As described in Section 2C, Araki (1980) found a relationship described in Equation 2.6 between a number of urinary analytes such as lead, creatinine, total solutes and coproporphyrin and urinary flow rate

$$U_i V^{b_i} = U_i^0$$  \hspace{1cm} (2.6)

where $U_i$ is the concentration of analyte "i" in the urine, $V$ is the urine flow, $U_i^0$ is the urinary concentration of i at a flow rate of 1 mL min⁻¹ and $b_i$ is the volume exponent or power coefficient. For 8 individuals exposed to lead, Araki found that separate plots of log $U_i$ versus log $V$ gave an average $b_i$ value for lead of 0.75; for total solutes, 0.63; for osmolarity, 0.73; and for creatinine, 1.01.

In the present study, a linear relationship was observed for plots of log $U_i$ versus log $V$ for the analytes creatinine, specific gravity (i.e. total solids), osmolality, lead and nickel (see Figures 2.8 to 2.12). The results from the regression plots using Equation 2.6 are summarized in Table 2.7. They are in close agreement with those found by Araki (1980). The regression coefficients $b_i$ for creatinine, solutes and osmolality are almost identical with Araki's
assignment (see Table 2.7). The value for lead in this study is also close to Araki's mean value. Thus both studies corroborate the validity of the relationship in Equation 2.6. The mean values for \( b_{Ni} \) and \( b_{\rho^-1} \) are identical (both 0.7 ± 0.2), whereas \( b_{\text{crea}} \) is much larger (1.0 ± 0.2). It is obvious from the data depicted in Figures 2.8 to 2.12, that a number of factors contribute to successful correlations of \( \log U_i \) versus \( \log V \). There must be a reasonable spread in \( V \) values (at least \( \Delta V > 1 \text{ mL min}^{-1} \), although \( \Delta V > 2 \text{ mL min}^{-1} \) is preferable) and an adequate number of data points (e.g., \( n \geq 4 \)). An even distribution of \( V \) values throughout the range studied is also mandatory (i.e. no grouped data). In the present study, the data from three donors were rejected for flow rate analysis because of errors made in the timing of the voids. However, data corresponding to these donors were included in the 24-h collections used later in this chapter and in Chapter 3. Furthermore, there were four donors who gave only 3-timed voids in a 24-h collection and this was considered to provide too few data points for regression analysis. Since no special steps were taken to hydrate the donors, it is not surprising that 14 failed to have an adequate spread in \( V \) values. Because of these limitations, only data from 11 of the 32 donors were suitable for examining the dependence of analyte concentrations on urine flow rates.

As shown in Figure 2.13, there is a similarity between \( b_{Ni} \) and \( b_{\rho^-1} \) and they both appear to be different from that
of \( b_{\text{creat}} \). However because of the scatter in the data, it is not clear how close individual values of \( b_{Ni} \) and \( b_{\rho^{-1}} \) are to each other. This dependence is more clearly illustrated in Figures 2.14 to 2.16, where this data was arbitrarily arranged so that the creatinine data formed a straight line. It was found that the nickel and solutes coefficients approximated straight lines both of which were below the creatinine line with almost identical slopes and intercepts (i.e.

\[
\begin{align*}
  b_{Ni} &= -0.0239(\text{donor}) + 1.02; \quad r = 0.67 \\
  b_{\rho^{-1}} &= -0.0251(\text{donor}) + 1.05; \quad r = 0.78.
\end{align*}
\]

These values are different from those found for the creatinine data (\( b_{\text{creat}} = -0.0322(\text{donor}) + 1.44; \quad r = 1.00 \)). For all donors for which \( b_i \) values are available, these equations yield \( b_{Ni}/b_{\text{creat}} = 0.7 \) and \( b_{Ni}/b_{\rho^{-1}} = 1 \). This illustrates the almost identical relationship that \( b_{Ni} \) and \( b_{\rho^{-1}} \) have to each other, and implies that specific gravity (solutions) adjustment of nickel levels in spot urine samples for urine-flow dependence is more appropriate than creatinine corrections.

When \( b_i = 1.0 \), the excretion rate of the analyte (amount excreted per unit time) is independent of secretion or reabsorption (i.e. is creatinine- or inulin-like) and simple dilution effects regulate its concentration. Under these conditions, Equation 2.6 reduces to Equation 2.7.

\[
V_i U_i = U_i^0
\]  (2.7)

If \( b_i \) is not equal to 1.0, the analyte excretion rate (i.e., \( UV \)) is dependent on either secretion or reabsorption. Evidence will be presented in Chapter 3 to show that nickel
is reabsorbed in the kidney in a similar manner to urea.

Equation 2.6 can be rearranged to give Equation 2.8.

\[ U_i = U_i^0 V^{-b_i} \quad (2.8) \]

Volume normalization usually compares the concentration of the analyte of interest \( (U_1) \) with that of an endogenous reference compound \( (U_2) \). Usually this endogenous reference compound has well-defined excretion parameters. Using Equation 2.8, the general equation for urine normalization becomes Equation 2.9.

\[ \frac{U_1}{U_2} = \frac{U_1^0}{U_2^0} b_2^{-b_1} \quad (2.9) \]

This expression illustrates the principle of volume normalization. The concentration quotient \( U_1/U_2 \) becomes flow-rate independent only when \( b_1 - b_2 = 0 \) (i.e. \( b_1 = b_2 \)), since under this condition \( V^{b_2^{-b_1}} = V^0 = 1 \) for all values of \( V \). In all other cases (i.e. \( b_1 \neq b_2 \)), the ratio \( U_1/U_2 \) has not been completely compensated for differences in the flow rate between different urine specimens. This leaves the term \( U_1/U_2 \) still with a functional dependence on \( V \) like the uncorrected quantity \( U_1 \). Thus a functional relationship between these quantities is expected and the analyte of interest \( (U_1) \) may be over- or under-compensated by the reference analyte \( (U_2) \), depending on whether \( b_1 - b_2 > 0 \) or \( b_1 - b_2 < 0 \).
(iii) CREATININE NORMALIZATION

For creatinine normalization of urinary nickel levels, Equation 2.9 may be written as in Equation 2.10

\[
\frac{U_{Ni}}{U_{creat}} = \frac{U_{Ni}^0}{U_{creat}^0} V^{b_{creat} - b_{Ni}} \quad (2.10)
\]

The mean value for \(b_{creat}\) for both the present and Araki's studies is 1.0, and thus Equation 2.10 reduces to

\[
\frac{U_{Ni}}{U_{creat}} = \frac{1}{U_{Ni}^0 V^{1-b_{Ni}}} \quad (2.11)
\]

which can be rearranged to give

\[
U_{Ni} = U_{Ni}^0 V^{1-b_{Ni}} \quad (2.12a)
\]

\[
= U_{Ni}^0 V^{0.3} \quad (2.12b)
\]

with \(b_{Ni} = 0.7\). The uncompensated volume dependence results in overcompensation of the urinary nickel levels for \(V > 1 \text{ mL min}^{-1}\) and undercompensation for \(V < 1 \text{ mL min}^{-1}\). A plot of \(U_{Ni}/U_{creat}\) versus \(U_{Ni}\) for the 24-h collections (Fig. 2.31) can be resolved into two lines A and B. This illustrates that even for 24-h collections there is still a residual flow rate dependence that has not been resolved by creatinine normalization. Curve A is consistent with 24-h collections
that have flow rates larger than 1 mL min\(^{-1}\) resulting in over-compensation by creatinine normalization. The mean ± SD of the flow rates for line A is 1.7 ± 0.4 mL min\(^{-1}\), while that of curve B is 0.8 ± 0.2 mL min\(^{-1}\) which thus is closer to 1 mL min\(^{-1}\). Table 2.9 summarizes additional data obtained from three other studies. The three curves represented by all the data in Table 2.9 are shown in Figure 2.37, where curves A and B are those from the present study (Fig. 2.31), and curve C is the mean of relationships 6 and 7 in Table 2.9. These data illustrate the three cases that appear to occur with creatinine normalization. Curve B represents data that is reasonably close to a flow rate of 1 mL min\(^{-1}\), whereas curve A represents data that have flow rates above 1 mL min\(^{-1}\); and it is reasonable to postulate that curve C represents data with flow rates less than 1 mL/min. The flow rate associated with curves A, B and C can be estimated from the slope (\(\frac{V}{U^0_{\text{creat}}}\), Eqn. 2.16a below) and employing the average value for \(U^0_{\text{creat}}\) (1.27 g L\(^{-1}\); Table 2.7). For Curve A, \(V=2.2\) mL min\(^{-1}\); Curve B, \(V=0.85\) mL min\(^{-1}\) and Curve C, \(V=0.43\) mL min\(^{-1}\). The flow rates for Curves A and B are in close agreement with the average values of the actual flow rates quoted earlier. This again supports the argument that the average flow rate associated with curve C is much less than 1 mL min\(^{-1}\).

The studies which form the basis of Relationships 6 and 7 in Table 2.9 did not provide any data on urine flow rates.
Table 2.9

EXPERIMENTAL RELATIONSHIPS OBSERVED FOR CREATININE NORMALIZED NICKEL IN BODY FLUIDS

<table>
<thead>
<tr>
<th>Observed Relationship(^{a,b})</th>
<th>Correlation Coefficient</th>
<th>Sample Number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{U_{Ni}}{U_{creat}} = 1.7U_{Ni} - 3.0 )</td>
<td>0.99</td>
<td>13</td>
<td>Current study Line A</td>
</tr>
<tr>
<td>( \frac{U_{Ni}}{U_{creat}} = 0.67U_{Ni} + 0.52 )</td>
<td>0.99</td>
<td>15</td>
<td>Current study Line B</td>
</tr>
<tr>
<td>( \frac{U_{Ni}}{U_{creat}} = 0.62U_{Ni} - 0.22 )</td>
<td>0.90</td>
<td>20</td>
<td>Jusys et al., 1982</td>
</tr>
<tr>
<td>( \frac{U_{Ni}}{U_{creat}} = 0.65U_{Ni} + 1.73 )</td>
<td>0.91</td>
<td>143</td>
<td>Bernacki et al., 1978</td>
</tr>
<tr>
<td>( \frac{U_{Ni}}{U_{creat}} = 0.65U_{Ni} - 3.0 )</td>
<td>0.98</td>
<td>26</td>
<td>Hagedorn-Gotz et al., 1977</td>
</tr>
<tr>
<td>( \frac{U_{Ni}}{U_{creat}} = 0.36U_{Ni} - 0.09 )</td>
<td>0.99</td>
<td>8</td>
<td>Jusys et al., 1982</td>
</tr>
<tr>
<td>( \frac{U_{Ni}}{U_{creat}} = 0.32U_{Ni} + 7.2 )</td>
<td>0.91</td>
<td>8</td>
<td>Hagedorn-Gotz et al., 1977</td>
</tr>
</tbody>
</table>

\(^{a}\) \( U_{Ni} \) are expressed in \( \mu g \ L^{-1} \), with \( U_{creat} \) in \( g \ L^{-1} \).

\(^{b}\) The average value for the slopes of lines 2 to 5 is 0.65, and for lines 6 and 7 is 0.34.
Figure 2.37. A composite diagram showing three possible linear relationships between $\frac{U_{\text{Ni}}}{U_{\text{Creat}}}$ and $U_{\text{Ni}}$. Curves A and B are from the present study, while curve C is the mean of the observed relationships 6 and 7 in Table 2.9.
Thus, it is not possible to assign curve C a definitive V value. However, Figure 2.32 illustrates that all the data of curve A in Figure 2.31 lie below $U_{\text{creat}}/S_{\text{creat}} = 100$, while curve B data have values above this arbitrary value. In terms of renal clearance, $C/V = U/S$ (see Chapter 3B), the ratio $U_{\text{creat}}/S_{\text{creat}}$ is viewed as the renal creatinine-concentration factor. As shown in Chapter 3, the average value for $C_{\text{creat}}/V$ for all workers corresponding to the data in Figure 2.32 is 98, with an average $C_{\text{creat}}$ of $122$ mL min$^{-1}$ and $V = 1.24$ mL min$^{-1}$. For $V < 1.2$ mL min$^{-1}$, $U_{\text{creat}}/S_{\text{creat}} > 100$; and conversely for $V > 1.2$ mL min$^{-1}$. Thus this plot is an independent verification that the data on curve A have a flow rate greater than 1 mL min$^{-1}$ and those on Curve B less than this reference value. The observation of three lines in Fig. 2.37 may therefore be interpreted to indicate that creatinine correction of $U_{\text{Ni}}$ results in inherent uncompensated urine flow-rate contributions. This can be illustrated in the following manner.

For creatinine and nickel, Equation 2.8 may be written and rearranged as in Equations 2.13 and 2.14.

\[
U_{\text{creat}} = U_{\text{creat}}^0 V^{-1.0} \tag{2.13}
\]

\[
U_{\text{Ni}} = U_{\text{Ni}}^0 V^{-b_{\text{Ni}}} \tag{2.14}
\]

If both sides of Equation 2.14 are multiplied by V, Equation 2.15 results.

\[
VU_{\text{Ni}} = U_{\text{Ni}}^0 V^{1-b_{\text{Ni}}} \tag{2.15}
\]
Therefore when combining Equations 2.12a and 2.15, Equation 2.16a results with Equation 2.16b as a special case.

\[
\frac{U_{Ni}}{U_{\text{creat}}} = \frac{1}{U_{\text{creat}}^0} U_{Ni} V \quad (2.16a)
\]

\[
\frac{U_{Ni}}{U_{\text{creat}}} = \frac{1}{U_{\text{creat}}^0} U_{Ni} \quad (V = 1) \quad (2.16b)
\]

Thus the slope of a plot of \(U_{Ni}/U_{\text{creat}}\) versus \(U_{Ni} V\) is \(1/U_{\text{creat}}^0\) and the term \(U_{Ni} V\) in effect is an estimate of the uncompensated volume dependence. Assuming \(V = 1\ \text{mL min}^{-1}\) (c.f., Eqn. 2.16b), the slope of curve B gives \(U_{\text{creat}}^0 = 1.5 \text{ g L}^{-1}\) which is not very different from \(U_{\text{creat}}^0 = 1.3 \text{ g L}^{-1}\) that was found from the regression plots according to Equation 2.6 (see Table 2.7). By contrast, the slopes of curves A and C of Fig. 2.37 yield 0.6 and \(2.9 \text{ g L}^{-1}\), respectively, which differ considerably from the regression value of \(1.3 \text{ g L}^{-1}\).

A plot of \(U_{Ni}/U_{\text{creat}}\) versus \(U_{Ni} V\) is expected to reduce the separation in the data observed in Figure 2.31 and Figure 2.37. This indeed is the case, because as shown in Figure 2.33 a single straight line is obtained for such a plot with \(U_{\text{creat}}^0 = 1.2 \text{ mg min}^{-1}\) which is in reasonable agreement with \(U_{\text{creat}}^0 = 1.3 \text{ g L}^{-1}\) obtained from the regression plots of Equation 2.6. Thus, the \(U_{Ni} V\) term in Equation 2.11 illustrates vividly the presence of a residual, uncompensated flow-rate dependence for creatinine adjustment of urinary nickel concentrations.
(iv) SPECIFIC GRAVITY NORMALIZATION

Equation 2.17 is used to normalize urinary analytes to a specific gravity of 1.024 in North America and to 1.016 in Europe (Levine and Fahy, 1945; Elkins and Pagnotto, 1965; Elkins et al., 1974; DHEW, 1974; Pryde, 1982; Sunderman et al., 1986b)

\[
U_i'(\text{at } \rho = 1.024) = 0.024 \frac{U_i}{\rho_i - 1.000} \quad (2.17)
\]

where \( U_i \) is the observed analyte concentration, \( U_i' \) is \( U_i \) normalized to a specific gravity of 1.024 and \( \rho_i \) is the observed specific gravity for the sample. In order to treat specific gravity in a manner similar to creatinine normalization, it is necessary to develop a relationship with total solutes.

Density measures the mass of a substance per unit volume, while specific gravity is the ratio of its mass to that of an equal volume of water at 4°C. Because water at 4°C has a density of 1.000 g mL\(^{-1}\), density and specific gravity thus defined are interchangeable. The following is a derivation of a formula for urinary solutes using the definition of specific gravity and the interchangeability of density and specific gravity which results in the weight (\( W \)) of water being numerically equal to its volume (\( V \)) at 4°C.

\[
\rho_i = \frac{(W_{\text{solute}} + W_{\text{solvent}})}{(\text{wt. of equal volume of water at 4°C})} \quad (2.18)
\]
\[
\rho_i = \frac{(W_{\text{solute}} + W_{\text{solvent}})}{V} \quad (2.19)
\]

\[
\rho_i = \frac{W_{\text{solute}}}{V} + \frac{W_{\text{solvent}}}{V} \quad (2.20)
\]

\[
\rho_i = \text{solute concentration} + 1.000 \quad (2.21)
\]

\[
\rho_i = U_{\rho^{-1}} + 1.000 \quad (2.22)
\]

\[
U_{\rho^{-1}} = \rho_i - 1.000 \quad (2.23)
\]

where \( U_{\rho^{-1}} \) denotes the urinary solute concentration in \( \text{g mL}^{-1} \). In this derivation, changes in volume due to dissolution of solutes (i.e., \( \partial V / \partial n \) the partial molar volume for both the solvent and solute) have been ignored. Multiplication of the right-hand side of Equation 2.23 by \( 1/(1-\beta) \) roughly corrects for this, with \( \beta = \partial V / \partial n_{\text{solute}} \) for the solute (Barber and Wallis, 1986). An approximate correction can thus be made to Equation 2.23 using \( \partial V / \partial n_{\text{solute}} = \beta = 17.82 \text{ mL mol}^{-1} = 0.32 \text{ mL g}^{-1} \) \( \text{NaCl} \), realizing that \( \partial V / \partial n_{\text{H}_2\text{O}} \) is approximately independent of solute concentration and assuming that all solutes in normal urine are similar to \( \text{NaCl} \) (Bromberg, 1980). This consideration suggests that \( U_{\rho^{-1}} \) is being underestimated by about 30%. Although the \( \beta \) correction is of unknown magnitude when considering all urine solutes, it may be assumed to be a constant and thus need not be considered further. By comparing Equations 2.17 and 2.23 it can be seen that
specific gravity adjustment is the same as normalization by urinary solute concentration (also called total solutes).

Creatinine normalization led to three curves when \( U_{Ni}/U_{\text{creat}} \) was plotted against \( U_{Ni} \) and this was interpreted that in creatinine normalization, uncompensated flow-rate residuals remain after normalization. If solute normalization is better for urinary nickel levels, then a plot of \( U_{Ni}/U_{\rho^{-1}} \) versus \( U_{Ni} \) should not give two lines as seen in creatinine adjustment (Fig. 2.31). Figure 2.34 is a plot of \( U_{Ni}/U_{\rho^{-1}} \) versus \( U_{Ni} \) for the same nickel data as in Figure 2.31. This results in a straight line \( (r = 0.93) \) instead of two lines seen in the creatinine data. Also, a plot of \( U_{Ni}/U_{\rho^{-1}} \) versus \( E_{Ni} (U_{Ni}V) \) (Fig. 2.35) is a straight line, but is somewhat less strongly correlated \( (r = 0.89) \). The derivation of a relationship similar to Equation 2.16a but for specific gravity normalization shows that the curve in Figure 2.35 should have a slope that is volume dependent \( \text{slope} = V^{\rho^{-1}} \text{j}^{1} /U_{\rho^{-1}} \), and presumably this explains the slightly poorer correlation coefficient. The values for \( U_{\rho^{-1}} \) from Figures 2.34 and 2.35 are 0.023 and 0.018 g mL\(^{-1}\), respectively which are in close agreement with those found from the regression plots employing Equation 2.6 (0.022 and 0.018 g mL\(^{-1}\) for the two groups of nickel workers studied; see Table 2.7). These results again indicate that urinary solute normalization is more appropriate for urinary nickel than creatinine adjustment.

In conclusion, the close agreement between \( b_{Ni} \) and \( b_{\rho^{-1}} \)
values (Table 2.7) along with the above mathematical and graphical verifications provide evidence that urinary solute correction is the most appropriate normalization procedure for nickel.

(v) BIOLOGICAL MONITORING

(a) Definition, Uses and Practices.

There are many definitions of this term. Waritz (1979) defines it as the "analysis of some biological fluid, such as urine, blood, tears, or perspiration, or analysis of some body component, such as hair or nails to evaluate past exposure to a chemical". These measurements may be carried out not only on body fluids and tissue (biopsies) of exposed populations during their normal life, but also on tissues taken during surgery or postmortem examination (Zielhuis, 1978). Olishifski (1983) includes in biological monitoring the objective of determining excessive absorption of a contaminant, while Lauwerys (1983) and Zielhuis (1978) include an assessment of the internal exposure (ie. the internal dose) to a chemical agent. This may relate to the amount of chemical recently absorbed, the amount stored in the body (body burden), or the amount of the active chemical species bound to a site of action. Ross (1984) has pointed out that biological monitoring provides an avenue for assessing the extent of environmental and occupational exposures and has the potential for early detection of toxic lesions at preclinical and reversible states. Nieboer et al.
(1984a) have essentially a similar perspective: "collection and analysis of biological samples for the purpose of determining exposure and health risk". The health risk assessment would be based on a toxicological outcome measurement (e.g., proteinuria).

Whatever the definition and goals of biological monitoring, it will involve the analysis of various biological samples (e.g., blood, serum, urine, hair, biopsy or autopsy tissue, saliva, expired air etc.). The most favoured body fluids used in nickel biological monitoring are serum, plasma and urine (Stoeppler, 1980; Stoeppler, 1984; Sunderman, 1984d). Urine is generally preferred to serum (plasma) because its collection is non-invasive. Collection of blood for nickel analysis requires venepuncture with a plastic intravenous cannula to avoid nickel contamination from needles. In addition, nickel concentrations are higher in urine than in serum, enhancing analytical accuracy and precision. Urinary nickel has the potential of early detection of toxic lesions at the preclinical state. For example, the Nieboer et al. (1984a) review of the data suggesting that high urinary levels of nickel generally reflect not only the extent of Ni(CO)₄ exposure, but also the clinical severity that may be expected. A urinary nickel level of more than 100 µg L⁻¹ on the day of exposure suggests that careful monitoring of the patient would be judicious even in the absence of initial symptoms. Nieboer et al. (1984a) also reported significant dose-response relationships
between total ambient nickel levels (both soluble and insoluble) and nickel in the urine and plasma (serum). This illustrates that urinary nickel is an appropriate medium for biological monitoring.

It is common to have timed 24-h urine collections when it is practical (e.g., in hospital patients and for research purposes). However, it is not feasible for routine 24-h urine collections to be requested in the biological monitoring of industrial workers (Levine and Fahy, 1945; Elkins et al., 1974; Lauwerys, 1983; Aitio and Jarvisalo, 1985). This has led to the common use of spot or grab samples of urine. In fact, spot samples may have a distinct advantage over 24-h collections. This is illustrated in the exposure of workers to nickel carbonyl. As pointed out on pages 29 and 30 of Chapter 1, immediate treatment of nickel carbonyl poisoning seems advisable and waiting for the traditional 8-h urine collection before initiating treatment may not be appropriate. Therefore, the collection of a reliable spot urine specimen may allow almost immediate treatment of the patient.

(b) Need for Urine-Flow Corrections.

A major objection to the use of urine as the fluid of choice is that it is subject to wide variations in analyte concentration because of dilution effects. Hence, this has led to the standardization by specific gravity or creatinine. The approach taken by some investigators to volume correction
has been somewhat empirical. Elkins et al. (1974) compared (in a graphical manner) the fluctuations of lead samples adjusted to a specific gravity of 1.024, to 1.8 g of creatinine or to mg Pb/osmol. They found that all adjusted values are better than unadjusted ones, but there was no general superiority for one of the normalizations investigated. Levine and Fahy (1945) compared the coefficient of variability for unadjusted urinary lead levels and the same urinary leads normalized to a specific gravity of 1.024. A decrease in variation of adjusted urinary lead excretion was found for a series of spot samples taken from subjects recovering from lead poisoning. Tola et al. (1979) corrected urinary nickel samples to a specific weight of 1.018 because this proved to result in the least variation.

The average coefficient of variation (CV = 100 SD/$\bar{X}$) found in the present study for the uncorrected urinary nickel ($U_{Ni}$) levels in the individual voids collected over a 24-h period from each donor is $39 \pm 21\%$ (32 donors). These same samples when normalized by creatinine ($U_{Ni}/U_{creat}$) yielded CV = $27 \pm 17\%$. For adjustment by solute (i.e., $U_{Ni}/U_{\rho}$), it was $24 \pm 12\%$. The solute-adjusted samples had the lowest average CV and were statistically different ($p<0.01$) from the uncorrected samples, indicating that solute (specific gravity) normalization is the most appropriate. It is interesting to consider the Thompson and Port-Colborne groups separately. For the Thompson study, the specific gravity corrections gave statistically significant reduction in CV
(p<0.01), while the creatinine did not. By contrast for the Port Colborne cohort, neither creatinine nor specific gravity adjustment yielded a significant improvement (p≥0.01), although the lowest CV value occurred for specific gravity correction. It is just this type of inconsistency that has marred the practices of urinary corrections, and emphasizes the need for a different perspective.

An interesting approach has been taken by Barber and Wallis (1986), who applied specific gravity, osmolality and creatinine corrections to the same set of urinary mercury data. They investigated the reproducibility of the corrected mercury concentration in a spot sample to predict a person's 24-h mercury excretion. Osmolality and specific gravity corrections were almost identical and creatinine was most effective by a small but statistically significant amount. They state that "it was not the purpose of this paper to recommend which correction is most appropriate", but they hoped that their work "will assist the physician in making a choice". Graul and Stanley (1982) emphasized that the adjusted concentration of a spot sample need not be representative of the 24-h concentration. Presumably agreement is only possible for substances that are excreted at a constant rate such as creatinine. Consequently, the approach suggested by Barber and Wallis is of limited applicability. It will be demonstrated in the next section that the perspective outlined in sections (ii), (iii) and (iv) above for nickel may be generalized for any analyte.
(c) **Standardization to Unitary Flow Rate.**

A more general approach to this problem can be developed using Equation 2.9.

\[
\frac{U_1}{U_2} = \frac{U_0^1}{U_0^2} v^{b_2-b_1} \tag{2.9}
\]

or

\[
\frac{U_1}{U_2} = \frac{U_0^1}{U_0^2} v^{\Delta b} \tag{2.24}
\]

For creatinine adjustment, the power coefficient term \((\Delta b = b_2-b_1)\) in Equation 2.9 becomes \(b_{\text{creat}}-b_{\text{Ni}}\) and in solute correction it is \(b_{\rho-1}-b_{\text{Ni}}\). A plot of \(U_1/U_2\) versus \(V\) (see Figs. 2.25 to 2.30) will give a measure of \(\Delta b\) and \(U_{\text{Ni}}^0/U_{\text{creat}}^0\) or \(U_{\rho-1}^0/U_{\text{Ni}}^0\). It is evident from the data in Figures 2.25 to 2.27, that the creatinine adjusted curves tend to increase rapidly at low flow rates and level off to a relatively constant \(U_{\text{Ni}}^0/U_{\text{creat}}\) value at high flow rates. By contrast, the solute-adjusted ratio \(U_{\text{Ni}}^0/U_{\rho-1}\) curves with negative \(\Delta b\) values tend to decrease rapidly at low flow rates and the curves level off to a relatively constant \(U_{\text{Ni}}^0/U_{\rho-1}\) value at high flow rates (see Figs. 2.28 to 2.30). In general, the solute curves appear to level more quickly than the corresponding creatinine plots. It is also obvious from these figures that the exact shape is donor dependent.

The shapes of the curves in Figures 2.25 to 2.30 can be rationalized by examining the family of theoretical curves.
plotted in Figure 2.38. Here $V^{\Delta b}$ (the uncompensated volume residual in Equation 2.24) has been plotted against $V$ for various values of $\Delta b$ and $V$. When $\Delta b > 0$, the curvature is very similar to the creatinine-normalized curves for nickel (Figs. 2.25 to 2.27), whereas when $\Delta b < 0$ the curves resemble some of the solute normalized curves (Figs. 2.28 to 2.30). It is apparent from Figure 2.38 that as $\Delta b$ approaches zero, the curves tend to flatten out rapidly. It is also clear from the point of convergence depicted in Figure 2.38, that the uncompensated (residual) volume factor, $V^{\Delta b}$ approaches unity when $\Delta b \rightarrow 0$ or $V \rightarrow 1.0$. Based upon the $b$ values summarized in Table 2.10, the mean value for $\Delta b = b_{\text{creat}} - b_{Ni} = 0.31$ and $\Delta b = b_{\rho}^{-1} b_{Ni} = 0.005$. Thus on the average, $V^{\Delta b} \rightarrow 1$ for specific gravity correction of urinary nickel, in contrast to creatinine normalization.

It is of interest to compare the relationship between the family of curves in Figure 2.38 and Equation 2.10. The right-hand side of this equation corresponds to the product $(U_1^0/U_2^0)V^{\Delta b}$, and thus is dependent on the individual donor. This follows since $U_1^0$ and $U_2^0$ are characteristic of a particular individual. A perusal of the $\Delta b$ values in Table 2.10 indicates that $b_i$ also exhibits a strong dependence on the individual donor. There are a number of consequences
Figure 2.38. The family of curves for the uncompensated residual flow factor ($V^{\Delta b}$) at various flow rates ($V$) and selected values of the power coefficient difference $\Delta b$. 
Table 2.10

ASSESSMENT OF THE FLOW-RATE POWER COEFFICIENT $b_i$ FOR CREATININE, $\rho - 1$ AND NICKEL, AND THE CORRESPONDING $\Delta b$ VALUES

<table>
<thead>
<tr>
<th>Donor</th>
<th>$b_{Ni}^a$, $r^a$</th>
<th>$b_{Creat}^a$, $r^a$</th>
<th>$b_{\rho - 1}^a$, $r^a$</th>
<th>$\Delta b = b_{\rho - 1}^a - b_{Ni}^a$</th>
<th>$\Delta b = b_{Creat}^a - b_{Ni}^a$, $r$</th>
<th>$\Delta b = b_{\rho - 1}^a - b_{Ni}^a$, $r$</th>
<th>$\Delta b = b_{\rho - 1}^a - b_{Creat}^a$, $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.14, 0.85</td>
<td>1.41, 0.81</td>
<td>0.89, 0.79</td>
<td>0.27</td>
<td>0.27, 0.42</td>
<td>-0.25</td>
<td>-0.25, 0.39</td>
</tr>
<tr>
<td>B</td>
<td>1.09, 0.95</td>
<td>1.17, 0.95</td>
<td>1.01, 0.95</td>
<td>0.08</td>
<td>0.08, 0.81</td>
<td>-0.08</td>
<td>-0.08, 0.55</td>
</tr>
<tr>
<td>C</td>
<td>0.68, 0.72</td>
<td>1.13, 0.96</td>
<td>0.89, 0.94</td>
<td>0.45</td>
<td>0.46, 0.73</td>
<td>0.21</td>
<td>0.21, 0.42</td>
</tr>
<tr>
<td>D</td>
<td>0.77, 0.92</td>
<td>1.08, 0.94</td>
<td>0.60, 0.93</td>
<td>0.31</td>
<td>0.26, 0.67</td>
<td>-0.17</td>
<td>-0.19, 0.58</td>
</tr>
<tr>
<td>E</td>
<td>0.44, 0.80</td>
<td>1.08, 0.98</td>
<td>0.89, 0.98</td>
<td>0.64</td>
<td>0.64, 0.88</td>
<td>0.45</td>
<td>0.44, 0.82</td>
</tr>
<tr>
<td>F</td>
<td>0.54, 0.96</td>
<td>1.05, 0.97</td>
<td>0.81, 0.94</td>
<td>0.51</td>
<td>0.52, 0.93</td>
<td>0.27</td>
<td>0.23, 0.77</td>
</tr>
<tr>
<td>G</td>
<td>0.67, 0.93</td>
<td>1.08, 0.99</td>
<td>0.69, 0.96</td>
<td>0.41</td>
<td>0.35, 0.85</td>
<td>0.02</td>
<td>0.01, 0.26</td>
</tr>
<tr>
<td>H</td>
<td>0.58, 0.86</td>
<td>1.01, 0.95</td>
<td>0.72, 0.92</td>
<td>0.43</td>
<td>0.50, 0.87</td>
<td>0.14</td>
<td>0.24, 0.75</td>
</tr>
<tr>
<td>I</td>
<td>1.01, 0.94</td>
<td>0.96, 0.96</td>
<td>0.74, 0.88</td>
<td>-0.05</td>
<td>-0.05, 0.20</td>
<td>-0.27</td>
<td>-0.26, 0.83</td>
</tr>
<tr>
<td>J</td>
<td>0.59, 0.86</td>
<td>0.85, 0.92</td>
<td>0.37, 0.94</td>
<td>0.26</td>
<td>0.26, 0.45</td>
<td>-0.22</td>
<td>-0.22, 0.49</td>
</tr>
<tr>
<td>K</td>
<td>0.49, 0.99</td>
<td>0.61, 0.99</td>
<td>0.43, 0.97</td>
<td>0.12</td>
<td>0.11, 0.90</td>
<td>-0.06</td>
<td>-0.07, 0.61</td>
</tr>
<tr>
<td>Mean±SDb</td>
<td>0.73 ± 0.24</td>
<td>1.04 ± 0.22</td>
<td>0.73 ± 0.20</td>
<td>0.31 ± 0.20</td>
<td>0.31 ± 0.21</td>
<td>0.004 ± 0.24</td>
<td>0.005 ± 0.24</td>
</tr>
</tbody>
</table>

a Evaluated from plots of log $U_i$ versus log $V$.

b $\Delta b$ was calculated by subtraction of $b_i$ values in columns 2, 3 and 1, 4.

c $\Delta b$ evaluated from plots of $\frac{U_{Ni}}{U_{Creat}}$ versus $V$ or $\frac{U_{Ni}}{U_{\rho - 1}}$ versus $V$.

d There was no statistical difference between measures of $\Delta b$ $p > 0.05$.

e The MEAN ± SD of $b_i$ or $\Delta b$. 
that follow from this. First of all, the position with respect to the Y-axis of the curves for plots of $U_1/U_2$ versus V (as in Figs. 2.25 to 2.30) is determined by $U_1^0/U_2^0$, while, the degree of curvature in these plots is determined by $\Delta b$.

Secondly $U_2^0$, which corresponds to an endogenous metabolite, depends on a number of personal factors (e.g. diet, activity, kidney function). In addition $U_1^0$, which corresponds to a xenobiotic or its metabolite, depends upon exposure and body burden. It is not obvious what determines the $b_1$ value for an individual, although dependence on kidney function must play a role (e.g. degree of tubular absorption or secretion). Nevertheless, for urinary excretion of metabolites such as nickel, creatinine and specific gravity common patterns of excretion may be discerned when examining group data, although considerable deviation from the average response can occur in any particular individual. As discussed later, this has implications for biological monitoring.

Consideration of the residual volume factor opens up an interesting perspective on normalization strategy. In Tables 2.11 and 2.12 and in Figure 2.39, numerical and graphical presentations are provided for the flow rates at which the uncompensated flow factor ($V^{\Delta b}$) deviates from unity by a predetermined amount. In addition, urinary creatinine concentrations, solute concentrations and specific gravity values corresponding to boundary flow rates ($V_{\text{min}}$ and $V_{\text{max}}$) are provided. It is clear from Figure 2.39, that the range of flow rates at which the residual flow factor does not
Figure 2.39. The boundary flow rates ($V_{\text{min}}$ ○ and $V_{\text{max}}$ □) for various $\Delta b$ values at which the uncompensated residual flow factor ($V^{\Delta b}$) deviates ± 10% (broken lines) or ± 20% (solid lines) from unity. The data plotted corresponds to that in columns 1 to 3 of Tables 2.11 and 2.12.
Table 2.11
BOUNDARY VALUES OF SELECTED URINARY PARAMETERS
CORRESPONDING TO $V^{\Delta b} = \pm 0.1$ FOR SELECTED $\Delta b$ VALUES

<table>
<thead>
<tr>
<th>$\Delta b$</th>
<th>$V_{\text{min}}$</th>
<th>$V_{\text{max}}$</th>
<th>$U_{\text{ creat, min}}$</th>
<th>$U_{\text{ creat, max}}$</th>
<th>$U_{\rho -1, \text{min}}$</th>
<th>$U_{\rho -1, \text{max}}$</th>
<th>$\rho_{\text{min}}$</th>
<th>$\rho_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.35</td>
<td>2.59</td>
<td>0.49</td>
<td>3.61</td>
<td>0.010</td>
<td>0.042</td>
<td>1.010</td>
<td>1.042</td>
</tr>
<tr>
<td>0.2</td>
<td>0.59</td>
<td>1.61</td>
<td>0.79</td>
<td>2.14</td>
<td>0.014</td>
<td>0.029</td>
<td>1.014</td>
<td>1.029</td>
</tr>
<tr>
<td>0.3</td>
<td>0.70</td>
<td>1.37</td>
<td>0.92</td>
<td>1.81</td>
<td>0.016</td>
<td>0.026</td>
<td>1.016</td>
<td>1.026</td>
</tr>
<tr>
<td>0.4</td>
<td>0.77</td>
<td>1.27</td>
<td>1.00</td>
<td>1.64</td>
<td>0.017</td>
<td>0.024</td>
<td>1.017</td>
<td>1.024</td>
</tr>
<tr>
<td>0.5</td>
<td>0.81</td>
<td>1.21</td>
<td>1.05</td>
<td>1.56</td>
<td>0.018</td>
<td>0.023</td>
<td>1.018</td>
<td>1.023</td>
</tr>
<tr>
<td>0.6</td>
<td>0.84</td>
<td>1.17</td>
<td>1.08</td>
<td>1.51</td>
<td>0.018</td>
<td>0.023</td>
<td>1.018</td>
<td>1.023</td>
</tr>
<tr>
<td>0.7</td>
<td>0.86</td>
<td>1.15</td>
<td>1.10</td>
<td>1.47</td>
<td>0.018</td>
<td>0.022</td>
<td>1.018</td>
<td>1.022</td>
</tr>
<tr>
<td>0.8</td>
<td>0.88</td>
<td>1.13</td>
<td>1.12</td>
<td>1.44</td>
<td>0.018</td>
<td>0.022</td>
<td>1.018</td>
<td>1.022</td>
</tr>
<tr>
<td>-0.1</td>
<td>0.39</td>
<td>2.87</td>
<td>0.44</td>
<td>3.24</td>
<td>0.010</td>
<td>0.039</td>
<td>1.010</td>
<td>1.039</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.62</td>
<td>1.69</td>
<td>0.75</td>
<td>2.04</td>
<td>0.014</td>
<td>0.028</td>
<td>1.014</td>
<td>1.028</td>
</tr>
<tr>
<td>-0.3</td>
<td>0.73</td>
<td>1.42</td>
<td>0.89</td>
<td>1.75</td>
<td>0.016</td>
<td>0.025</td>
<td>1.016</td>
<td>1.025</td>
</tr>
<tr>
<td>-0.4</td>
<td>0.79</td>
<td>1.30</td>
<td>0.97</td>
<td>1.60</td>
<td>0.017</td>
<td>0.024</td>
<td>1.017</td>
<td>1.024</td>
</tr>
<tr>
<td>-0.5</td>
<td>0.83</td>
<td>1.23</td>
<td>1.03</td>
<td>1.52</td>
<td>0.017</td>
<td>0.023</td>
<td>1.017</td>
<td>1.023</td>
</tr>
<tr>
<td>-0.6</td>
<td>0.85</td>
<td>1.19</td>
<td>1.06</td>
<td>1.49</td>
<td>0.018</td>
<td>0.022</td>
<td>1.018</td>
<td>1.022</td>
</tr>
<tr>
<td>-0.7</td>
<td>0.87</td>
<td>1.16</td>
<td>1.09</td>
<td>1.45</td>
<td>0.018</td>
<td>0.022</td>
<td>1.018</td>
<td>1.022</td>
</tr>
<tr>
<td>-0.8</td>
<td>0.89</td>
<td>1.14</td>
<td>1.11</td>
<td>1.42</td>
<td>0.020</td>
<td>0.022</td>
<td>1.020</td>
<td>1.022</td>
</tr>
</tbody>
</table>

$a$ $V_{\text{min}}$ = the flow rate (V) at which $V^{\Delta b} = 0.9$ or $V^{\Delta b} = 1.1$

$b$ $V_{\text{max}}$ = the flow rate (V) at which $V^{\Delta b} = 1.1$ or $V^{\Delta b} = 0.9$

$c$ $U_{i, \text{max}} = U_{i, \text{min}} - b_i$, with $b_{\text{creat}} = 1.0$, $b_{\rho -1} = 0.7$

$d$ $U_{i, \text{min}} = U_{i, \text{max}} - b_i$, with $b_{\text{creat}} = 1.0$, $b_{\rho -1} = 0.7$

$e$ $\rho_{\text{min}} = U_{\rho -1, \text{min}} + 1$; $\rho_{\text{max}} = U_{\rho -1, \text{max}} + 1$
Table 2.12
BOUNDARY VALUES OF SELECTED URINARY PARAMETERS
CORRESPONDING TO $V^{\Delta b} = \pm 0.2$ FOR SELECTED $\Delta b$ VALUES

<table>
<thead>
<tr>
<th>$\Delta b$</th>
<th>$V_{\text{min}}^a$ (mL min$^{-1}$)</th>
<th>$V_{\text{max}}^b$ (mL min$^{-1}$)</th>
<th>$U_{\text{creat}, \text{min}}^c$ (g L$^{-1}$)</th>
<th>$U_{\text{creat}, \text{max}}^d$ (g L$^{-1}$)</th>
<th>$U_{\rho, \text{min}}^e$ (g mL$^{-1}$)</th>
<th>$U_{\rho, \text{max}}^e$ (g mL$^{-1}$)</th>
<th>$\rho_{\text{min}}^e$</th>
<th>$\rho_{\text{max}}^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.11</td>
<td>6.19</td>
<td>0.20</td>
<td>11.50</td>
<td>0.006</td>
<td>0.094</td>
<td>1.006</td>
<td>1.094</td>
</tr>
<tr>
<td>0.2</td>
<td>0.33</td>
<td>2.49</td>
<td>0.51</td>
<td>3.83</td>
<td>0.011</td>
<td>0.044</td>
<td>1.011</td>
<td>1.044</td>
</tr>
<tr>
<td>0.3</td>
<td>0.48</td>
<td>1.84</td>
<td>0.69</td>
<td>2.64</td>
<td>0.013</td>
<td>0.033</td>
<td>1.013</td>
<td>1.033</td>
</tr>
<tr>
<td>0.4</td>
<td>0.57</td>
<td>1.58</td>
<td>0.80</td>
<td>2.22</td>
<td>0.015</td>
<td>0.030</td>
<td>1.015</td>
<td>1.030</td>
</tr>
<tr>
<td>0.5</td>
<td>0.64</td>
<td>1.44</td>
<td>0.88</td>
<td>1.98</td>
<td>0.016</td>
<td>0.027</td>
<td>1.016</td>
<td>1.027</td>
</tr>
<tr>
<td>0.6</td>
<td>0.69</td>
<td>1.36</td>
<td>0.93</td>
<td>1.83</td>
<td>0.016</td>
<td>0.026</td>
<td>1.016</td>
<td>1.026</td>
</tr>
<tr>
<td>0.7</td>
<td>0.73</td>
<td>1.30</td>
<td>0.97</td>
<td>1.73</td>
<td>0.017</td>
<td>0.025</td>
<td>1.017</td>
<td>1.025</td>
</tr>
<tr>
<td>0.8</td>
<td>0.76</td>
<td>1.26</td>
<td>1.00</td>
<td>1.66</td>
<td>0.017</td>
<td>0.024</td>
<td>1.017</td>
<td>1.024</td>
</tr>
<tr>
<td>-0.1</td>
<td>0.16</td>
<td>9.31</td>
<td>0.14</td>
<td>7.91</td>
<td>0.004</td>
<td>0.072</td>
<td>1.004</td>
<td>1.072</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.40</td>
<td>3.05</td>
<td>0.42</td>
<td>3.16</td>
<td>0.009</td>
<td>0.038</td>
<td>1.009</td>
<td>1.038</td>
</tr>
<tr>
<td>-0.3</td>
<td>0.54</td>
<td>2.10</td>
<td>0.60</td>
<td>2.34</td>
<td>0.012</td>
<td>0.031</td>
<td>1.012</td>
<td>1.031</td>
</tr>
<tr>
<td>-0.4</td>
<td>0.63</td>
<td>1.75</td>
<td>0.72</td>
<td>2.01</td>
<td>0.014</td>
<td>0.028</td>
<td>1.014</td>
<td>1.028</td>
</tr>
<tr>
<td>-0.5</td>
<td>0.69</td>
<td>1.56</td>
<td>0.81</td>
<td>1.83</td>
<td>0.015</td>
<td>0.026</td>
<td>1.015</td>
<td>1.026</td>
</tr>
<tr>
<td>-0.6</td>
<td>0.74</td>
<td>1.45</td>
<td>0.87</td>
<td>1.71</td>
<td>0.015</td>
<td>0.025</td>
<td>1.015</td>
<td>1.025</td>
</tr>
<tr>
<td>-0.7</td>
<td>0.77</td>
<td>1.38</td>
<td>0.92</td>
<td>1.64</td>
<td>0.016</td>
<td>0.024</td>
<td>1.016</td>
<td>1.024</td>
</tr>
<tr>
<td>-0.8</td>
<td>0.80</td>
<td>1.32</td>
<td>0.96</td>
<td>1.58</td>
<td>0.017</td>
<td>0.023</td>
<td>1.017</td>
<td>1.023</td>
</tr>
</tbody>
</table>

a $V_{\text{min}}$ = the flow rate ($V$) at which $V^{\Delta b} = 0.8$ or $V^{\Delta b} = 1.2$

b $V_{\text{max}}$ = the flow rate ($V$) at which $V^{\Delta b} = 1.2$ or $V^{\Delta b} = 0.8$

c $U_{i, \text{max}} = U_{i}^{\rho, \text{min}}$; with $b_{\text{creat}} = 1.0$, $b_{\rho, 1} = 0.7$

d $U_{i, \text{min}} = U_{i}^{\rho, \text{max}}$; with $b_{\text{creat}} = 1.0$, $b_{\rho, 1} = 0.7$

e $\rho_{\text{min}} = U_{\rho, \text{min}}^+ 1$; $\rho_{\text{max}} = U_{\rho, \text{min}}^+ 1$
differ from unity by more than ±10 or ±20% is strongly dependent on $\Delta b$. As $\Delta b$ increases, the boundary values $V_{\text{min}}$ and $V_{\text{max}}$ approach each other. For example, providing a 10% error is acceptable $V_{\text{min}} = 0.59$ and $V_{\text{max}} = 1.6 \text{ mL min}^{-1}$ when $\Delta b = 0.2$; compared to $V_{\text{min}} = 0.84$ and $V_{\text{max}} = 1.1 \text{ mL min}^{-1}$ when $\Delta b = 0.6$. The corresponding boundary concentrations follow a similar pattern (see Tables 2.11 and 2.12). Thus once the $b_i$ values are known for both the metabolite of interest and the reference substance, it is possible to recommend concentration ranges of the reference material for which uncorrected spot samples do not differ from $U^o_1$ by more than a predetermined percentage. Taking nickel and creatinine as examples ($\Delta b = 0.3$), for an uncertainty of ±10% as shown in Table 2.11 these "boundary values" are: $V_{\text{min}} = 0.70$ and $V_{\text{max}} = 1.37 \text{ mL min}^{-1}$; $U_{\text{creat, min}} = 0.92$ and $U_{\text{creat, max}} = 1.81 \text{ g L}^{-1}$; and $\rho_{\text{min}} = 1.016$ with $\rho_{\text{max}} = 1.026$. Similarly for a ±20% error: $V_{\text{min}} = 0.48$ and $V_{\text{max}} = 1.84 \text{ mL min}^{-1}$; $U_{\text{creat, min}} = 0.69$ and $U_{\text{creat, max}} = 2.64 \text{ g L}^{-1}$; $\rho_{\text{min}} = 1.013$ and $\rho_{\text{max}} = 1.033$. If these error levels are acceptable, rejection of specimens with specific gravity or creatinine concentrations outside of these ranges would assure that on the average $U^o_{Ni}(U^o_{\text{creat}}/U_{\text{creat}})$ approximates $U^o_{Ni}$ or $U_1/U_2 = U^o_1/U^o_2$.

A slightly more sophisticated approach is possible when taking into account the uncertainty in the $\Delta b$ value. Equation 2.24 can be interpreted in terms of $\Delta b \pm \text{SD}$ as shown in Equation 2.25.
\[ \frac{U_1}{U_2} = \frac{U_1^0}{U_2^0} \sqrt{\Delta \bar{b} + SD} \]  

(2.25)

Here, SD is the standard deviation corresponding to the mean of the observed \( \Delta b \) values. It is evident from the data in Table 2.10, that for specific gravity and nickel \( \Delta \bar{b} \pm SD \) has the value 0.005 \( \pm 0.24 \). Now the data in Tables 2.11 and 2.12 and Figure 2.39 can be employed to establish specific gravity ranges for which \( \sqrt{\Delta \bar{b} \pm SD} \) will not differ from unity by more than 10 or 20%, regardless of inter-personal variation in \( b_i \) values. Thus when \( \Delta \bar{b} = 0 \) and \( SD = \pm 0.2 \), \( \rho_{\min} \) and \( \rho_{\max} \) are calculated to be 1.014 and 1.029 respectively for a \( \pm 10\% \) deviation from unity of \( \sqrt{\Delta \bar{b} \pm SD} \). Rejecting samples outside of this specific gravity range assures that specimens from individuals with \( \Delta b \) quite different from \( \Delta \bar{b} \) are still properly compensated within the preselected allowable error.

A normal distribution is assumed for the statistics employed in the remainder of this chapter. Boundary values for the urinary parameters in Tables 2.11 and 2.12 can be selected based upon a preselected confidence level (CL) of \( \Delta \bar{b} \) which corresponds to \( \Delta \bar{b} \pm t(\text{SD}/\sqrt{n}) \) when considering small sampling theory, with \( t = \text{Student t factor} \) and \( n \) the number of donors (McClave and Dietrich, 1985). Taking nickel and creatinine as examples \( (\Delta \bar{b} = 0.3 \pm 0.2) \), for 20 subjects the 95% confidence limits of \( \Delta \bar{b} \) would be 0.2 to 0.4. It is obvious from the data in Figure 2.39 that of these two \( \Delta b \) values, \( \Delta b = 0.4 \) imposes the greatest restriction and thus
determines $V_{\text{min}}$ and $V_{\text{max}}$. To facilitate a comparison of the relative effectiveness of creatinine and specific gravity adjustment protocols, it is helpful to consider the boundary concentrations for both these metabolites. The pertinent urinary parameters for ±10% uncertainty as shown in Table 2.11 are: $U_{\text{creat}, \text{min}} = 1.00$ and $U_{\text{creat}, \text{max}} = 1.64 \, \text{g L}^{-1}$; similarly, $\rho_{\text{min}} = 1.017$ and $\rho_{\text{max}} = 1.024$. By comparison for nickel and specific gravity, the 95% confidence limit of $\Delta b$ for 20 donors is -0.1 to +0.1. The corresponding urinary boundary parameters for ±10% uncertainty (Table 2.11) are: $U_{\text{creat}, \text{min}} = 0.49$ and $U_{\text{creat}, \text{max}} = 3.24 \, \text{g L}^{-1}$; $\rho_{\text{min}} = 1.010$ and $\rho_{\text{max}} = 1.039$. Thus if the spot voids are within the above boundary values, we would expect that at the 95% confidence level the mean flow rate for the group of 20 individuals would fall within the range predetermined by an error of ±10% in the residual flow factor. For urinary nickel correction, the range of both $U_{\text{creat}}$ and $\rho$ is larger when specific gravity adjustment is employed than for creatinine. This illustrates an important advantage of using specific gravity to correct $U_{\text{Ni}}$ values: namely, the number of samples that would have to be rejected at the 95% confidence level to meet the ±10% error specification is fewer. For the 144 voids collected from the 20 donors in the Thompson study, 63% of all the specimens fall outside of the confidence interval specified if creatinine adjustment were adopted; while for specific gravity correction only 15% of the specimens would have been rejected. Similarly, creatinine normalization
would require the rejection of 50% of all the first voids in a shift compared to only 6% for specific gravity adjustment. For the larger group of both cohorts studied (Port Colborne and Thompson; 32 donors) creatinine adjustment would result in 62% of the 242 voids collected falling outside of the confidence interval; while for specific gravity adjustment 15% of the specimens would have been rejected.

For a much smaller group of 3 donors, the 95% confidence interval for \( \Delta b \) increases to -0.3 to +1.0 for creatinine and -0.7 to +0.7 for specific gravity correction. This results in much narrower ranges for values of \( U_{\text{creat}} \) and specific gravity that allow for a \( \pm 10\% \) error. The range of \( U_{\text{creat}} \) would be 1.14 to 1.41 g L\(^{-1}\), while \( \rho = 1.019 \) to 1.022 for creatinine correction. Specific gravity correction would have \( U_{\text{creat}} = 1.10 \) to 1.47 g L\(^{-1}\) and \( \rho = 1.018 \) to 1.022. These narrow ranges would result in a large number of voids being rejected. For small groups it may prove more advantageous to collect 24-h urine specimens (but see below). As illustrated above in groups larger than 20, the accepted range of urinary parameters would only broaden further minimally. Groups of 20 or more would facilitate biological monitoring since only a small number of voids would require rejection to have the mean flow rate falling within the acceptable confidence limits. Similar arguments as above can be employed for a \( \pm 20\% \) acceptable uncertainty-in the residual flow factor. The corresponding urinary parameters are compiled in Table 2.12.

Unlike creatinine, specific gravity can be easily
measured with a hand held refractometer shortly after collection. If the specific gravity observed falls within the predetermined uncertainty, the specimen can be accepted for further processing (i.e., nickel analysis). By contrast if it is outside this range, then the specimen should be rejected and another obtained.

It is appropriate to mention that specific gravity adjustment may be influenced by fluctuation of salt intake and yields erroneous results in the presence of glucosuria or proteinuria (Sunderman et al., 1986b). Nephrotoxic metals are capable of producing the above symptoms (Hook and Hewitt, 1986) and hence may interfere with specific gravity measurements. However, even though nickel may interfere with amino acid excretion (see Chapter 4) the amount of renal abnormality observed in workers of this study is minimal (see Chapter 3). Because of these limitations, along with the fact that the above arguments for normalization presume normal kidney function, it seems appropriate that spot urine voids be screened for diagnostic markers of abnormal kidney function (e.g. presence of glucose and protein). This can be conveniently achieved in a semiquantitative manner with "dip-sticks" (see Chapter 3). If there is evidence of any urinary abnormality, a 24-h collection seems advisable.

The above approach to normalization can be generalized for any urinary analyte of interest. As in the case of nickel, the appropriate $b_i$ value of the exogenous substance or its metabolite should be determined before a normalization protocol is designed. Such evaluation of $b_i$ and of $\Delta b \pm SD$
will allow the setting of appropriate ranges of creatinine or specific gravity for the accepted error level. As will be shown in Chapter 3, the choice of an endogenous substance for normalization is basically determined by the need to match the renal excretion pattern (i.e., clearance) of the xenobiotic with that of the reference metabolite. Since such matching assures that $\Delta b = 0$ and thus $V^{\Delta b} = 1$, the standardization process effectively corrects the urinary concentration to the value that may be expected at $V = 1$ mL min$^{-1}$ since $V^{\Delta b} = 1$ under this condition. An alternative approach to urinary normalization is thus to adjust the analyte of interest to a flow rate that approaches 1 mL min$^{-1}$. This perspective is especially important when a small number of donors is considered. For example, when $n = 3$ then for nickel with specific gravity adjustment $V_{\text{min}} = 0.81$ and $V_{\text{max}} = 1.23$ mL min$^{-1}$, at the 95% confidence level for a 10% error. For a 20% error, the flow rates would be 0.69 to 1.44 ml min$^{-1}$. In the case of 4 people, $V_{\text{min}} = 0.73$ and $V_{\text{max}} = 1.37$ mL min$^{-1}$, for a 10% error; while they have values of $V_{\text{min}} = 0.54$ and $V_{\text{max}} = 1.84$ for 20% error. Unfortunately, urine flow rates are not available for untimed spot specimens. However, as illustrated in Figure 2.17 and Table 2.8, the flow rate distribution for 24-h collections is centered about 1 mL min$^{-1}$ and shows a smaller variation when compared to individual voids (c.f., data in Fig. 2.17 and Table 2.8). Therefore by taking a 24-h collection, the flow rate is automatically closer to the ideal or hypothetical state of 1 mL min$^{-1}$. Of the 32 24-h collections summarized
in Table 2.1, 17 fall within the range mentioned above of $V_{\text{min}} = 0.73$ to $V_{\text{max}} = 1.37 \, \text{mL min}^{-1}$ (10% uncertainty range), and 25 fall within the values $V_{\text{min}} = 0.54$ to $V_{\text{max}} = 1.84 \, \text{mL min}^{-1}$ (20% uncertainty range). This confirms the plausibility of the recommended approach. It also follows from the rationale employed that for spot samples, any correction is better than none, providing $|b_i| \gg |\Delta b|$ (c.f., Eqn's 2.6 and 2.24; also see Fig. 2.38).

Using the above approach, it is not obvious what the protocol for a single donor should be. However, using the standard sample score $= (\text{observation} - \text{mean})/\text{SD}$ (Moore, 1979) and a percentile table it is possible to estimate boundaries for an individual 24-h or spot-sample collection. For the data in this study (Fig. 2.18) the flow rate ($V$) for a 24-h collection is expected to be within the range of 0.5 to 2.0 mL min$^{-1}$ 86 percent of the time ($\bar{V} = 1.2 \pm 0.5 \, \text{mL min}^{-1}$). (These boundaries are selected to keep the uncompensated residual $V^{\Delta b}$ to a reasonable magnitude; see Figure 2.38.) Using the standard score, it can be estimated for individual 24-h collections adjusted by specific gravity, that the $\Delta b$ term will be in the range $-0.4$ to $0.4$ 95% of the time ($\Delta \bar{b} = 0.0 \pm 0.2$). From these ranges of $V$ and $\Delta b$, it is possible to calculate that the uncompensated volume residual term ($V^{\Delta b}$) will have an error of between -24 and 32%. Therefore the standard score predicts a maximum error of $\approx 30\%$ can be expected for an individual using a 24-h collection corrected by specific gravity if $V$ is within the boundary values of 0.5 and 2.0 mL min$^{-1}$. This would seem an acceptable error and
rejection rate (i.e., corresponding to 5/32 (16%) of 24-h specimens in the present study). Similarly for the same boundary values of V and the 95% confidence interval for Δb, the rejection rate for the 210 spot-sample voids depicted in Figure 2.17 would be 64/210 (30%). This indicates that if a 30% error is acceptable then a specific-gravity adjusted urinary nickel concentration in a spot sample is a suitable measure of nickel excretion in a single individual, providing the specific gravity value falls between \( \rho = 1.015 \) (at \( V = 0.50 \text{ mL min}^{-1} \) and \( \Delta b = 0.4 \); or \( V = 2.0 \text{ mL min}^{-1} \) and \( \Delta b = -0.4 \)) and \( \rho = 1.026 \) (at \( V = 2.0 \text{ mL min}^{-1} \) and \( \Delta b = 0.4 \); or \( V = 0.50 \text{ mL min}^{-1} \) and \( \Delta b = -0.4 \)). Consequently, it may be concluded that specific-gravity adjustment of urinary nickel concentrations in spot samples when combined with appropriate flow-rate restrictions (expressed in terms of specific gravity range) provide a suitable estimate of \( U_{Ni}^0 / U_{\rho-1}^0 \) at a specified error and confidence interval.

The question of whether to normalize, and if so with what, should not be left to the discretion of the individual investigator. As pointed out above, any correction in most cases would be better than none; however if this work is to be used in a meaningful manner it is essential that investigators be consistent and a normalization protocol have general acceptance and implementation. Only when this happens will it be possible for urinary data to be compared, to be employed as indices of exposure and to be useful in risk assessments.
CHAPTER 3 RENAL CLEARANCE OF NICKEL AND BIOCHEMICAL INDICES OF KIDNEY FUNCTION IN ELECTROLYTIC REFINERY WORKERS

A INTRODUCTION

As indicated in the general introduction to this thesis there are a small number of reports of nickel induced nephrotoxicity in humans. Proteinuria has been reported for people who chronically drank water from a well contaminated with nickel (Gitlitz et al., 1975). Sunderman and Horak (1981) reported a significant association between increased urine concentration of $\beta_2^\text{micro}-\text{globulin} (\beta_2^-\mu)$ ($>240 \, \mu\text{g} \, \text{L}^{-1}$) and hypernickeluria ($>100 \, \mu\text{g} \, \text{L}^{-1}$) for 30 electrolytic nickel refinery workers ($p<0.05$ Mann-Whitney U test) with the largest $\beta_2^-\mu$ concentration of 450 $\mu\text{g} \, \text{L}^{-1}$. These urinary $\beta_2^-\mu$ levels were not, however, reported per mole or g of creatinine as is common in cadmium studies. Recently, Elinder et al. (1985a) reported unadjusted urinary $\beta_2^-\mu$ levels of between 40 and 20100 $\mu\text{g} \, \text{L}^{-1}$ for 19 workers exposed to cadmium. High levels of $\beta_2^-\mu$ appear to be quite common in cadmium workers and are taken as a marker for kidney tubular damage. Lauwerys (1983) considered renal function of Cd-exposed workers to be abnormal when total protein in the urine exceeded 250 mg/g-creatinine. Wall and Calnan (1980) found that there was no evidence of proteinuria in seventeen nickel-process workers in an electroforming plant during an outbreak of occupational dermatitis. Other than two cases of nickel carbonyl poisoning (Sunderman, 1977), no other reports of proteinuria, or of other markers of renal
disease have been reported with workers exposed to nickel. Recently, there has been one report of IgA nephropathy associated with dental nickel alloy sensitization (Strauss and Eggleston, 1985). These authors suggest that their patient may represent an example of nickel-induced sensitization associated with IgA glomerulopathy. There are also isolated reports indicating slight elevations in kidney and prostate cancer in nickel workers. However, these are not consistent, nor statistically significant, and their importance has yet to be clarified (Nieboer et al., 1984a; Roberts et al., 1984). The apparent lack of renal damage attributed to nickel is in direct contrast to the well established nephrotoxic and specific damage caused by a number of other metals such as uranium, cadmium, lead and mercury (Nieboer and Sanford, 1985). Metal-ion induced kidney damage is specific for individual metals.

In contrast to the inconclusive evidence of renal damage in humans, there is much better evidence of nickel-induced nephropathy in animal studies. For example, poisoning of dogs and cats by nickel nitrate was reported to produce acute renal injury with proteinuria and hyaline casts. Inhalation or injection of nickel carbonyl was found to cause transient azotemia, with pathological evidence of glomerular and tubular damage in various species including man (Sunderman et al., 1975; Gitlitz et al., 1975; Sunderman, 1977; Sunderman and Horak, 1981). Acute nephropathy developed in rats after single ip injection of NiCl₂ with a 4-fold increase in urinary total proteins, 2-fold increase of urine NAG activity
and a 20- to 25-fold increase of valine excretion (Sunderman and Horak, 1981). Mathur and Tandon (1981) also found significant elevation of urine and plasma α-amino acid levels in rats 7 days after ip injection of NiSO₄·6H₂O. A more detailed outline of renal damage found in animal studies will be presented in Chapter 4.

There has only been one detailed study of the renal excretion of nickel by humans. Mertz et al. (1970) looked at the renal excretion of Ni under varying conditions of diuresis in adults of both sexes with normal and impaired kidney function. Great variability in Ni excretion was observed within the individual experimental groups that could not be related to different functional states of the kidneys. However, nickel excretion was shown to be dependent on urine flow rates.

It is interesting to look at the work done by Tossavainen et al. (1980) to describe the excretion pattern of 4 electroplaters exposed to water-soluble nickel sulphate and chloride. Air, urine and plasma samples were collected during one workweek immediately after the workers' summer vacation. Urine and plasma samples were taken at 0700 and 1600 h each day of the week. Air samples were collected with personal samplers for the am and pm periods during the work shift from Monday to Friday inclusive. The urinary nickel levels were corrected to a specific gravity of 1.018. They were able to fit this experimental data to a mathematical model that employed a simple linear one-component kinetic model with one input and one output variable. It was assumed
that the nickel uptake is proportional to the ambient nickel level and can be described as a series of consecutive doses during the workweek. Elimination was also assumed to be governed by first order kinetics (see Figure 3.1). This model gave estimates of elimination half-time ($t_{1/2}$) ranging from 17 to 39 h for nickel in the urine and 20 to 34 h for nickel in the plasma. These values are in good agreement with those reported in Chapter 1 for exposure to soluble nickel salts. Both the mathematical model and the experimental data indicate that the urine (or plasma) concentrations increase significantly not only during the work day, but also gradually during the workweek.

The research described in this chapter investigates urinary nickel excretion pattern(s), its renal clearance and evidence for renal damage in electrolytic nickel refinery workers. It will also look at the change in urinary nickel levels after a one month lay-off and its fluctuations during a workshift.

B THEORETICAL CONSIDERATIONS OF RENAL CLEARANCE

(i) INTRODUCTION

The following derivations are based on the classical treatment of clearance of an analyte (i) as depicted in Equations 3.1 and 3.2.
Figure 3.1. Urinary nickel concentrations (adjusted to a specific gravity of 1.018) for subject A during a work week. Bar diagrams represent nickel concentrations in air (am and pm). Reproduced from Tossavainen et al. (1980). The model used to predict the urinary concentration due to exposure may be summarized by the following expression:

$$U_{Ni} = k_3 + k_1 C_{air} - k_1 C_{air} \exp[-k_2(T-t_1)]$$

Here $U_{Ni}$ is the urinary nickel concentration ($\mu g$ L$^{-1}$); $k_1$ is a scaling constant relating air and urinary concentration $[(\mu g$ L$^{-1})/(\mu g$ m$^{-3})]$ and $k_2$ a scaling constant related to biological half-life $T_{1/2}$ ($k_2 = \ln 2/T_{1/2}$); $C_{air}$ is the measured air concentration (mg m$^{-3}$); $t_1$ is the time of onset of exposure; $T$ is the time of observation and $k_3$ is constant baseline concentration (dietary uptake and body burden). $U_{Ni}$ is determined by a term which is independent of the biological half-life (2nd from right) and another (1st from right) which depends on it. Changes in $C_{air}$ can be accommodated in the above formula by adding similar terms for the new values of $C_{air}$ (positive or negative) and summing the various contributions.
\[ C_i S_i (\text{amount cleared from serum}) = U_i V (\text{amount excreted in urine}) \quad (3.1) \]

where

\[ C_i = \frac{U_i}{S_i} V \quad (3.2) \]

\[ \text{where} \]

- \( C_i \) = clearance of analyte (i) in mL min\(^{-1}\) (the hypothetical volume of plasma or serum that has theoretically been completely cleared of analyte (i))
- \( U_i \) = urine concentration of the analyte (i) with the same units as the plasma or serum
- \( V \) = urine flow rate in mL min\(^{-1}\)
- \( S_i \) (or \( P_i \)) = the serum or plasma concentration of the analyte (i) with the same units as in the urine.

It is common for metals to have only a fraction of the total amount in the serum available for filtration through the glomerulus of the kidney. For example, serum calcium is recognized to have three distinct fractions: (a) protein-bound, (b) low molecular-mass complexes, and (c) free \( \text{Ca}^{2+} \) (Weissman and Pileggi, 1974). These authors state that the total amount of ultrafilterable calcium (free ion and low molecular-mass complexes) is 60.5%. When the fraction of a metal that is ultrafilterable \( (\alpha_i) \) is included in the clearance expression, Equation 3.2 becomes:

\[ C_i = \frac{U_i}{\alpha_i S_i} V \]

(3.3)
and Equation 3.1 should be written as in 3.4.

\[ U_i V \text{ (amount excreted)} = \alpha_i C_i S_i \text{ (amount cleared)} \quad (3.4) \]

The clearance \( C_i \) now represents the volume from which the ultrafilterable component of substance \( i \) is removed and \( \alpha_i C_i \) is the hypothetical volume from which all of substance \( i \) is removed.

(ii) FRACTIONAL CLEARANCE

In Chapter 2 it was shown that nickel specific gravity (solutes) and creatinine adhere to Equation 3.5

\[ U_i^b_i = U_i^0 \quad (3.5) \]

where \( U_i^0 \) is the urinary concentration of \( i \) at a flow rate of \( 1 \text{ mL min}^{-1} \) and \( b_i \) is the volume exponent or power coefficient. The parameters \( U_i \) and \( V \) are as previously defined. When \( V = 1 \text{ mL min}^{-1} \) Equation 3.3 becomes

\[ U_i = \alpha_i C_i^0 S_i \quad (3.6) \]

where \( C_i^0 \) is the clearance of the metal at a flow rate of \( 1 \text{ mL min}^{-1} \). If both sides of Equation 3.4 are divided by \( V^{1-b} \), Equation 3.7 is obtained.

\[ \frac{U_i V}{V^{1-b_i}} = \frac{\alpha_i C_i}{V^{1-b_i}} S_i \quad (3.7) \]

which reduces to

\[ U_i^b_i = \alpha_i C_i^b_i V^{1-b_i-1} S_i \quad (3.8) \]

Equation 3.9 is obtained by combining Equations 3.5, 3.6 and 3.8.
\[ U_i^b_i = \alpha_i C_i^b_i V_i^b_i \quad \therefore \quad S_i = U_i^0 = \alpha_i C_i^0 \]  

\[ C_i^{b_i-1} = C_i^0 \]  

or

\[ C_i = C_i^0 V_i^{1-b_i} \]  

Considering two analytes 1 and 2, Equations 3.12 and 3.13 then follow.

\[ C_1 = C_1^0 V_1^{1-b_1} \]  

\[ C_2 = C_2^0 V_2^{1-b_2} \]  

The general equation for the relative clearance (RC) for any analyte 1 being normalized by a second analyte 2 is

\[ RC_{1,2} = \frac{C_1}{C_2} = \left[ \frac{C_1^0}{C_2^0} \right] \times \left[ \frac{V_1^{1-b_1}}{V_2^{1-b_2}} \right] \]  

\[ = \left[ \frac{C_1^0}{C_2^0} \right] V_2^{b_2-b_1} \]  

For creatinine-normalization of urinary nickel Equation 3.15 takes the form 3.16, which constitutes the fractional clearance; Equation 3.17 represents the relative clearance for specific-gravity adjustment.

\[ FC_{\text{Ni,creat}} = \frac{C_{\text{Ni}}}{C_{\text{creat}}} = \left[ \frac{C_{\text{Ni}}^0}{C_{\text{creat}}^0} \right] V_{\text{creat}}^{b_{\text{creat}}-b_{\text{Ni}}} \]  

\[ RC_{\text{Ni,}\rho-1} = \frac{C_{\text{Ni}}}{C_{\rho-1}} = \left[ \frac{C_{\text{Ni}}^0}{C_{\rho-1}^0} \right] V_{\rho-1}^{b_{\rho-1}-b_{\text{Ni}}} \]
The expression in Equation 3.16 denotes the fractional clearance because creatinine is used as an estimate of the glomerular filtration rate (GFR) (see Section 3C). $\text{FC}_{\text{Ni,creat}}$ thus represents the fraction of filtered nickel appearing in the urine.

(iii) SERUM/URINE RELATIONSHIP

Equation 3.4 can be rearranged to highlight the serum term as in Equation 3.18

$$S_i = \frac{U_i V}{\alpha C_i} \quad (3.18)$$

or

$$S_i = \frac{1}{\alpha C_i} U_i V \quad (3.19)$$

or

$$S_i = \frac{1}{\alpha C_i} E_i \quad (3.20)$$

where $U_i V = E_i$ = excretion rate or excretion per unit time.

C  REVIEW OF LABORATORY METHODS FOR SERUM CREATININE AND SPECIFIC GRAVITY DETERMINATION

A review of the literature indicates that there is not a definitive method to determine concentrations of creatinine in serum. The most common approach employs Jaffé's reagent which is know to be nonspecific in that colour develops with noncreatinine chromogens such as cepha antibiotics, the hypoglycemic agent acetohexamide (Kroll et al., 1987),
acetone and glucose (Cook, 1975). Kroll et al. (1987) also postulated that a co-planar charge-transfer complex is formed between Jaffé's reagent (picrate) and creatinine (or other ketones) which accounts for the observed reactions with noncreatinine chromogens. Three approaches have been followed in attempts to improve the specificity of the creatinine assay: (1) modification by adsorption with Lloyd's reagent; (2) use of alternative methods to the Jaffé reaction such as high pressure liquid chromatography (HPLC) (Okuda et al., 1983) and (3), use of creatinine-splitting enzymes (Cook, 1975). Two of these alternative methods highlighted in the current literature are briefly described. The "proposed selected method" of Haeckel (1981) is a modification of that by Jaffé (1886). This method uses Fuller's earth (kaoline containing a hydrated aluminum/magnesium silicate; also referred to as Lloyd's reagent) to eliminate interference by other chromogens. The second approach by Jaynes et al. (1982) employs an enzyme assay. The change in absorbance at 340 nm represents the utilization of NADH in a coupled-enzyme assay for creatinine.

Rapoport and Husdan (1968) compared three different sample pretreatments before colour development by the Jaffé method. These included: (1) deproteinization of serum which results in a small amount of non-creatinine chromogen being detected; (2) adsorption of creatinine on Lloyd's reagent; and (3), dialysis as part of a Technicon Auto-Analyzer unit. They found that the Auto-Analyzer method gave the best
correlation between creatinine clearance and the clearance of inulin. The residual amount of noncreatinine chromogens measured in plasma by this method appears to balance the creatinine appearing in the urine due to tubular secretion. This compensation is illustrated in the following mathematical formula employed in the calculation of clearance of endogenous creatinine (Duarte et al., 1980).

\[
\text{Creatinine clearance} = \frac{\text{Urine creatinine (filtered + secreted) mg dL}^{-1} \times V \text{ ml min}^{-1}}{\text{Plasma (creatinine + noncreatinine chromogens) mg dL}^{-1}}
\]

Simultaneous measurements of inulin clearance, the actual creatinine clearance (creatinine determined in absence of other chromogens), and the clearance of creatinine assessed by the Auto Analyzer/dialysis method have demonstrated that for normal glomerular filtration rates (GFR), actual creatinine clearance overestimates GFR by a mean amount of 46%, while creatinine clearance by Auto Analyzer/dialysis overestimates by 8% (Anderson et al., 1970). Since creatinine is the only known endogenous substance in the body whose clearance approximates the clearance of inulin (Duarte et al., 1980; Epstein, 1977), this makes its use for the estimate of GFR practical for the current study. Because the Auto Analyzer/dialysis approach gave results closest to that of the true GFR as measured by inulin clearance, this method was selected for the
measurement of serum and urine creatinine.

The two most common methods of measuring urinary specific gravity is by a urinometer and refractometry (Faulkner and King, 1976). The urinometer method involves the measurement of specific gravity by a hydrometer in a cylinder containing urine. A number of disadvantages are associated with this method: (i) it is time consuming, (ii) a large volume of urine is needed, (iii) requires correction for large quantities of protein and glucose, and (iv) needs to be corrected for temperature (Faulkner and King, 1976). By contrast, measurement of urinary specific gravity by a temperature compensated refractometer (American Optical Corporation) overcomes many of the above problems. This method is rapid, only requires a small drop of urine and does not need to be compensated for temperature. However, large amounts of protein and or glucose will still interfere.

Refractometry is based on the linear relationship between refractive index and the total concentration of dissolved solids (Glover and Goulden, 1963). Such relationship even occurs for a complex solution such as urine (Rubini and Wolf, 1957). Deviations from linearity are negligible for urinary specific gravity values below 1.035, which corresponds to the upper end of the range normally encountered (Cannon, 1974). Because of its simplicity, speed of measurement and general acceptance in Clinical Chemistry laboratories, the refractometric method was chosen for the measurement of urinary specific gravity.
D MATERIALS AND METHODS

The same serum and urine samples considered in Chapter 2 also form the data base here. Thus a number of the laboratory solutions and procedures are the same as described in Chapter 2. Therefore they will not be described in detail here and the reader is referred to Chapter 2.

(i) MATERIALS

(a) Chemical Reagents

Pertinent information about the routine chemical reagents used is summarized in Table 2.1.

(b) Laboratory Solutions (Auto Analyzer)

Details about the source and preparation of solutions are described in Chapter 2.

(c) Laboratory Solutions (Nickel Determination)

Full details of the preparation of these solutions are described in Chapter 2.

(d) Laboratory Solutions (Total Protein Determination)

Veronal Buffer (pH = 7.4). Stock buffer solution contained 0.015 M barbital, 0.0045 M sodium barbital and 0.77 M NaCl in DDW. Before use it was diluted four-fold (100 mL to 400 mL) with DDW giving a final pH of 7.4.

Stock Total Protein Standard (4.5 mg mL⁻¹). Into a 50 ml
beaker, exactly 0.1125 g of Human Serum Albumin (HSA) was added. Approximately 10 mL of veronal buffer was added and HSA was allowed to dissolve. The solution was transferred to a 25 mL volumetric flask and brought to volume with veronal buffer. This solution was stored at 4°C.

(e) Decontamination of Plastic and Glasswares

The same protocol as described in Chapter 2 was employed. In Table 2.2, details are provided about the type of glassware and plasticware employed.

(ii) DONOR POPULATIONS

As described in Chapter 2, this study was carried out on 26 subjects occupationally exposed to nickel at INCO's electrolytic refining operations in Port Colborne (Ontario, Canada) and Thompson (Manitoba, Canada). A collection involving twenty workers was conducted at Thompson over a single 24-h period involved three shifts. Two sera samples were collected for each donor. The sera for the workers from the day shift (7am - 3pm) and afternoon shift (3pm - 11pm) were collected at the start of the shift. The donors from the midnight shift (11pm - 7am) had their sera taken at the end of a shift. Two exceptions to this were the General Foreman (Refinery) and the General Foreman (Safety and Production) who although they worked between 7am and 4pm, were not able to have their sera taken until 11:00am and 12:00 (noon) because of their workload. The sera for the
collections at Port Colborne were taken at the beginning of the day shift at 8:00 am (1st collection) and between 8:00 and 9:00 am for the second collection.

Detailed job classifications for both donor groups are given in Table 3.1.

During all 24-h multi-void urine collections there was no attempt to restrict diet (i.e., fasting) or increase liquid intake (i.e., water loading) above their normal level. Furthermore, there was no attempt to restrict alcohol intake.

(iii) METHODS

(a) Urine Collection Procedures

As described in Chapter 2, each void was analyzed for nickel, creatinine and specific gravity ($\rho$) and its pH was measured. In addition, dip-stick analysis for ketones, bilirubin, glucose, nitrite, blood, urobilinogen and protein were also performed. Selected specimens were analyzed for total protein (Bradford, 1976) and $\beta_2$-microglobulin (RIA assay).

(b) Serum Collection Procedure

Serum samples were collected as described in Chapter 2B (iii) b. Each sample was analyzed for nickel, creatinine and $\beta_2$-microglobulin (RIA assay).
Table 3.1

JOB DESCRIPTION OF WORKERS

<table>
<thead>
<tr>
<th>JOB</th>
<th>Number of Workers</th>
<th>Port Colborne</th>
<th>Thompson</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreman (Refinery)</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Foreman (Safety &amp; Protection)</td>
<td></td>
<td>0</td>
<td>1(^a)</td>
</tr>
<tr>
<td>Process Assistant</td>
<td></td>
<td>1(^b)</td>
<td>0</td>
</tr>
<tr>
<td>Boxman</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Scrapwash</td>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Plating Tankman</td>
<td></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Cobalt Recovery</td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Copper Recovery</td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Purification/Thickener</td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Equipment Man</td>
<td></td>
<td>0</td>
<td>1(^c)</td>
</tr>
<tr>
<td>Labourer</td>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Electrician (Refinery)</td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) Present job does not require spending significant time in the electrolytic refinery. Previously he had worked for 10 y as a foreman in the electrolytic refinery.

\(^b\) This job involves collecting samples of electrolyte throughout the refinery for laboratory analysis.

\(^c\) This job requires the worker to be in and out of the refinery.
(c) Determination of Serum and Urinary Nickel

These procedures are identical to those described in Chapter 2B (iii)c and e.

(d) Determination of Serum and Urinary Creatinine

These were measured by the Jaffé reaction employing a single-channel Technicon Auto Analyzer with a dialysis block; a detailed description is found in Chapter 2B (iii) f.

(e) Determination of Specific Gravity

Specific gravity was measured on a hand-held, temperature-compensated, refractometer (American Optical Corporation). The accuracy of this instrument was comparable to established equipment in the Clinical Chemistry Laboratory at the Chedoke-McMaster Medical Centre.

(f) Determination of Osmolality

This parameter was measured by the Clinical Chemistry Laboratory of the Chedoke-McMaster Hospital by freezing-point depression of centrifuged urine samples using an Advanced Instruments Osmometer Model No. 68-L.

(g) Determination of Urinary Parameters by Dip-Sticks

This is a semiquantitative method for the simultaneous determination of up to six or seven chemical properties of urine. This is done by dipping into the specimen of urine a strip of hydrophobic plastic that has test areas impregnated
with various chemical indicators. The reagent test areas are completely immersed in fresh, well mixed, uncentrifuged urine and then removed immediately to avoid leaching out of any chemical. Excess urine is removed by running the edge of the strip against the rim of the urine container. The strip is held in a horizontal position to prevent mixing of chemicals, and read at specific time intervals by comparing the developed colours of a test area to standard colour charts.

The detailed instructions for the use of these test strips is given by the manufacturer in an information sheet that is included with every package.

(h) Determination of Total Protein

The total protein content of urine was measured by the Bio-Rad protein assay. Bradford (1976) first demonstrated the usefulness of this method. It is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein. Nesbett et al. (1978) found that for urine-protein determinations it is necessary to make sure the urine pH is below 8.0. Their results were comparable if not superior to sulfosalicylic (turbidimetric) measurements. This is consistent with the results found in the present study where the Bio-Rad assay performed in a comparable manner to the sulfosalicylic acid standard method adopted by the American Society of Clinical Pathologists (code ST-63 1973). Bio-Rad protein determination has been
shown to have a linear standard curve and is relatively free from most of the interferences that limit the application of the earlier Lowry protein assay. It has been shown that Ni\(^{2+}\) does not interfere with the Bio-Rad method (Maxwell, 1984).

The total protein working standards were made up fresh daily by pipeting accurately aliquots (with a fixed volume 100-\(\mu\)L Eppendorf pipettor) of an HSA standard solution (4.5 mg mL\(^{-1}\)) into a 5 mL volumetric to give final concentrations between 4.5 and 45 mg dL\(^{-1}\). The working standards were brought to volume with veronal buffer (pH = 7.4). The purchased dye reagent was diluted (5x) with DDW and filtered prior to use. This reagent was made up fresh each day. In the assay, 100 \(\mu\)L (from a fixed volume 100-\(\mu\)L Eppendorf pipettor) of standards, urines and blanks (veronal buffer) were placed in dry test tubes. Then 5.0 mL (from a Brinkman Dispensette) of diluted dye reagent are added. The solution was mixed by gentle inversion to avoid excess foaming. After a period of \(\approx\) 30 min, the absorbance was measured at 595 nm. The unknowns were read from a standard curve.

The total protein was measured for all the urine samples that showed trace amounts by the dip-stick method and on all samples selected for \(\beta_2\)-microglobulin determination.

(i) Determination of \(a_{Ni}\)

The ultrafiltrable fraction of nickel in serum \((a_{Ni})\) was determined by ultracentrifugation and nickel analysis of the supernatant by electrothermal atomic absorption spectrometry
The serum was thawed at room temperature and 5.0 mL (1mL Gilson pipettor) were added to a threaded-top polycarbonate ultracentrifuge bottle. The tubes were put in a fixed angle titanium rotor (Type 70.1 Ti) and ultracentrifuged at 230 000 g for 17 h at 4°C on a Beckman Ultracentrifuge (model L8-80). The tubes were then removed from the rotor in a cold room and the volume of supernatant was measured. The supernatant was collected without disturbing the pellet and the low-molecular-mass lipids at the top of the tube. The protein content of the supernatant was determined by the Bio-Rad procedure.

The nickel content of the supernatant was measured by EAAS. The value was calculated by comparing the amount of nickel in the ultrafiltrate (i.e., the supernatant) to that found in the serum before centrifugation.

(j) Determination of urinary pH

The pH was measured by a Radiometer model M84 pH meter, or similar instrument, that had been standardized against two buffers.

(k) Determination of $\beta_2$-microglobulin

Human $\beta_2$-microglobulin ($\beta_2$-μ) is a low molecular mass protein (relative molecular mass of 11800). It is produced by the nucleated cells of the body and eliminated via the kidneys. After filtration through the glomeruli, it is
reabsorbed by the proximal tubular cells, where it is rapidly catabolized within the lysosomes (Bernier and Conrad, 1969; Conway and Poulit, 1977). Normally, only trace amounts of \( \beta_2 \)-microglobulin are excreted in the urine. However, exposure to metals such as Hg and Cd, or organic chemicals (e.g., aminoglycosides) that damages the proximal tubule, can lead to larger amounts in the urine due to tubulointerstitial disorders resulting in decreased reabsorption of this protein.

Two different RIA kits from Pharmacia were used because the Phadebas \( \beta_2 \)-micro (RIA-1) kit used initially was phased out by Pharmacia during the course of this work and was replaced by the RIA (RIA-2) test kit. Both employ a competitive radioimmunoassay. The \( \beta_2 \)-microglobulin in the sample competes with a fixed amount of added \(^{125}\)I-labelled \( \beta_2 \)-microglobulin (\(^{125}\)I-\( \beta_2 \)-\( \mu \)) for the binding sites of anti-\( \beta_2 \)-microglobulin antibodies. After the appropriate incubation time, the unreacted \( \beta_2 \)-\( \mu \) is separated from that bound to the anti-\( \beta_2 \)-\( \mu \) antibody by two methods. In the RIA-1 kit, the anti-\( \beta_2 \)-\( \mu \) antibodies are covalently bound to Sephadex particles which are removed from the "free" \( \beta_2 \)-\( \mu \) by centrifugation followed by decantation and subsequent washes. The RIA-2 kit employs the addition of a second antibody which precipitates the \( \beta_2 \)-\( \mu \)/primary antibody complex. Separation is achieved by centrifugation, which is followed by decantation and washing to remove the "free" \( \beta_2 \)-\( \mu \) from the bound \( \beta_2 \)-\( \mu \). The bound \(^{125}\)I-labeled \( \beta_2 \)-\( \mu \) is measured by a
gamma counter. The measured radioactivity due to $^{125}\text{I}$ is inversely related to the concentration of unlabelled $\beta_2\mu$ in the unknown or standard. The percent of bound activity

$$\% \text{ activity bound} = \frac{\text{counts of standard/unknown}}{\text{counts of zero standard}} \times 100$$

is plotted against the standard $\beta_2\mu$-microglobulin concentrations or amount on semi-log paper. The urinary $\beta_2\mu$ concentrations were obtained from the standard curve employing an appropriate dilution factor as required. Table 3.2 compares the reagents and methods employed in both methods.

Immediately after collection, urine specimens were adjusted with 1.0 M NaOH to a pH of between 6 and 8 and were promptly frozen to prevent degradation of the $\beta_2\mu$ (Lauwarys et al., 1984). Transport to the laboratory was in ice-packed freezer containers. The transport from Thompson also included placement of containers in air-plane cargo areas at $-10^\circ\text{C}$. On arrival at the laboratory, the frozen urine samples were immediately placed in a freezer ($<-10^\circ\text{C}$). For both Port Colborne collections, the $\beta_2\mu$ was measured using the RIA-1. However, when the Thompson collection was made Pharmacia started to phase out this test kit and replaced it with the RIA-2 kit. The main difference in the standards provided in these kits is that in the RIA-2 method they are more concentrated than in the RIA-1 procedure. The lowest standard provided with the RIA-1 method was 10 $\mu\text{g L}^{-1}$ while
for the RIA-2 it was 400 \( \mu g \text{ L}^{-1} \). Since the highest value of \( \beta_2-\mu \) measured in the Thompson collection was 178 \( \mu g \text{ L}^{-1} \), modifications of the recommended procedure were necessary to permit the analysis of the samples with such "low" values. Details are provided below.

Low working standards for the RIA-2 assay were made up by diluting the high standards with DDW to make 25, 50, 100, 300, and 400 \( \mu g \text{ L}^{-1} \) working standards (mixing was by gentle inversion). Next 200 \( \mu L \) of standard, blank (DDW) and unknown were pipetted into the bottom of polystyrene centrifuge tubes (12mm x 75 mm; round bottom). The \( ^{125}\text{I-}\beta_2-\mu \) was pipetted (500 \( \mu L \)) into each standard, unknown and blank followed by 50 \( \mu L \) of antibody. Test tubes were vortexed if necessary until they were a uniform green colour. The remainder of the steps were the same as listed in Table 3.2. After the prescribed centrifugation and decantation, the radioactivity of all test tubes was measured. To avoid non-specific contributions to the measured counts, the test tubes were then washed with a 0.05% (w/v) Tween and 0.15 M NaCl buffer, centrifuged, decanted and the \( ^{125}\text{I} \) measured again. The \( \beta_2-\mu \) values for the unknowns were read off a semi-log plot of % bound versus \( \beta_2-\mu \) (ng).
### Table 3.2

**β₂-MICROGLOBULIN RADIOIMMUNOASSAY REAGENTS AND ASSAY PROTOCOLS**

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RIA-1 (β₂-micro)</strong></td>
<td><strong>RIA-2</strong></td>
</tr>
<tr>
<td><strong>Buffer Solution</strong></td>
<td>Standards</td>
</tr>
<tr>
<td>Added 1 vial of Tween solution to 300 mL of DDW; then added 1 vial (8.3 g) of buffer powder.</td>
<td>Antibody</td>
</tr>
<tr>
<td><strong>Sephadex-anti-β₂-µ complex</strong></td>
<td><strong>β₂-µ-globulin (¹²⁵I)</strong></td>
</tr>
<tr>
<td>Added 7.0 mL DDW to the purchased vial and the suspension was allowed to stand for ~2 min, then mixed (magnetic stirrer).</td>
<td><strong>Decanting Solution</strong></td>
</tr>
<tr>
<td><strong>β₂-µ Standards</strong></td>
<td></td>
</tr>
<tr>
<td>1000 µL of DDW was added to each vial and after standing for ~1 min was mixed to give the following standards: 10; 25; 75; 200 and 500 µg/L.</td>
<td></td>
</tr>
<tr>
<td><strong>β₂-µ-globulin (¹²⁵I)</strong></td>
<td>5.5 mL of DDW was added to the vial and after standing for ~1 min was mixed.</td>
</tr>
</tbody>
</table>
Table 3.2 Continued

**METHOD**

<table>
<thead>
<tr>
<th>RIA-1 (β₂-micro)</th>
<th>RIA-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> (a) Blanks. Pipetted 50 μL of buffer solution into dry test tubes.</td>
<td><strong>1</strong> Pipetted 50 μL of β₂-μ standards into test tubes.</td>
</tr>
<tr>
<td>(b) Standards. Pipetted 50 μL of β₂-μ standards into the test tubes.</td>
<td></td>
</tr>
<tr>
<td>(c) Samples. Pipetted 50 μL of diluted unknowns into test tubes.</td>
<td><strong>2</strong> Pipetted 50 μL of unknown (serum or urine) samples (DO DILUTION).</td>
</tr>
<tr>
<td><strong>NOTE:</strong> Serum samples were diluted 1/51; urine samples were diluted 1/5 with buffer solution.</td>
<td></td>
</tr>
<tr>
<td><strong>2</strong> Pipetted 50 μL of (^{125}\text{I-β₂-μ}) solution into all the test tubes in step 1.</td>
<td><strong>3</strong> Pipetted 50 μL of (^{125}\text{I-β₂-μ}).</td>
</tr>
<tr>
<td><strong>3</strong> Pipetted 50 μL of Sephadex anti-β₂-μ complex suspension into all test tubes.</td>
<td><strong>4</strong> Pipetted 50 μL of antibody.</td>
</tr>
<tr>
<td><strong>NOTE:</strong> The Sephadex anti-β₂μ complex suspension was stirred continuously on a magnetic stirrer during pipetting.</td>
<td><strong>5</strong> Each test tube was mixed by vortexing until solution turned a homogenous green colour.</td>
</tr>
<tr>
<td><strong>4</strong> Each test tube was mixed by vortexing until the solution turned a homogeneous green colour. The test tubes were then covered with Parafilm and incubated on a horizontal shaker for 90 min at room temperature.</td>
<td><strong>6</strong> 2.0 mL of decanting suspension was added to each test tube (5 mL Gilson).</td>
</tr>
<tr>
<td></td>
<td><strong>NOTE:</strong> The suspension was periodically mixed to ensure homogeneity.</td>
</tr>
</tbody>
</table>
Table 3.2 Continued

<table>
<thead>
<tr>
<th>METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RIA-1 (β₂-micro)</strong></td>
</tr>
</tbody>
</table>
| 5 Separation  
(a) Added 2.0 mL buffer to each test tube.  
(b) Centrifuged the test tubes on a swingout bucket for 10 min at 2000xg.  
(c) Decanted the supernatant by gently turning the tubes upside down with a smooth uninterrupted movement; the inverted tubes were placed on absorbent paper for 5s. | 7 The test tubes were then covered with Parafilm and incubated for 45 min at room temperature on a horizontal shaker.  
8 Centrifuged all the test tubes on a swingout bucket for 10 min at 2000xg.  
9 The test tubes were decanted as described in step 5 (c) of the RIA-1 method. |
| 6 The bound reactivity in the tubes were measured on a LKB Universal 1282 gamma counter with one minute counting. The background was measured by using empty test tubes.  
The % activity bound obtained from the β₂-μ standards were plotted against the β₂-μ concentration (µg/L) on semi-log paper to construct a standard curve. The unknowns were read off the standard curve and multiplied by the appropriate dilution factor. | 10 The bound reactivity in the tubes were measured on a LKB Universal 1282 gamma counter with one minute counting. The background was measured by using empty test tubes.  
The % activity bound obtained from the β₂-μ standards were plotted against the β₂-μ concentration (µg/L) on semi-log paper to construct a standard curve. The unknowns were read off the standard curve. |
E RESULTS

(i) URINARY $\beta_2$-MICROGLOBULIN

The RIA-1 ($\beta_2$-micro) kit required an extra-washing step of the sample-equilibrated Sephadex to attain acceptable accuracy and reproducibility. The RIA-2 method, modified for low standards, was found to have comparable accuracy, reproducibility and limit of detection as the RIA-1 kit. Also, there did not seem to be a large difference between the values of $\beta_2$-µ obtained by the RIA-2 procedure when the pellets were not washed and when they were washed once with a 0.05% (w/v) Tween and 0.15 M NaCl buffer.

(ii) BIOCHEMICAL INDICES

The data in Tables 3.3 and 3.4 summarize the results of the biochemical indices of nephrotoxicity measured in the Port Colborne group. They suggest that the concentrations of indicator substances were within the normal range. A similar pattern was observed in the Thompson workers, although a few anomalies appear evident (Tables 3.5 and 3.6). Two workers had abnormally low creatinine clearances, 29 and 41 mL min$^{-1}$, adjusted for body surface area of 1.73 m$^2$. Three workers were found to have elevated urinary $\beta_2$-microglobulin in individual voids. However, when averaged over three or more voids, the average values were below 200 µg g$^{-1}$ creatinine and within the normal 24-h excretion range. Tables 3.7 and 3.8 summarize these results for the combined donor groups. Statistical evaluation of the results in the above tables was
## Table 3.3

### LABORATORY TEST RESULTS
(Port Colborne Study Group)

#### URINE

<table>
<thead>
<tr>
<th>Substance</th>
<th>Observed Range</th>
<th>Normal Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k, n)</td>
<td>((\bar{x} \pm s))</td>
<td>((\bar{x} \pm s))</td>
<td></td>
</tr>
<tr>
<td>(No. of donors,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of specimens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (6, 11)</td>
<td>19 - 27 (24 ± 2)</td>
<td>21 - 26 (^c)</td>
<td>mg kg(^{-1}) d(^{-1})</td>
</tr>
<tr>
<td>Total Protein (6, 26)</td>
<td>53 - 143 (97 ± 37)</td>
<td>&lt;150 (^d)</td>
<td>mg d(^{-1})</td>
</tr>
<tr>
<td>Total Protein(^j) (6, 26)</td>
<td>16 - 253 (^a) (41 ± 44)</td>
<td>&lt;95 (^e)</td>
<td>mg g(^{-1}) creat</td>
</tr>
<tr>
<td>(\beta_2)-microglobulin(^j) (5, 9)</td>
<td>&lt;50 - 85 (62 ± 14)</td>
<td>&lt;250 (^f)</td>
<td>(\mu g) L(^{-1})</td>
</tr>
<tr>
<td>(\beta_2)-microglobulin(^j) (5, 9)</td>
<td>&lt;30 - 70 (44 ± 13) (60 ± 40) (^i)</td>
<td>&lt;200 (^g)</td>
<td>(\mu g) g(^{-1}) creat</td>
</tr>
<tr>
<td>Specific Gravity(^j) (6, 97)</td>
<td>1.011 - 1.032 (1.018 ± 0.0008)</td>
<td>1.002-1.030 (^h)</td>
<td>—</td>
</tr>
<tr>
<td>Osmolality(^j) (3, 31)</td>
<td>110 - 881 (530 ± 230)</td>
<td>50 -1200 (^d)</td>
<td>mosmol kg(^{-1})</td>
</tr>
<tr>
<td>pH(^j) (6, 97)</td>
<td>5.0 - 7.7</td>
<td>4.8 - 7.8 (^h)</td>
<td>—</td>
</tr>
</tbody>
</table>
Table 3.3 Continued

<table>
<thead>
<tr>
<th>Substance</th>
<th>Observed Range</th>
<th>Normal Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>0.73 - 1.15</td>
<td>0.6 - 1.20</td>
<td>mg dL⁻¹</td>
</tr>
<tr>
<td></td>
<td>(0.93 ± 0.15)</td>
<td>(0.99 ± 0.12)</td>
<td></td>
</tr>
</tbody>
</table>

---

*a* One void for one donor was above 95 mg g⁻¹ creat when this void was combined with the 4 other voids analyzed for this donor the average was 57.5 mg g⁻¹ creat.

*b* The creatinine clearance was 148 ± 32 mL min⁻¹ (range 100 - 211 mL min⁻¹) or 132 ± 28 mL min⁻¹ (range 84 - 190) adjusted to a standard surface area of 1.73 m².

*c* Di Giorgio, 1974.

*d* Clinical Chemistry Laboratory, Adult Reference Ranges, Chedoke-McMaster Medical Centre.

*e* Herber, 1984.

*f* Pharmacia β₂-μ instruction book.

*g* Lauwerys, 1983.

*h* Tietz, 1976.

*i* Sunderman and Horak, 1981.

*j* Spot urine samples.

**NOTE:** None of the observed body-fluid concentrations differed significantly from the normal values (p>0.05) tested relative to the mid-point of the normal range and/or mean ± SD when given, see text.
Table 3.4

DIP STICK RESULTS FOR URINE SAMPLES

Port Colborne Study Group

(k = donors\textsuperscript{a}, n = total analysis)

<table>
<thead>
<tr>
<th>Test</th>
<th>Negative  ( k(n) )</th>
<th>Positive ( k(n) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketones</td>
<td>6(85)</td>
<td>4(8)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>6(83)</td>
<td>6(10)</td>
</tr>
<tr>
<td>Glucose</td>
<td>6(93)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Nitrite</td>
<td>6(93)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Blood</td>
<td>6(93)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>6(84)</td>
<td>5(9)</td>
</tr>
<tr>
<td>Protein</td>
<td>6(53)</td>
<td>6(40)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Ages: 31, 35, 48, 56, 57, 59 years.

\textsuperscript{b}Only trace levels were indicated in all cases.
Table 3.5

LABORATORY TEST RESULTS
(Thompson Study Group)

URINE

<table>
<thead>
<tr>
<th>Substance</th>
<th>Observed Range</th>
<th>Normal Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($\bar{x} \pm s$)</td>
<td>($\bar{x} \pm s$)</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>14 - 25g</td>
<td>21 - 26</td>
<td>mg kg$^{-1}$ d$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>(20 ± 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein</td>
<td>28 - 129</td>
<td>&lt;150</td>
<td>mg d$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>(100 ± 38)g</td>
<td>(77 ± 26)</td>
<td></td>
</tr>
<tr>
<td>Total Protein$^c,e$</td>
<td>12 - 116</td>
<td>&lt;95</td>
<td>mg g$^{-1}$ creat</td>
</tr>
<tr>
<td></td>
<td>(46 ± 19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_2$-microglobulin$^e$</td>
<td>ND - 178</td>
<td>&lt;250</td>
<td>$\mu$g L$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>(80 ± 37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_2$-microglobulin$^d,e$</td>
<td>ND - 442</td>
<td>&lt;200</td>
<td>$\mu$g g$^{-1}$ creat</td>
</tr>
<tr>
<td></td>
<td>(91 ± 87)</td>
<td>(60 ± 40)</td>
<td></td>
</tr>
<tr>
<td>Specific Gravity$^e$</td>
<td>1.003 - 1.030</td>
<td>1.002-1.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.018 ± 0.0007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH$^e$</td>
<td>4.9 - 7.8</td>
<td>4.8 - 7.8</td>
<td></td>
</tr>
</tbody>
</table>

$^a$For sources see Table 3.3.

$^b$Excluding two donors with low creatinine clearances (See text for details)

$^c$One void was above 95 mg g$^{-1}$ creat (116) when this void was combined with other from that donor the average was 59.4 mg g$^{-1}$ creat.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Observed Range</th>
<th>Normal Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{x} ± s)</td>
<td>(\bar{x} ± s)</td>
<td></td>
</tr>
<tr>
<td>Creatinine(^f)</td>
<td>0.79 - 1.36</td>
<td>0.6 - 1.20</td>
<td>mg dL(^{-1})</td>
</tr>
<tr>
<td>(20, 40)</td>
<td>(1.10 ± 0.15)(^g)</td>
<td>(0.99 ± 0.12)</td>
<td></td>
</tr>
<tr>
<td>(\beta_2)-microglobulin</td>
<td>0.85 - 2.05(^g)</td>
<td>0.8 - 2.5(^h)</td>
<td>mg L(^{-1})</td>
</tr>
<tr>
<td>(20, 20)</td>
<td>(1.44 ± 0.33)(^i)</td>
<td>(1.6 ± 0.4)(^i)</td>
<td></td>
</tr>
</tbody>
</table>

\(^d\)Three donors had individual voids with \(\beta_2\)-microglobulin levels above 200 \(\mu g\) g\(^{-1}\) (i.e., 258, 283, 442 and 265 \(\mu g\) g\(^{-1}\)). When the results for all voids analyzed were combined, the average was less than 200 \(\mu g\) g\(^{-1}\) in each case.

\(^e\)Spot urine samples.

\(^f\)The creatinine clearance was 101 ± 25 mL min\(^{-1}\) (all values) and 107 ± 16 mL min\(^{-1}\) (range 84 - 145 mL min\(^{-1}\)) when excluding the two low values discussed in the text. The creatinine clearances adjusted to 1.73 m\(^2\) were 87 ± 22 mL min\(^{-1}\) (all values) and 93 ± 13 (range 70 - 117 mL min\(^{-1}\)) when excluding the two low values discussed in the text.

\(^g\)Statistically significant, p<0.05; relative to normal range or mean ± SD.

\(^h\)Pharmacia RIA instruction book.

\(^i\)Sunderman and Horak, 1981.
Table 3.6  
DIP STICK RESULTS FOR URINE SAMPLES  
Thompson Study Group  
(k = donors\textsuperscript{a}, n = total analysis)

<table>
<thead>
<tr>
<th>Test</th>
<th>Negative $k(n)$</th>
<th>Positive $k(n)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketones</td>
<td>20(132)</td>
<td>1(3)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20(132)</td>
<td>3(3)</td>
</tr>
<tr>
<td>Glucose</td>
<td>19(120)</td>
<td>7(15)</td>
</tr>
<tr>
<td>Nitrite</td>
<td>20(127)</td>
<td>4(8)</td>
</tr>
<tr>
<td>Blood</td>
<td>20(133)</td>
<td>1(2)</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>20(124)</td>
<td>6(11)</td>
</tr>
<tr>
<td>Protein</td>
<td>19(100)</td>
<td>14(35)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The average age of the workers was 45 \pm 8 y with the range 32 - 57 y.

\textsuperscript{b} Only trace levels were indicated in all cases.
done by a one-group comparison of the mid point of the normal range to the mean ± SD of the observed serum or urinary parameter using small sampling theory and the student t statistic \( t = \frac{[\bar{X} - \mu]}{s} \sqrt{n} \), where \( \bar{X} \) is the mean of a sample of size \( n \) and \( \mu \) is the mean of a normal population (mid point of the normal range). Each serum or urinary parameter was tested using the null hypothesis \( H_0: \mu = \bar{X} \) and the alternative hypothesis \( H_a: \mu \neq \bar{X} \). In the few cases where reference values were available as mean ± SD, the significance test consisted of small-sample t-test for independent samples (McClave and Dietrich, 1985).

(iii) **NICKEL CLEARANCE AND \( \alpha_{Ni} \)**

Nickel clearances (the volume of serum from which ultrafilterable nickel is removed in one minute) were calculated using the formula \( C_{Ni} = U_{Ni} V / \alpha_{Ni} S_{Ni} \), with \( U_{Ni} \) = nickel concentration in the 24-h collection, \( V \) = the flow rate in mL min\(^{-1}\) based on the 24-h volume; \( \alpha_{Ni} \) the ultrafiltrable fraction of nickel in serum; and \( S_{Ni} \) = average nickel concentration of two serum specimens, one collected at the beginning and the other at the end of the 24-h collection period. The clearance of nickel when divided by the creatinine clearance (= \( U_{creat} V / S_{creat} \)) yields the fractional clearance of nickel (see Figures 3.2 and 3.3). The creatinine clearance may be taken as an estimate of the glomerular filtration rate (GFR). Figures 3.2 and 3.3 illustrate that the fractional clearance of nickel has a
Figure 3.2. Fractional clearance of nickel(%) \[ \frac{C_{Ni}}{C_{creat}} \times 100 \]
as a function of urinary flow rate. Each symbol denotes a different donor; grouping of donors is arbitrary.
Figure 3.3. Fractional clearance of nickel(%) $\left( \frac{C_{Ni}}{C_{creat}} \times 100 \right)$ as a function of urinary flow rate. Each symbol denotes a different donor; grouping of donors is arbitrary.
flow-rate dependence. It can be shown (see Theoretical Aspects and Discussion, Section B), that the exponent of the curves in Figures 3.2 and 3.3 represent the term $\Delta b = b_{\text{creat}} - b_{\text{Ni}}$ (or on average $1 - b_{\text{Ni}}$). This allows a separate evaluation of $\Delta b$ from that described in Chapter 2. All the nickel clearances were less than the creatinine clearances and the fractional clearances of nickel were less than 100 percent (see Figures 3.2 and 3.3; Table 3.7). Table 3.8 provides a comparison of different assessments of $\Delta b = b_{\text{creat}} - b_{\text{Ni}}$. The fractional clearance values vary from 21 to 46 percent of the GFR.

The ultracentrifugation experiments with serum established that $24 \pm 6$ percent of the nickel was not bound to high-molecular-mass molecules, which constitutes the fraction of serum nickel that is available for glomerular filtration (i.e., $a_{\text{Ni}} = 0.24 \pm 0.06; n = 6$).

In Figure 3.4, the 24-h average urinary nickel excretion rate ($E_{\text{Ni}} = U_{\text{Ni.V}}$) is compared with the serum nickel levels. The observed relationship conforms to the equation $S_{\text{Ni}} = 0.10 E_{\text{Ni}} + 0.40, \ r = 0.97$; here $E_{\text{Ni}}$ is the nickel excreted in ng min$^{-1}$, calculated from the 24-h nickel excretion ($U_{\text{Ni.V}}$).

(iv) **URINARY OSMOLALITY**

For the first collection at Port Colborne the relationship between urinary values of specific gravity and osmolality was found to comply with the relationship
Table 3.7
CLEARANCE SUMMARY

<table>
<thead>
<tr>
<th>Donor Group</th>
<th>Nickel Clearance</th>
<th>Creatinine Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN±SD</td>
<td>MEAN±SD</td>
</tr>
<tr>
<td></td>
<td>(RANGE)</td>
<td>(RANGE)</td>
</tr>
<tr>
<td>Port Colborne</td>
<td></td>
<td>148±32 (100-211)</td>
</tr>
<tr>
<td>(n=7; nickel)</td>
<td>25±7 (17-35)</td>
<td>(n=12; creatinine)</td>
</tr>
<tr>
<td>(n=12; creatinine)</td>
<td>21±5 (15-29)</td>
<td></td>
</tr>
<tr>
<td>Thompson</td>
<td></td>
<td>102±19 (84-145)</td>
</tr>
<tr>
<td>(n=20; nickel)</td>
<td>41±12 (25-32)</td>
<td>(n=18; creatinine)c</td>
</tr>
<tr>
<td>(n=18; creatinine)c</td>
<td>36±9 (22-66)</td>
<td></td>
</tr>
<tr>
<td>Both Collections</td>
<td></td>
<td>124±31 (84-211)</td>
</tr>
<tr>
<td>(n=27; nickel)b</td>
<td>37±13 (17-82)</td>
<td>(n=30; creatinine)c</td>
</tr>
<tr>
<td>(n=30; creatinine)c</td>
<td>32±11 (15-66)</td>
<td></td>
</tr>
</tbody>
</table>

a The clearances adjusted to a body surface area of 1.73 m² were calculated by the following formula employed by Tietz (1976).

\[ C_i = \frac{U_i V_i}{S_i} \times \frac{1.73}{A} \]

Where: \( A = \) body surface area (m²) calculated from:

\[ \log(A) = (0.425 \log(W)) + (0.725 \log(H)) - 2.144 \]

\( W = \) weight (kg); \( H = \) height (cm)

\( C_i = \) clearance of analyte (i) in mL min⁻¹ (the hypothetical volume of serum that has theoretically been cleared of analyte (i); in the case of nickel it is the volume from which ultrafiltrable nickel is removed).

\( U_i = \) urine concentration of analyte (i) with the same units as in serum.

\( S_i = \) serum concentration of analyte (i) with the same units as in urine.

\( V_i = \) Urine flow rate in mL min⁻¹.

b There were 5 collections without serum nickel.

c Excluding two donors with low creatinine clearances (29 and 41 mL min⁻¹ per 1.73 m²).
Table 3.8
PREDICTION OF CREATININE CLEARANCE
THOMPSON STUDY GROUP

<table>
<thead>
<tr>
<th>Donor</th>
<th>Predicted Clearance (mL min⁻¹)</th>
<th>Predicted Clearance (mL min⁻¹)</th>
<th>Observed Clearance (mL min⁻¹)</th>
<th>Observed Clearance (mL min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>112</td>
<td>92</td>
<td>123</td>
<td>101</td>
</tr>
<tr>
<td>B</td>
<td>95</td>
<td>91</td>
<td>113</td>
<td>109</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>90</td>
<td>106</td>
<td>82</td>
</tr>
<tr>
<td>D</td>
<td>106</td>
<td>100</td>
<td>105</td>
<td>100</td>
</tr>
<tr>
<td>E</td>
<td>105</td>
<td>76</td>
<td>84</td>
<td>70</td>
</tr>
<tr>
<td>F</td>
<td>123</td>
<td>113</td>
<td>95</td>
<td>87</td>
</tr>
<tr>
<td>G</td>
<td>79</td>
<td>69</td>
<td>112</td>
<td>98</td>
</tr>
<tr>
<td>H</td>
<td>73</td>
<td>65</td>
<td>91</td>
<td>80</td>
</tr>
<tr>
<td>I</td>
<td>120</td>
<td>97</td>
<td>145</td>
<td>117</td>
</tr>
<tr>
<td>J</td>
<td>74</td>
<td>60</td>
<td>98</td>
<td>80</td>
</tr>
<tr>
<td>K</td>
<td>105</td>
<td>91</td>
<td>111</td>
<td>96</td>
</tr>
<tr>
<td>L</td>
<td>91</td>
<td>80</td>
<td>119</td>
<td>105</td>
</tr>
<tr>
<td>M</td>
<td>78</td>
<td>70</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>N</td>
<td>108</td>
<td>90</td>
<td>49</td>
<td>41</td>
</tr>
<tr>
<td>O</td>
<td>145</td>
<td>117</td>
<td>96</td>
<td>77</td>
</tr>
<tr>
<td>P</td>
<td>116</td>
<td>95</td>
<td>134</td>
<td>110</td>
</tr>
<tr>
<td>Q</td>
<td>107</td>
<td>86</td>
<td>121</td>
<td>97</td>
</tr>
<tr>
<td>R</td>
<td>110</td>
<td>98</td>
<td>86</td>
<td>76</td>
</tr>
<tr>
<td>S</td>
<td>105</td>
<td>91</td>
<td>105</td>
<td>91</td>
</tr>
<tr>
<td>T</td>
<td>102 ± 18</td>
<td>88 ± 15</td>
<td>101 ± 26</td>
<td>87 ± 22</td>
</tr>
<tr>
<td>U</td>
<td>103 ± 18</td>
<td>89 ± 15</td>
<td>102 ± 19</td>
<td>93 ± 13</td>
</tr>
</tbody>
</table>

Note: Predicted clearance values are calculated using a certain method, and observed clearance values are measured directly. The table shows a comparison between predicted and observed values for each donor, along with mean and standard deviation (SD) values for all donors and for donors excluding those with values N & 0.
Table 3.8 Continued

PREDICTION OF CREATININE CLEARANCE

PORT COLBORNE STUDY GROUP

<table>
<thead>
<tr>
<th>Donor</th>
<th>Predicted(^a) Clearance (mL min(^{-1}))</th>
<th>Predicted(^b) Clearance (mL min(^{-1}))</th>
<th>Observed Clearance (mL min(^{-1}))</th>
<th>Observed Clearance (mL min(^{-1}))(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>87</td>
<td>72</td>
<td>100</td>
<td>84</td>
</tr>
<tr>
<td>B</td>
<td>99</td>
<td>90</td>
<td>138</td>
<td>124</td>
</tr>
<tr>
<td>C</td>
<td>90</td>
<td>80</td>
<td>135</td>
<td>119</td>
</tr>
<tr>
<td>D</td>
<td>79</td>
<td>73</td>
<td>109</td>
<td>102</td>
</tr>
<tr>
<td>E</td>
<td>125</td>
<td>111</td>
<td>132</td>
<td>117</td>
</tr>
<tr>
<td>F</td>
<td>123</td>
<td>113</td>
<td>153</td>
<td>140</td>
</tr>
<tr>
<td>A'</td>
<td>136</td>
<td>114</td>
<td>185</td>
<td>155</td>
</tr>
<tr>
<td>B'</td>
<td>137</td>
<td>123</td>
<td>211</td>
<td>190</td>
</tr>
<tr>
<td>C'</td>
<td>105</td>
<td>98</td>
<td>132</td>
<td>123</td>
</tr>
<tr>
<td>D'</td>
<td>94</td>
<td>84</td>
<td>143</td>
<td>126</td>
</tr>
<tr>
<td>E'</td>
<td>149</td>
<td>132</td>
<td>176</td>
<td>156</td>
</tr>
<tr>
<td>F'</td>
<td>155</td>
<td>143</td>
<td>167</td>
<td>154</td>
</tr>
<tr>
<td>MEAN±SD</td>
<td>115 ± 26</td>
<td>103 ± 23</td>
<td>148 ± 32</td>
<td>132 ± 28</td>
</tr>
</tbody>
</table>

\(^a\) CREATININE CLEARANCE (mL min\(^{-1}\)) = \frac{(140 - \text{age in y}) \times (\text{mass in kg})}{(\text{serum creatinine; mg dL}^{-1}) \times 72}

This formula is based on serum creatinine determination by Auto-Analyzer/dialysis protocol (Cockroft and Gault, 1976).

\(^b\) CREATININE CLEARANCE = \left[ \frac{\text{predicted clearance}}{\text{column 1}} \right] \times \frac{1.73}{A \text{ (m}^2\text{)}} \text{ (mL min}^{-1}\text{per 1.73 m}^2\text{)}

A = \text{body surface area (m}^2\text{)} \text{ calculated from:}

\log(A) = (0.425\log(W)) + (0.725\log(H)) - 2.144

W = \text{weight (kg)}; H = \text{height (cm)}; \text{from Teitz (1976).}

\(^c\) The unprimed and the primed donor notation correspond to the first and second 24-h collection, respectively, of the same donor.
Figure 3.4. Relationship between serum nickel concentrations and nickel excretion ($E_{Ni}$). Data correspond to the 24-h collections reported in Chapter 2 (Figs. 2.31 to 2.35) for the 20 donors of the Thompson collection. The insert illustrates the regression line including the two data points above 80 ng min$^{-1}$. The regression parameters are included in the figure ($p<0.001$).
Figure 3.5. Observed relationship between specific gravity of spot urine samples and the corresponding osmolality for three donors (k) with a total of 31 voids (n) for the Port Colborne study group (p<0.001).
\[ \rho = 2.8 \times 10^{-5} \text{(osmolality)} + 1.0, \ r = 0.99 \] (see Figure 3.5).

(v) EFFECT OF EXPOSURE ON \( U_{\text{Ni}}^0 \)

When the Port Colborne urinary nickel levels were normalized with specific gravity, the resulting \( U_{\text{Ni}}^0 \) values appeared to increase during the shift with a subsequent decrease during the post-shift period (See Figs. 3.6 and 3.7). The values of \( U_{\text{Ni}}^0 \) observed after the layoff were lower by approximately 36 - 70 percent, compared to pre-layoff values. Interestingly, \( U_{\text{Ni}}^0 \) values (for individual voids) in the second 24-h collection at Port Colborne were found to be more uniform throughout the day as illustrated in Figures 3.6 and 3.7. In the Thompson collection, there was not always an indication of an increase in \( U_{\text{Ni}}^0 \) values during the shift, nor was a subsequent post-shift decrease always evident (see Figs. 3.8 to 3.10).

F DISCUSSION

(i) BIOCHEMICAL INDICES OF KIDNEY FUNCTION

Two markers of renal status usually associated with Cd nephrotoxicity, total protein and \( \beta_2-\mu \), were evaluated in the current investigation of electrolytic refinery workers. Normally only trace amounts of \( \beta_2-\mu \) and total protein are present in the urine. Excretion of \( \beta_2-\mu \) is noticeably
Figure 3.6. Fluctuations in standardized urine nickel concentrations ($U_{Ni}^o$) for a nickel electrolytic refinery worker (donor $E_{PC}$) during two 24-h multi-void collections (Port Colborne cohort). The diagonal lines indicate the day shift, which started at 7:00 am and ended at 3:00 pm.

**Top Curve:** $U_{Ni}^o$ (-o-o-) estimated from $U_{Ni}$, $V$ and $b_{Ni}$ (Equation 2.6) and $U_{Ni}$ adjusted to $\rho = 1.021$ (-A-A-) prior to a one month layoff.

**Bottom Curve:** $U_{Ni}^o$ (-●-●-) estimated from $U_{Ni}$, $V$ and $b_{Ni}$ (Equation 2.6) and $U_{Ni}$ adjusted to $\rho = 1.021$ (-▲-▲-) at the end of a one month layoff (prior to resuming work).
Figure 3.7. Fluctuations in standardized urine nickel concentrations ($U_{Ni}^o$) for a nickel electrolytic refinery worker (donor A_{PC}) during two 24-h multi-void collections (Port Colborne cohort). The diagonal lines indicate the day shift, which started at 7:00 am and ended at 3:00 pm.

**Top Curve:** $U_{Ni}^o$ (-o-o-) estimated from $U_{Ni}$, V and $b_{Ni}$ (Equation 2.6) and $U_{Ni}$ adjusted to $\rho = 1.021$ (-A-A-) prior to a one month layoff.

**Bottom Curve:** $U_{Ni}^o$ (-•-•-) estimated from $U_{Ni}$, V and $b_{Ni}$ (Equation 2.6) and $U_{Ni}$ adjusted to $\rho = 1.021$ (-A-A-) at the end of a one month layoff (prior to resuming work).
Figure 3.8. Fluctuations in standardized urine nickel concentrations for a nickel electrolytic refinery worker (donor A_T) during a 24-h multi-void collection. \( U^o_{NI} \) (-o-o-) estimated from \( U_{NI} \) adjusted to \( \rho = 1.018 \). The diagonal lines indicate the day shift at Thompson, which started at 7:00 am and ended at 3:00 pm.
Figure 3.9. Fluctuations in standardized urine nickel concentrations for a nickel electrolytic refinery worker (donor E_T) during a 24-h multi-void collection. U_{Ni}^c (-o-o-) estimated from U_{Ni} adjusted to ρ = 1.018. The diagonal lines indicate the midnight shift at Thompson, which started at 11:00 pm and ended at 7:00 am.
Figure 3.10. Fluctuations in standardized urine nickel concentrations for a nickel electrolytic refinery worker (donor N_T) during a 24-h multi-void collection. \( U_{Ni}^o \) (-o-o-) estimated from \( U_{Ni} \) adjusted to \( \rho = 1.018 \). The diagonal lines indicate the midnight shift at Thompson, which started at 11:00 pm and ended at 7:00 am.
increased in nephrotubular disorders. This change can be due to exposure to heavy metals such as Cd (Lauwerys, 1983), Au based anti-rheumatic compounds (Merle et al., 1980), or to urinary tract infections (Schardijn et al., 1979). In renal diseases, $\beta_2$-micro level in the serum reflects alterations in GFR more accurately than serum creatinine (Wibell et al., 1973 and Shea et al., 1981). Usually, the amount of protein excreted in the urine from normal donors rarely exceeds $150 \text{ mg day}^{-1}$. Increased excretion of urinary protein is an early sign of renal damage. Proteins cross the glomerular filter by a complex process that depends on the endothelial pore size, electrical charge and the binding characteristics of the protein molecule as well as on the absolute value of the glomerular filtration rate (Lauwerys, 1984). Proteins with molecular mass below 40000 to 60000 pass easily through the glomerular filter and are reabsorbed by the proximal tubular cells of the nephron. When there is glomerular damage, the glomerular permeability is usually increased and therefore larger quantities of high molecular mass proteins enter the glomerular filtrate and appear in the urine (Lauwerys, 1984). Low-molecular mass proteinuria is usually not increased because their reabsorption has not changed. If there is tubular dysfunction, the amount of protein filtered through the glomeruli is not increased. However, in this instance the high- and low-molecular mass proteins normally filtered are not reabsorbed to the same degree. Hence larger quantities of the low-molecular mass proteins appear in the
urine (Lauwerys, 1984). In the present study, the urinary levels of \( \beta_2^-\mu \) are used as a marker of renal tubular damage, while total protein is used as an indicator of glomerular dysfunction (high-molecular mass proteinuria). Chapter 4 gives a more complete description of the anatomy and physiology of the kidney and its functional unit the nephron.

In this study there were two donors (one from each collection) who had voids with a total protein level above 95 mg g\(^{-1}\)creat and only one void was above 200 mg g\(^{-1}\)creat. When these voids with high levels of total protein were combined with other voids from the same donor the "average" was below 95 mg g\(^{-1}\)creat, which is much below the level of 250 mg g\(^{-1}\)creat that is considered to be an indication of abnormal kidney function associated with Cd exposure (Lauwerys, 1983; Herber, 1984). The urinary concentration of \( \beta_2^-\mu \) in four voids derived from three donors exceeded 200 \( \mu g \) g\(^{-1}\)creat. However, when these results for each donor were combined with those for the remaining voids, the 24-h "average" was below 200 \( \mu g \) g\(^{-1}\)creat in each case. Values exceeding this level are usually considered an indication of Cd nephrotoxicity (Lauwerys, 1983). Since high levels of the tubulo-disorder marker \( \beta_2^-\mu \) and elevated total protein levels were not apparent in this study of electrolytic refinery workers, glomerular or tubulonephrotoxicity appears not to occur.

The serum \( \beta_2^-\mu \) levels for the Thompson collection were within the normal range (not significant, \( p > 0.05 \) when means are compared). There were 7 donors in the Thompson
collection with one of the two sera creatinine levels above 1.2 mg dL\(^{-1}\) and none had levels below 0.60 mg dL\(^{-1}\). This is reflected in the mean which was significantly different from the normal mean (p<0.05). This indicates slightly elevated levels of serum creatinine in the Thompson collection, while in the Port Colborne collection they are within the normal range (not significant p>0.05; with no donors outside the normal range). For the Thompson group, the highest serum creatinine concentration was 1.36 mg dL\(^{-1}\). Levels <1.4 mg dL\(^{-1}\) are considered minor elevations (Lilis et al., 1980) or even the upper range of normal (Thun and Clarkson, 1986) In the Port Colborne collection, 3/11 of the 24-h urinary creatinine excretions (mg kg\(^{-1}\) d\(^{-1}\)) were outside the accepted normal range. Because there was 1 above and 2 below the range, these outliers did not cause the creatinine excretion to be significantly different from the normal values (p>0.05). The Thompson collection was statistically different (p<0.05) with 9/20 below and none above the accepted range. Consequently, the Thompson subgroup has relatively low 24-h urinary creatinine excretion and slightly increased serum creatinine levels.

The pH values of all the urine voids were within the normal range of 4.8 to 7.8 cited by Faulkner and King (1976). This indicates that the kidneys in both study groups maintain normal hydrogen ion balance under the conditions of the studies.

The osmolality of random urine samples is usually within
a normal range of 50 to 1200 mosmol kg\(^{-1}\) (H\(_2\)O) (private communication, Clinical Chemistry Laboratory, Chedoke-McMaster Medical Centre). Since specific gravity can be used as a measure of urine osmolality even in the presence of proteinuria (Gault, 1982), the regression equation in Figure 3.4 can be used to estimate the urine osmolality in this study. The appropriate specific gravity range calculated from the normal osmolality range is 1.001 to 1.034. As evident in Tables 3.3 and 3.5 the specific gravity and hence the osmolality of the urines collected in this study are as expected and thus renal concentrating ability was normal in the work and home environments.

Each urine void was screened for indicators of general health effects and abnormal kidney function in a semi-quantitative manner by dip sticks. This was used as a guideline for further laboratory test of total protein. Specimens for which dip-stick tests showed trace levels of urinary protein were analyzed by the Bio-Rad protein assay. Both study groups exhibited a low incidence of positive trace levels for the parameters measured. Below is a brief summary of each test and its significance for kidney function or general health. The appropriate biochemical/physiological information is found in a number of sources such as Green (1976), Leaf and Cotran (1980), Matta and Wilbraham (1981) and Vander et al. (1985).

**Ketones.** Ketone bodies (acetoacetic acid, \(\beta\)-hydroxybutyric acid and acetone) are produced during the oxidation of fatty
acids. Accumulation (ketosis) results from the incomplete metabolism of fatty acids, generally because of carbohydrate deficiency or low carbohydrate metabolism as in untreated diabetes mellitus. Normal urine specimens ordinarily yield negative results with this method. In both groups of this study, there were a total of 11 voids (5%) from 5 donors (Table 3.6) that had trace levels of ketones. This illustrates that there is a low incidence of ketonuria in both study groups and that it is to be expected that the donor population is essentially free of renal complications such as papillary necrosis or nephrotic syndrome associated with diabetes mellitus (Leaf and Cotran, 1980).

**Bilirubin.** This is the orange-coloured or yellowish pigment in the bile. It is produced from the haem moiety (without iron) of the haemoglobin molecule during the catabolism of erythrocytes that occurs mainly in the reticuloendothelial cells in the bone marrow and the spleen. Any blockage or failure of the bilirubin excretory pathway (e.g. blockage of bile-ducts by a gall stone) will result in its accumulation in the blood causing the condition known as jaundice and excretion in the urine rather than in the faeces. For both collections, nine donors (13 voids; 6%) had detectable trace levels of bilirubin in their urine. No donors had bilirubin in all of their voids, indicating that this occurrence was transient and of minimal importance.

**Glucose.** This sugar is usually entirely reabsorbed in the kidney and hence not present in the urine. If present, it is
usually an indication of impaired glucose metabolism associated with diabetes mellitus or an indication of poor reabsorption of glucose in the kidney. In the Port Colborne sub-group, there was no indication of glucose in the urine; while there were 7 donors at Thompson who had 15 voids (11%) with a trace of glucose. It was not found in all the voids of any one donor, indicating that the incidence of low levels of glucosuria was transient in this population even though there was no attempt to restrict food intake.

**Nitrite.** Normally no nitrite is detectable in the urine. A positive test indicates significant bacteriuria that may indicate pyelonephritis, cystitis and urethritis. In this study there was no indication of nitrite in the Port Colborne subgroup, while there was for 4 donors (8 voids; 6%) at Thompson. No individual had nitrite present in all voids. Therefore, the incidence of bacteriuria is low in this population.

**Blood.** Haematuria is associated with many kidney disorders such as acute nephritis, calculi, renal carcinoma or chronic kidney infection. In this study there was no evidence of the presence of blood in the Port Colborne group, while only 1 donor (2 voids out of 4) had a trace at Thompson. This illustrates the low occurrence of haematuria in this study.

**Urobilinogen.** Bilirubin is oxidized in the large intestine by bacterial action to colourless urobilinogen, some of which is reabsorbed and excreted in the urine. A small amount in urine is normal and increased levels are associated with
hepatic disease while decreased levels indicate biliary obstruction. The dip stick method will only detect high and normal levels. Low levels of urobilinogen or its complete absence in the specimens being tested cannot be determined. There was no indication of increased urinary urobilinogen in both groups.

**Protein.** Proteinuria may be caused by increased permeability of the glomerular filter associated with a number of kidney diseases such as acute glomerulonephritis, or by pyelonephritis, a tubulointerstitial disease. In this study none of the voids indicated more than trace levels of urinary protein.

In summary, the trace levels and transient nature of the results from the dip-stick screening of urine specimens indicate that there was no evidence of complicating factors in kidney function such as bacterial infection nor any renal indication of other major health perturbations in the populations examined. The only abnormality observed in the study was that of slightly increased serum creatinine and decreased urinary creatinine excretion in the Thompson group.

**(ii) CREATININE CLEARANCE**

The elevated serum creatinine levels are within the upper limit cited by other authors, but as pointed out by Gault (1982) and Thun and Clarkson (1986), 50 to 67% of normal GFR may be lost in some patients before the serum creatinine concentration exceeds the upper normal limit of 1.4 mg dL\(^{-1}\).
Creatinine clearance is known to decrease with age (e.g., Gault, 1982). The age dependence of creatinine clearance for both donor groups was calculated from a formula given by Gault (1982) which employs age, body mass and serum creatinine levels to predict creatinine clearances (Table 3.8). There are two Thompson donors (N and O) with observed clearances much below their predicted values. The mean of the predicted values in columns 1 and 2 of Table 3.8 for the Thompson cohort are in good agreement with the mean of the observed values (columns 3 and 4) both including and excluding the two donors (N and O) with low creatinine clearances. However, the standard deviation in the observed group is larger when these low values are included while there was no change in the predicted standard deviation. This illustrates that the low clearances observed for donors N and O is not dependent on age. The Port Colborne cohort has larger, but not significant (p≥0.05), observed creatinine clearances than the predicted values. This is attributed to the higher 24-h flow rate found in the Port Colborne (1.3 ± 0.6 ml min⁻¹) group in comparison to the Thompson (1.1 ± 0.4 mL min⁻¹) (see Table 2.8). Since the creatinine clearances for the Thompson group were normal (donors N and O excluded), the slightly elevated creatinine serum levels and depressed urinary creatinine excretion found (Table 3.5) may well be due to an inappropriate selection of normal values for this metabolite. The principal value in the determination of urinary excretion of creatinine is not the evaluation of
renal function (unless part of a creatinine clearance test). Because creatinine excretion is relatively constant for an individual, it can be used as a check on the completeness of a 24-h collection (Faulkner and King, 1976). Therefore the low creatinine excretion and clearance values observed for donors N and 0 could be due to the 24-h collection being incomplete and is not necessarily due to renal dysfunction. As pointed out by Faulkner and King (1976), a number of factors can cause errors in the measurement of creatinine clearances. Among the most common are faulty timing, vigorous exercise during the collection period and improper hydration of the patient (a urine flow of 2 mL min$^{-1}$ is ideal). This flow-rate tends to eliminate retention of urine in the bladder as a source of negative error. During the present study, there was no effort made to water-load any donors to ensure a high urine flow rate. It is interesting to compare the biochemical indices of kidney function for the donors with low creatinine clearances (N and 0) and other donors with $V<0.6$ mL min$^{-1}$ (Table 3.9). From the data in this table it is clear that there is not one parameter that is common to all of the donors in this subgroup. This suggests that there may not be a single underlying cause. Because of the possible errors associated with the 24-h urine collections of donors N and 0, it seems advisable that measurement of their GFR should be re-evaluated.

It is interesting to note that both acute renal failure and primary glomerulonephritis (acute nephritis) are
Table 3.9

BIOCHEMICAL INDICIES OF KIDNEY FUNCTION
(DONORS WITH 24-h FLOWRATE LESS THAN 0.6 mL/UNUSUALLY LOW CREATININE CLEARANCES)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Age (y)</th>
<th>Weight (lb)</th>
<th>Job</th>
<th>Creatinine 21 - 26 h (mg kg⁻¹ d⁻¹)</th>
<th>Total Protein (g 10⁶ mg d⁻¹)</th>
<th>Creatinine Clearance U (mg d⁻¹)</th>
<th>Specific Gravity</th>
<th>24-h Flowrate ml min⁻¹</th>
<th>Creatinine Clearance U (mg d⁻¹)</th>
<th>Specific Gravity</th>
<th>24-h Flowrate ml min⁻¹</th>
<th>Creatinine Clearance U (mg d⁻¹)</th>
<th>Specific Gravity</th>
<th>24-h Flowrate ml min⁻¹</th>
<th>Creatinine Clearance U (mg d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nv</td>
<td>54</td>
<td>170</td>
<td>Section Leader</td>
<td>7.3</td>
<td>32</td>
<td>1.002 - 1.030</td>
<td>0.50</td>
<td>1.002 - 1.030</td>
<td>0.43</td>
<td>1.002 - 1.030</td>
<td>0.43</td>
<td>1.002 - 1.030</td>
<td>0.43</td>
<td>1.002 - 1.030</td>
<td>0.43</td>
</tr>
<tr>
<td>Ov</td>
<td>62</td>
<td>200</td>
<td>Task Aerostat</td>
<td>9.7</td>
<td>42</td>
<td>1.008 - 1.026</td>
<td>0.64</td>
<td>1.008 - 1.026</td>
<td>0.64</td>
<td>1.008 - 1.026</td>
<td>0.64</td>
<td>1.008 - 1.026</td>
<td>0.64</td>
<td>1.008 - 1.026</td>
<td>0.64</td>
</tr>
<tr>
<td>Lv</td>
<td>34</td>
<td>178</td>
<td>Refinery Laborer</td>
<td>18</td>
<td>66</td>
<td>1.027 - 1.030</td>
<td>0.84</td>
<td>1.027 - 1.030</td>
<td>0.84</td>
<td>1.027 - 1.030</td>
<td>0.84</td>
<td>1.027 - 1.030</td>
<td>0.84</td>
<td>1.027 - 1.030</td>
<td>0.84</td>
</tr>
<tr>
<td>*pc</td>
<td>21</td>
<td>165</td>
<td>Boomer</td>
<td>27</td>
<td>4.8</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
</tr>
<tr>
<td>*fpc</td>
<td>21</td>
<td>165</td>
<td>Boomer</td>
<td>27</td>
<td>4.8</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
<td>1.024 - 1.024</td>
<td>0.70</td>
</tr>
</tbody>
</table>

*The mean ± SD for both collections excluding donors Np and Gp is 10.5 ± 2.8 ml min⁻¹ per 1.73 m².

The range for both collections excluding donors Np and Gp is 10 to 150 ml min⁻¹ per 1.73 m².

*Org a number of voids with no parameter detected.

*Org a number of voids with only one trace level present.

*Subscript T is Thompson; PC is Port Collector.

* pc and *fpc are two 24-h collections from the same donor who took 7.5 mg Transaxone per day. This tranquilizer is known to make urination difficult and the results are included in this table for comparison only.

*pc and *fpc are two 24-h collections from the same donor who took 7.5 mg Transaxone per day. This tranquilizer is known to make urination difficult and the results are included in this table for comparison only.

*pc and *fpc are two 24-h collections from the same donor who took 7.5 mg Transaxone per day. This tranquilizer is known to make urination difficult and the results are included in this table for comparison only.

*pc and *fpc are two 24-h collections from the same donor who took 7.5 mg Transaxone per day. This tranquilizer is known to make urination difficult and the results are included in this table for comparison only.

*pc and *fpc are two 24-h collections from the same donor who took 7.5 mg Transaxone per day. This tranquilizer is known to make urination difficult and the results are included in this table for comparison only.

*pc and *fpc are two 24-h collections from the same donor who took 7.5 mg Transaxone per day. This tranquilizer is known to make urination difficult and the results are included in this table for comparison only.
associated with a reduction in the GFR and an increase in serum creatinine (Thomson et al., 1982; Clark, 1982; Thun and Clarkson, 1986). The significance of these abnormalities will be discussed in more detail in Chapter 4.

Certainly in comparison with comparable exposures to a known nephrotoxic metal such as Cd (Elinder et al., 1985a,b), any changes in renal function caused by nickel exposure in electrorefining appear to be minimal. Chapter 4 and 5 will discuss the observed nephrotoxic effects of nickel on rats which support the observed low incidence of renal dysfunction in electrolytic refinery workers.

(iii) Determination of $\alpha_{Ni}$

As described in Section C(ii) of Chapter 1 there does not appear to be any agreement on the exact size of the ultrafiltrable or high molecular mass fractions of nickel(II) in human serum. The work by Lucassen and Sarkar (1979) found only 4.2% of the total Ni$^{2+}$ bound to L-histididine which comprises the ultrafiltrable fraction. Their experiment was done by adding $^{63}$NiCl$_2$ to 2.0 mL of serum and diluting to a constant volume of 2.5 mL before ultracentrifugation. Therefore, this estimate does not necessarily represent the endogenous nickel distribution. In the present work, six determinations were made of the actual endogenous low- and high-molecular-mass fractions of nickel(II) in human serum. The low molecular mass fraction of 24% ($\alpha_{Ni} = 0.24 \pm 0.06$) is substantially higher than the 4.2% observed by Lucassen and
Sarkar (1979). However it is greater than the 15% observed by Asato et al. (1975) for rabbit serum and less than the 40% ultrafiltrable fraction attributed to human serum by Sunderman et al. (1972) and Nomoto (1980). It has been shown that there are large species variations in the proportions of ultrafiltrable and protein-bound serum nickel (Hendel and Sunderman, 1972). New Zealand rabbit sera are known to have a larger concentration of nickel than human sera, which may explain the different distribution between high- and low-molecular-mass components. The species distribution work done by Sunderman et al. (1972) on human sera also involved direct measurement of nickel. However, the nickel concentrations found for the sera from healthy unexposed subjects (2.3 μg L\(^{-1}\)) was much higher than currently being reported (0.46±0.26 μg L\(^{-1}\); Sunderman et al., 1984), and thus the value for \(a_{Ni}\) may not be correct because of the dubious nickel(II) measurements.

For all calculations of nickel clearance in the next section, \(a_{Ni} = 0.24\) was employed.

(iv) CLEARANCE OF NICKEL

The creatinine and nickel clearances based on the combined voids for 24-h collections were calculated from Equations 1 and 2 of Section B and adjusted to a body surface area of 1.73 m\(^2\). They are summarized in Table 3.10. Observed nickel clearances were less than the corresponding creatinine clearances, including the two donors with
abnormally low creatinine clearances (Tables 3.9). Because creatinine clearance is a measure of glomerular filtration rate (GFR) (Duarte et al., 1980; Epstein, 1977) any substance that has a clearance below GFR is being reabsorbed in the kidney (Green, 1976). The low nickel clearance may thus be interpreted as evidence for its reabsorption in the kidney. The animal data reported in Chapter 4 are consistent with this conclusion. This is also supported by the fractional clearances of nickel \( \frac{C_{Ni}}{C_{creat}} \) being less than 100 percent. The reabsorption process is a function of the urine flow rate as illustrated by the variation in fractional clearance of nickel with flow rate (see Figures 3.2 and 3.3). The nickel clearance varies from individual to individual as indicated by the different sets of curves depicted in Figure 3.2 (c.f., values of \( FC_{Ni} \) at \( V = 1 \) mL min\(^{-1} \)).

In the Theoretical Considerations of Renal Clearance (Section B), it was illustrated that the general equation for the fractional clearance (FC) of nickel may be written as shown in Equation 3.16.

\[
FC = \frac{C_{Ni}}{C_{creat}} = \frac{C_{Ni}^0}{C_{creat}^0} \frac{b_{creat} - b_{Ni}}{V} \tag{3.16}
\]

This equation is very similar to Equation 2.4 with \( b_1 = b_{Ni} \) and \( b_2 = b_{creat} \). Therefore, a plot of \( C_{Ni}/C_{creat} \) versus \( V \) (see Figs. 3.1 and 3.2) should yield estimates of \( \Delta b = b_{creat} - b_{Ni} \) and \( C_{Ni}^0/C_{creat}^0 \). It is clear from Figures 3.2
and 3.3, that the fractional clearances of nickel tend to increase rapidly at low flow rates and level off somewhat at high flow rates. The shape of these FC curves can be rationalized by examining the family of theoretical curves plotted in Figure 2.38, where $V^\Delta b$ (the uncompensated volume residual in Equation 3.16) has been plotted against $V$ for various values of $\Delta b$ and $V$. Thus the nickel FC curves belong to the sub-group of curves with $\Delta b > 0$. Table 3.10 compares estimates of $\Delta b$ for log $U_i$ versus log $V$ plots, $U_{Ni}/U_{creat}$ versus $V$ plots, and $C_{Ni}/C_{creat}$ versus $V$ plots. No statistical difference occurs between these alternative estimates.

Figure 3.4 displays the relationship between urinary excretion rate ($E_{Ni} = U_{Ni} V$) and serum nickel levels. As shown in Section B (Eqn. 3.20), the slope of this equation is a measure of the effective clearance of nickel (i.e. slope = $1/aC_{Ni}$; unadjusted to surface area). Consequently, the volume of serum from which available nickel is removed per unit time, $C_{Ni} = 1/a_{Ni} \times$ slope = $1/(0.24 \times 0.10) = 42 \text{ mL min}^{-1}$, and thus about 65% of the nickel in the glomerular filtrate [i.e. $100(1 - 42/GFR)$] is reabsorbed taking the GFR = 120 mL min$^{-1}$. This alternative method of estimating nickel clearance yields a value close to that found in the Thompson collection ($41 \pm 12 \text{ mL min}^{-1}$) and reasonably close to the overall average ($37 \pm 13 \text{ mL min}^{-1}$); but is higher than that observed for the Port Colborne collections ($25 \pm 7 \text{ mL min}^{-1}$);
Table 3.10

ASSESSMENT OF THE URINE FLOW-RATE POWER COEFFICIENT \( b_1 \) FOR CREATININE, NICKEL AND THE CORRESPONDING \( \Delta b \) VALUES

<table>
<thead>
<tr>
<th>Donor</th>
<th>( b_{Nt} ), r</th>
<th>( b_{creat} ), r</th>
<th>( \Delta b = b_{creat} - b_{Ni} )</th>
<th>( \Delta b = b_{creat} - b_{Ni} ), c, g, r</th>
<th>( \Delta b = b_{creat} - b_{Ni} ), d, g, r</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.14, 0.85</td>
<td>1.41, 0.81</td>
<td>0.27</td>
<td>0.27, 0.42</td>
<td>0.21, 0.45</td>
</tr>
<tr>
<td>B</td>
<td>1.09, 0.95</td>
<td>1.17, 0.95</td>
<td>0.08</td>
<td>0.08, 0.81</td>
<td>0.10, 0.90</td>
</tr>
<tr>
<td>C</td>
<td>0.88, 0.72</td>
<td>1.13, 0.96</td>
<td>0.45</td>
<td>0.46, 0.73</td>
<td>0.56, 0.78</td>
</tr>
<tr>
<td>D</td>
<td>0.77, 0.92</td>
<td>1.06, 0.94</td>
<td>0.31</td>
<td>0.26, 0.67</td>
<td>0.26, 0.66</td>
</tr>
<tr>
<td>E</td>
<td>0.44, 0.80</td>
<td>1.05, 0.98</td>
<td>0.64</td>
<td>0.64, 0.86</td>
<td>0.64, 0.86</td>
</tr>
<tr>
<td>F</td>
<td>0.64, 0.96</td>
<td>1.05, 0.97</td>
<td>0.51</td>
<td>0.52, 0.93</td>
<td>0.52, 0.93</td>
</tr>
<tr>
<td>G</td>
<td>0.67, 0.93</td>
<td>1.08, 0.99</td>
<td>0.41</td>
<td>0.35, 0.85</td>
<td>0.35, 0.86</td>
</tr>
<tr>
<td>H</td>
<td>0.58, 0.86</td>
<td>1.01, 0.95</td>
<td>0.43</td>
<td>0.50, 0.87</td>
<td>0.50, 0.87</td>
</tr>
<tr>
<td>I</td>
<td>1.01, 0.94</td>
<td>0.96, 0.96</td>
<td>0.05</td>
<td>-0.05, 0.20</td>
<td>0.03, 0.17</td>
</tr>
<tr>
<td>J</td>
<td>0.59, 0.86</td>
<td>0.85, 0.92</td>
<td>0.26</td>
<td>0.26, 0.45</td>
<td>0.57, 0.67</td>
</tr>
<tr>
<td>K</td>
<td>0.49, 0.99</td>
<td>0.61, 0.99</td>
<td>0.12</td>
<td>0.11, 0.90</td>
<td>0.25, 0.98</td>
</tr>
<tr>
<td>MEAN±SD f</td>
<td>0.70 ± 0.20</td>
<td>1.04 ± 0.22</td>
<td>0.26 ± 0.18</td>
<td>0.25 ± 0.18</td>
<td>0.28 ± 0.18</td>
</tr>
</tbody>
</table>

a Evaluated from plots of \( \log(U_t) \) versus \( \log(V) \).

b \( \Delta b \) was calculated by subtraction of \( b_1 \) values in columns 2 and 3.

c \( \Delta b \) was evaluated from plots of \( \frac{U_{Ni}}{U_{creat}} \) versus \( V \).

d \( \Delta b \) was evaluated from plots of \( \frac{C_{Ni}}{C_{creat}} \) versus \( V \).

e Serum nickel was not evaluated for donors E and H.

f MEAN ± SD does not include donors E and H.

g There was no statistical difference between these measures of \( \Delta b = b_{creat} - b_{Ni} \); \( p > 0.05 \).
Table 3.9).

The shape of the fractional clearance plots for nickel has a close resemblance to that for the fractional clearance of urea (Smith, 1964). When \( V \) is larger than 2 mL min\(^{-1} \), \( C_{\text{urea}} \approx 75 \text{ mL min}^{-1} \), but when \( V \) is less than 2 mL min\(^{-1} \), \( C_{\text{urea}} \) decreases (Green, 1976; Pickford and Lambie, 1976). Tubular reabsorption of urea is by passive diffusion down a concentration gradient created by the reabsorption of water associated with the active reabsorption of NaCl (Pickford and Lambie, 1976). Therefore, by analogy to urea it is reasonable to postulate that the reabsorption of nickel in the kidney has a large passive component.

\((v)\) **EFFECT OF EXPOSURE ON \( U_{\text{Ni}}^{\circ} \)**

From the discussion it is clear that nickel is reabsorbed in the kidney and thus a substance like urea or total solutes will have a very similar excretion pattern. In the previous Chapter, total solutes \( (\rho-1) \) was selected to be more appropriate for adjustment of urinary nickel than creatinine. It is known that soluble nickel to which electrolytic-refinery workers are exposed is rapidly absorbed into the body and is excreted with a half-life near 24 h. Therefore, it is reasonable to expect to see fluctuations in nickel levels that represent a worker's exposure during the work shift resulting in an increase in \( U_{\text{Ni}}^{\circ} \) (\( U_{\text{Ni}} \) adjusted to \( \rho = 1.018 \); see Chapter 2). After the shift, when the worker is no longer exposed to nickel, a decrease in \( U_{\text{Ni}}^{\circ} \) is
expected. The model depicted in Figure 3.1 illustrates this.

Figures 3.6 to 3.10 illustrate the occurrence of changes in $U_{Ni}^0$ throughout the 24-h collection period. For donors in both study groups (Figs. 3.6 to 3.8), the $U_{Ni}^0$ values generally appear to increase during the shift with a subsequent decrease during the post-shift period. These results follow the same pattern reported by Tola et al. (1979) and Tossavainen et al. (1980) who found an increase in the levels of urinary nickel adjusted by specific gravity when workers were exposed to soluble nickel in electroplating processes and a decrease when they were off work (see Fig. 3.1). As explained in the introduction to this chapter, a linear one-compartment kinetic model gave estimates of the half-life of $Ni^{2+}$ of between 17 and 39 h in urine, and from 20 to 34 h in plasma (Tossavainen et al., 1980). It is not surprising therefore that the maximum $U_{Ni}^0$ value is reached post-shift. Since air concentrations were not available in the present study, modelling of the data in a quantitative manner was not possible.

The post-layoff curves in Figures 3.6 and 3.7, obtained just before the workers returned to their jobs after a one month layoff, are approximately 36 to 70% lower than the pre-layoff values. Nevertheless, these urinary levels are in the range of 6 to 27 $\mu g L^{-1}$ which is above the range 0.5 to 6.0 $\mu g L^{-1}$ expected for nonexposed healthy adults (Sunderman et al., 1986b). This indicates that there is a slow release of nickel from pools stored in the body which causes nickel
to have an effective half life considerably larger than 24-h. This has been observed in other studies for retired nickel workers exposed to particulate forms of nickel (Nieboer et al., 1984a) and is discussed further in Chapter 5. The suggestion of variation in \( \Delta U_{\text{Ni}}^{\text{O}} \) during the second Port Colborne collection as illustrated in Figure 3.7 is unexpected. Examination of the data corresponding to all 6 donors indicates either invariance of \( \Delta U_{\text{Ni}}^{\text{O}} \) (as in Fig. 3.6) during the 24-h period or some suggestion of a systematic change (as in Fig. 3.7). Since these workers were not at work and had been off for one month, some diurnal variation is suggested.

An interesting application of the specific gravity adjustment of urinary nickel concentrations described in Chapter 2 is that \( \Delta U_{\text{Ni}, \text{max}}^{\text{O}} \) (maximum of \( U_{\text{Ni}}^{\text{O}} \) [\( U_{\text{Ni}} \) adjusted to 1.018] - minimum of \( U_{\text{Ni}}^{\text{O}} \) [\( U_{\text{Ni}} \) adjusted to 1.018]) observed during the 24-h collection period may be taken as an estimate of current exposure according to the model described in the legend to Figure 3.1. Table 3.11 summarizes by job description the \( \Delta U_{\text{Ni}, \text{max}}^{\text{O}} \) levels found in the Thompson Cohort. A number of comments seem appropriate about the distribution of these values. The foreman, electrician, equipment man and scrapwash supervisor (see footnote b, Table 3.11) all spent more than half of the work shift away from the electrolytic tankhouse. This is in agreement with the relatively low \( \Delta U_{\text{Ni}, \text{max}}^{\text{O}} \) values observed. The remaining jobs or work areas represent workers more closely associated with the
electrolytic refining process. All have elevated $\Delta U_{Ni,\text{max}}^0$ values of comparable magnitude with few exceptions. In the purification/thickener area there was one donor who had approximately 2.4x the nickel levels of the mean, while in the plating subgroup there were two donors who had $\Delta U_{Ni,\text{max}}^0$ levels $\simeq 1.4x$ than the mean. Because there was only one worker from the cobalt recovery area it is not appropriate to speculate if the relatively high $\Delta U_{Ni,\text{max}}^0$ is representative of this area. Personal air sampling data are required to rationalize more fully the range of $\Delta U_{Ni,\text{max}}^0$ observed. Generally, there is a good correlation between the rise in urinary nickel concentration during the 24-h collection period which included a workshift as a result of exposure at work and job description.
Table 3.11

ESTIMATE OF CURRENT EXPOSURE ($\Delta U_{Ni,max}^0$) IN THE THOMPSON COHORT IN RELATION TO JOB CLASSIFICATION

<table>
<thead>
<tr>
<th>JOB DESCRIPTION</th>
<th>$\Delta U_{Ni,max}^0$ ($\mu g \text{ L}^{-1}$)</th>
<th>Individual Donors</th>
<th>mean $\pm$ SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreman (Safety and Protection)</td>
<td>6.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equipment man</td>
<td>7.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foreman (Refinery)</td>
<td>8.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrican</td>
<td>11.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper recovery</td>
<td>19.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boxman</td>
<td>36.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt recovery</td>
<td>59.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scrapwash</td>
<td>32.0, 29.5, 5.76 b</td>
<td>22.4 $\pm$ 14.5</td>
<td></td>
</tr>
<tr>
<td>Purification/Thickener</td>
<td>12.4, 62.1 c, 23.6, 5.04 d</td>
<td>25.8 $\pm$ 25.4</td>
<td></td>
</tr>
<tr>
<td>Plating Tankman</td>
<td>47.0, 39.6, 29.0</td>
<td>37.7 $\pm$ 11.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.1, 53.8 e, 25.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ $\Delta U_{Ni,max}^0 = U_{Ni,max}^0 - U_{Ni,min}^0$ (at the beginning or end of the 24-h period; adjusted to $\rho=1.018$).

$^b$ This donor was the scrapwash supervisor.

$^c$ $\approx 2.4 \times$ higher than mean.

$^d$ Average $U_{Ni}^0$ for this donor was 81.5 $\mu g \text{ L}^{-1}$, but there was small change during the 24-h collection.

$^e$ $\approx 1.4 \times$ higher than mean.
CHAPTER 4  UPTAKE AND DISTRIBUTION OF NICKEL IN THE RAT KIDNEY

A  INTRODUCTION

(i) Renal Toxicity of Metals

The nickel clearance study of Chapter 3 concludes that nickel is reabsorbed in the human kidney in a manner very similar to urea. Since urea is largely reabsorbed by passive diffusion down a concentration gradient generated by the reabsorption of water (Pickford and Lambie, 1976), it was postulated that renal reabsorption of nickel has a large passive component. In this chapter, a rat model will be used to investigate the relative contributions of the passive and active components of the nickel reabsorption process in the nephron.

The biochemical indices evaluated for electrolytic nickel-refinery workers in Chapter 3 illustrate that compared to known nephrotoxic metals such as cadmium, the changes observed in renal function due to nickel exposure are minimal. Thus it is likely that the accumulations of nickel found in post-mortem kidney specimens in adults without known occupational or iatrogenic exposures to nickel compounds are medically insignificant. The highest concentrations of nickel are usually found in the lung, followed by kidney and liver (Nomoto, 1974; Sunderman, 1984d; Zober et al., 1984; Kollmeier et al., 1985; EPA, 1986; Rezuke et al., 1987). Animal studies show the same general trend in that, for
nickel(II) salts administered parenterally to a variety of animals, the largest accumulation was found in the lung, kidney, liver and endocrine gland, with relatively little nickel found in neural tissue (Mushak, 1980). Such studies also show that after several half-lives (h to d) there is little permanent evidence for long-term tissue accumulation. Thus both animal and human data illustrate a potential of short-term accumulation of nickel in the kidney and elevation with age in the lung, the organ exposed to particulate nickel compounds (EPA, 1986). The work described in this chapter will use an animal model to explore the manner in which the kidney handles nickel and exhibits its toxicity when present in excess.

As summarized by Nieboer and Sanford (1985), metal-ion-induced kidney damage is not a general cytotoxic effect, but is specific for individual metals. For example, \( \approx 50\% \) of the uranyl ion (UO\(_2^{2+}\)) is bound to bicarbonate which is filtered through the glomerulus and becomes attached to the membrane of the proximal tubule cells when it dissociates from HCO\(_3^-\) concomitant with a drop in pH. The UO\(_2^{2+}\) ion inhibits tubular reabsorption of many substances and epithelial cell death follows. Cadmium is largely excreted in the urine complexed with the low-molecular-mass protein metallothionein (CdMT). In a healthy kidney, the CdMT complex is reabsorbed in the proximal tubule and is degraded intracellularly within the lysosomes. Released Cd\(^{2+}\) is thought to be scavenged by de novo synthesized
metallothionein in the kidney (Lauwerys, 1983). The current hypothesis is that saturation of the induction of renal metallothionein results in free Cd\(^{2+}\) which is toxic. Cd\(^{2+}\) ion can impair a cell by inhibiting the function of lysosomes and the nucleus, and induces tubular loss of protein and suppresses peritubular amino acid transport (Foulkes, 1983; Fowler, 1983). Pb\(^{2+}\) is taken up in the proximal tubule cells where it inhibits the mitochondria. It also accumulates as inclusion bodies in cell nuclei. However, it is thought that chronic industrial exposure to lead can affect the glomerulus resulting in reduction of the effective filtration capacity (Lilis et al., 1980). A complication of the treatment of rheumatoid arthritis with gold salts is the development of a membranous glomerulonephropathy in humans (Duke et al., 1982). This is usually characterized by the presence of electron-dense deposits on the epithelial side of the glomerular capillary basement membrane. Duke et al. (1982) conclude that high doses of gold salts in rats appear to result in direct toxic damage to the renal tubular epithelium, a condition not found in humans. Low doses in man are known to cause an immune-complex type of glomerulonephritis. From the above description it is clear that not only is metal-ion-induced kidney damage not a general cytotoxic phenomenon, but it tends to be site specific, often depending on dose.

Metals may also have a regulatory role in kidney function, including Ca\(^{2+}\), Na\(^{+}\) and K\(^{+}\). In addition, Phillips
et al. (1983) postulate that vanadium, a possible essential element in animals, may play a regulatory role (as the vanadate anion) in salt and water excretion by modification of the Na\(^+\) pump in the kidney. However, these authors also recognise that at high concentrations vanadate has toxic renal effects.

From this brief review of the action of metals on the kidney, it is clear that there is no simple common response. Their roles may be essential, essential and toxic, or simply toxic. In this chapter an attempt is made to clarify the uptake, toxic effects and excretion of Ni\(^{2+}\) in the male-rat kidney.

(ii) Experimental Approaches to Nephrotoxicity

The remainder of this introduction will give a brief review of the more common types of animal studies used in the evaluation of transport mechanisms (e.g., p-aminohippuric acid, PAH, uptake) and the action of nephrotoxins (e.g., Pb\(^{2+}\)). In vivo studies usually involve the evaluation of kidney function or toxicity by various methods such as histology, excretion of proteins, amino acids or N-acetyl-\(\beta\)-D-glucosaminidase (NAG) following the injection of a toxic xenobiotic. This approach usually consists of multiple evaluations over an extended time period (e.g., 24-h) rather than a single measurement. However, these types of studies would not lead to more information on the passive/active components of nickel uptake in the nephron
than the human work already described in Chapters 2 and 3. Nevertheless, they may supply information on why the kidney does not appear to be a major target organ for Ni\textsuperscript{2+}.

In vitro studies usually involve one of the following methods: examination of renal slices, ex vivo perfusion of isolated kidneys, and studies with isolated glomeruli or proximal-tubule fragments. Techniques involving isolated tissues offer the general advantage of being able to study purely nephrogenic changes that are difficult to assess in the whole animal due to a number of extra-renal effects (Bach and Lock, 1982). These difficulties, which include undesirable changes in renal haemodynamics, generalized changes in the cardiovascular function, and problems of maintenance of whole animal body temperature, are minimized in isolated-tissue studies (Berndt, 1987). Renal slices of kidney tissue can be prepared free hand, with the aid of a Stadie-Riggs microtome (Berndt, 1981), or with a mechanised device in which tissue is kept in an oxygenated medium for the duration of the cutting process (Krumdieck et al., 1980). These slices (≈ 0.2 to 0.5 mm thick) are incubated in a balanced salt solution that usually has added nutrients such as glucose as well as the chemical being investigated. The uptake of a chemical is measured by the so called slice to medium (s/m) ratio: i.e., the ratio of the concentration of the substance being investigated in the renal slice per unit tissue weight to that of the substance per unit volume of bathing solution (Berndt, 1987). Renal slices have also been
used in transport and metabolism studies (e.g., PAH transport; Foulkes and Miller, 1959). However, this technique does involve an altered organ in which the functional and a portion of its structural integrity have been partially damaged because of mechanical injury (Bach and Lock, 1982).

The isolated perfused-kidney technique involves removal of the kidney after a catheter has been inserted through the superior mesenteric artery, across the aorta and into the right renal artery of the rat (Berndt, 1987). This permits continuous perfusion of the kidney to be maintained during the isolation process. The kidney is then placed into an apparatus that includes a pump for movement of the perfusate (containing the chemical of interest), an oxygenator and a mechanism for control of temperature (Bach and Lock, 1982). Although the isolated perfused-kidney maintains the structural integrity of the kidney, the system is perfused with an artificial medium that does not always maintain all functional properties such as the ability to concentrate urine normally (Bach and Lock, 1982). The perfused kidney assesses the whole organ and cannot directly differentiate the exact site of change or transport along a nephron, identify the population of nephrons affected, nor reveal any subtle changes in renal "haemodynamics" or cardiovascular function that are related to a toxic action (Bach and Lock, 1982; Berndt, 1987).

Studies involving isolated proximal tubules and
glomeruli have the same general advantages and disadvantages described above for renal slices.

In the current work, in vitro isolated renal slices and proximal-tubule studies are combined with in vivo time-course studies involving histology, autoradiography and subcellular fractionation to investigate the renal uptake and toxicity of nickel.

B MATERIALS AND METHODS

(i) MATERIALS

(a) Chemical Reagents

Pertinent information about the routine chemical reagents and animal diets used is summarized in Table 4.1 and of the radiolabelled reagents in Table 4.2.

(b) Nickel-injection Solutions

The 1.5, 3 and 6 mg Ni$^{2+}$ kg$^{-1}$ injection solutions were made up from NiCl$_2$·6H$_2$O; 5 ml of DDW was added to appropriate amounts of this nickel salt so that a 0.7 mL injection of the solution contained the appropriate amount of Ni$^{2+}$ per kg of body weight.

(c) Laboratory Solutions (Renal Slices)

HEPES Incubation Medium (pH = 7.4 and 5.5). The following reagents were added to a 500-mL beaker and brought close to volume with DDW. The pH was then adjusted to 7.4 or 5.5 with
Table 4.1
CHEMICAL REAGENTS

<table>
<thead>
<tr>
<th>Chemical Reagent</th>
<th>Source</th>
<th>Grade</th>
<th>Use or Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid (Glacial)</td>
<td>BDH</td>
<td>Aristar</td>
<td>Na/K ATP-ase</td>
</tr>
<tr>
<td>Ammonium molybdate</td>
<td>BDH</td>
<td>AnalR</td>
<td>Na/K ATP-ase</td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>BDH</td>
<td>AnalR</td>
<td>Na/K ATP-ase</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>BDH</td>
<td>AnalR</td>
<td>Renal slice incubation medium</td>
</tr>
<tr>
<td>DNP</td>
<td>BDH</td>
<td>AnalR</td>
<td>uncoupler of oxidative phosphorylation renal slice study</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>BDH</td>
<td>AnalR</td>
<td>Na/K ATPase (Ethylenediaminetetraacetic acid disodium salt)</td>
</tr>
<tr>
<td>EDTA</td>
<td>BDH</td>
<td>AnalR</td>
<td>Na/K ATPase</td>
</tr>
<tr>
<td>Ethanol</td>
<td>James Burrough</td>
<td>absolute</td>
<td>99.86% (v/v) minimum</td>
</tr>
<tr>
<td>Glucose</td>
<td>BDH</td>
<td>AnalR</td>
<td>Renal slice incubation medium</td>
</tr>
<tr>
<td>HCl</td>
<td>BDH</td>
<td>Aristar</td>
<td>Na/K ATP-ase ampoule of pure HCl (1 ampoule prepares 1 L of 1 M HCl)</td>
</tr>
<tr>
<td>HEPES</td>
<td>Sigma</td>
<td></td>
<td>Renal slice incubation medium</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>Sigma</td>
<td></td>
<td>L-histidine monohydrochloride (monohydrate)</td>
</tr>
<tr>
<td>KCl</td>
<td>Fisons</td>
<td>Analytical Reagent</td>
<td>Renal slice incubation medium and Na/K ATPase</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>Sigma</td>
<td>≥99%</td>
<td>Monopotassium dihydrogen phosphate</td>
</tr>
</tbody>
</table>
Table 4.1 Continued

<table>
<thead>
<tr>
<th>Chemical Reagent</th>
<th>Source</th>
<th>Grade</th>
<th>Use or Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂·6H₂O</td>
<td>BDH</td>
<td>AnalaR</td>
<td>Renal slice incubation medium and Na/K ATPase and Alkaline phosphatase</td>
</tr>
<tr>
<td>Na₂ATP</td>
<td>Sigma</td>
<td></td>
<td>Na/K ATP- ase</td>
</tr>
<tr>
<td>NaCl</td>
<td>Fisons</td>
<td>Analytical Reagent</td>
<td>Renal slice incubation medium and Na/K ATPase</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sigma</td>
<td>Anhydrous pellets</td>
<td>Isolation of Glomeruli and Tubules/Alkaline phosphatase</td>
</tr>
<tr>
<td>NiCl₂·6H₂O</td>
<td>BDH</td>
<td>AnalaR</td>
<td>Renal slice incubation medium and histological study</td>
</tr>
<tr>
<td>p-Nitrophenol</td>
<td>BDH</td>
<td>≥99%</td>
<td>Alkaline phosphatase standard</td>
</tr>
<tr>
<td>p-Nitrophenol</td>
<td>Sigma</td>
<td>Phosphate substrate</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>phosphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouabain</td>
<td>Sigma</td>
<td></td>
<td>Na/K ATP- ase</td>
</tr>
<tr>
<td>PAH</td>
<td>Sigma</td>
<td></td>
<td>p-aminohippuric acid (sodium salt crystalline) for renal slice study</td>
</tr>
<tr>
<td>Percoll</td>
<td>Pharmacia</td>
<td></td>
<td>Gradient used for the isolation of basolateral and brush-border membranes</td>
</tr>
<tr>
<td>PMSF</td>
<td>Sigma</td>
<td></td>
<td>Na/K ATP- ase protease inhibitor (Phenylmethylsulfonyl fluoride)</td>
</tr>
<tr>
<td>Chemical Reagent</td>
<td>Source</td>
<td>Grade</td>
<td>Use or Comment</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
<td>---------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Probenecid</td>
<td>Sigma</td>
<td></td>
<td>anhydrous p-[dipropylsulfamoyl] benzoic acid</td>
</tr>
<tr>
<td>Sodium acetate trihydrate</td>
<td>Fisons</td>
<td>Analytical Reagent</td>
<td>Renal slice incubation medium</td>
</tr>
<tr>
<td>Sodium arsenate</td>
<td>BDH</td>
<td>AnalaR</td>
<td>Na/K ATP-ase</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Sigma</td>
<td></td>
<td>Isolation of P₃ pellet</td>
</tr>
<tr>
<td>Tris-Buffer</td>
<td>BDH</td>
<td>Aristar</td>
<td>Na/K ATP-ase and Alkaline phosphatase (2-Amino-2- (hydroxymethyl) propane-1,3-diol)</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>BDH</td>
<td>AnalaR</td>
<td>Na/K ATP-ase</td>
</tr>
</tbody>
</table>

**DIET**

<table>
<thead>
<tr>
<th>Type of Diet</th>
<th>Source</th>
<th>Grade</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>Labsure</td>
<td>LAD1</td>
<td>Christopher Hill Group Ltd. Poole Dorset</td>
</tr>
<tr>
<td>Pellets</td>
<td>Spratt</td>
<td>Lab Diet No. 1</td>
<td>Spratts, Barking, U.K.</td>
</tr>
</tbody>
</table>
### Table 4.2

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Source</th>
<th>Grade</th>
<th>Use or Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scintillation Cocktail</td>
<td>United Technologies</td>
<td>PICO-FLUOR 30</td>
<td>Scintillation solution for aqueous and non-aqueous samples.</td>
</tr>
<tr>
<td>(³H)L-Histidine</td>
<td>Amersham</td>
<td></td>
<td>40-60 Ci/mmol sterilized aqueous solution containing 2% ethanol.</td>
</tr>
<tr>
<td>⁶³NiCl₂</td>
<td>Amersham</td>
<td></td>
<td>1 mCi mL⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80 μg Ni²⁺ mL⁻¹</td>
</tr>
<tr>
<td>³H-PAH</td>
<td>Amersham</td>
<td></td>
<td>1 mCi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p-amino[³H] hippuric acid</td>
</tr>
<tr>
<td>³H-Proline</td>
<td>Amersham</td>
<td></td>
<td>10-20 Ci/mmol sterilized aqueous solution.</td>
</tr>
<tr>
<td>³H-Thymidine</td>
<td>Amersham</td>
<td></td>
<td>20-30 Ci/mmol sterilized aqueous solution containing 10% ethanol.</td>
</tr>
</tbody>
</table>
1M NaOH or HCl as required and brought to volume in a 500-mL volumetric flask.

HEPES 11.92 g (100 mM HEPES)
CaCl₂ (as CaCl₂·2H₂O) 0.13 g (1.8 mM Ca²⁺)
MgCl₂·6H₂O 0.024 g (0.17 mM Mg²⁺)
Glucose 0.50 g (5.6 mM)
Sodium acetate trihydrate 0.68 g (10 mM acetate)
Na⁺ (as NaCl) 3.22 g (110 mM Na⁺)
K⁺ (as KCl) 1.49 g (40 mM K⁺)

The prepared medium was stored in a refrigerator in a beaker covered with Parafilm until use.

(d) Preparation of Labelled Solutions

\[ ^{63}\text{Ni}^{2+} \quad (100 \mu g \text{ Ni} \ L^{-1}) \]. The stock nickel solution was made by adding 0.4487 g of NiCl₂·6H₂O to a 50-mL volumetric flask, dissolved in and brought to volume with HEPES buffer (pH = 7.4). This solution (0.03775 M Ni²⁺) was further diluted one hundred-fold with HEPES buffer (100 μL to 10 mL) to yield the secondary stock. The labelled working solution was made up by delivering 100 μL of the secondary stock solution to a 25-mL volumetric flask and then adding enough buffer so that it was ~3/4 full. Subsequently, 250 μL of diluted \[ ^{63}\text{Ni}^{2+} \] stock (50 μL \[ ^{63}\text{NiCl}_2 \] stock, Table 4.2, diluted with 3.5 ml HEPES buffer) was added and the mixture was brought to volume with HEPES. Stock solutions were stored in a refrigerator (4°C) when not in use. The working nickel solution was made up fresh every day.
Histidine. The stock histidine solution was made up by dissolving 0.2288 g of L-histidine (monochloride, monohydrate) in a 50-mL volumetric flask. The working solution was derived from this by diluting 200 µL of the stock solution to 10 mL. The stock solution was stored in a refrigerator (4°C) when not in use. The working histidine solution was made up fresh daily.

$^{63}\text{Ni}(\text{His})_2 (100 \mu g \text{ Ni L}^{-1})$. An aliquot (200 µL) of the working histidine solution was added to a 25-mL volumetric flask and brought to ~3/4 volume with HEPES buffer (pH = 7.4). After mixing, 250 µL of the diluted $^{63}\text{Ni}^{2+}$ stock and 100 µL of the ‘cold’ Ni$^{2+}$ secondary stock solution were added as described above in the final step of the preparation of the working $^{63}\text{Ni}^{2+}$ solution. The solution was brought to volume with HEPES buffer (pH = 7.4), mixed, and allowed to stand for 2 h at room temperature before it was used. Only freshly prepared solutions were employed in experiments.

$^{63}\text{Ni}(\text{His})_1 (100\mu g \text{ Ni L}^{-1})$. An aliquot (101 µL) of the working histidine solution (pH = 7.4) was added to a 25-mL volumetric and brought to ~3/4 volume with HEPES buffer (pH = 5.5). After mixing, 250 µL of diluted hot $^{63}\text{Ni}^{2+}$ solution (pH = 7.4) and 100 µL of the ‘cold’ secondary stock Ni$^{2+}$ solution (pH = 7.4) were added as before. The solution was brought to volume with HEPES buffer (pH = 5.5), mixed, and allowed to stand for 2 h at room temperature before it was used. Only
freshly prepared solutions were employed in experiments.

\(^3\text{H-PAH (0.25 mM)}.\) Both labelled (20 \(\mu\text{L}\)) and 'cold' (0.00484 g; sodium salt) PAH were added to a 100-mL volumetric flask. They were dissolved in HEPES or Tyrodes buffer (pH = 7.4) and brought to volume. When not in use, this solution was stored in a refrigerator (4°C).

(e) Preparation of Unlabelled Solutions

**Probenecid (0.04 M).** Probenecid (0.0571 g) was dissolved in ethanol in a 5-mL volumetric flask. This gave a final concentration of \(8 \times 10^{-4} \text{ M}\) when 20 \(\mu\text{L}\) of this solution was added to 1 mL of incubation medium.

**2,4-Dinitrophenol (DNP, 20 mM).** DNP (0.0184 g) was dissolved in ethanol in a 5-mL volumetric flask. This gave a final concentration of 1 mM of DNP when 50 \(\mu\text{L}\) of this solution was added to 1 mL of incubation medium.

**Na/K ATP-ase.** Solution A was made up by dissolving 1 g of ammonium molybdate in a total of 10 mL of DDW. Then 6 g of ascorbic acid was dissolved in 70 mL of DDW to which 100 mL of 1 M HCl was added. (Note the 1 M HCl was accurately made from a standard ampoule of Aristar (BDH) HCl.) The ascorbic acid solution was then cooled to 0°C on an ice bath. Just before the end of the first incubation (see below) the molybdate and ascorbic solutions were mixed. The final
solution is a characteristic yellow colour.

Solution B was made up by dissolving 10 g of sodium arsenite (in a fume hood), 10 g sodium citrate and 9.5 mL of glacial acetic acid in DDW. The resulting solution was then made up to 500 mL (volumetric flask) and thoroughly mixed.

The assay mixture was made up by dissolving the following substances in DDW and bringing to a total volume of 100ml with DDW and thoroughly mixing:

- **NaCl**: 0.842 g
- **KCl**: 0.228 g
- **MgCl\(_2\)**: 0.096 g
- **TRIS**: 0.872 g
- **EDTA**: 0.044 g
- **Na\(_2\)ATP**: 0.264 g.

To 50 mL of the above solution, 0.0436 g of ouabain was added.

**Alkaline Phosphatase.** The buffer + activator solution was prepared by making up a TRIS buffer (0.25 M, pH = 8.8) and adding enough MgCl\(_2\) (activator) to the buffer so that it was 5 mM.

The substrate consisted of a solution of 0.05 M p-nitrophenylphosphate and 0.1 M NaOH (with EDTA 0.05 M).
METHODS

(a) NiCl₂ Injection Study

Injection Protocol. The experimental animals were 12 male Wistar Albino rats. The rats were housed individually in plastic metabolic cages and were permitted free access to powdered diet (Labsure, LAD1) and distilled water. All rats were allowed to acclimatize to the metabolic cages for 12 h before injections. Subcutaneous injections (0.7 mL) were given between 9:00 and 11:00 am. Dosages of 1.5, 3 and 6 mg Ni kg⁻¹ were given to groups of 3 rats. The rats were sacrificed by cervical dislocation 48-h after injection. Urine samples (24-h) were collected and the distilled water consumed in each 24-h period was measured for all groups. The mass of faeces produced and powdered diet consumed were monitored for the two 24-h periods following injection.

Histology. The kidneys were removed from all animals and placed in 10% (v/v) formalin for fixation. Slices 2-4 mm thick were prepared with a single-edge razor to include the papilla. They were washed in 0.1 M phosphate buffer (pH = 7.4) for four 30-min. periods. Fresh buffer was used for each washing. The tissue was dehydrated using gradually more concentrated solutions of ethanol as follows:

- 50 % (v/v) ethanol 1 h
- 85 % (v/v) ethanol 1 h
- 100 % (v/v) ethanol 2x1.5 h.

The washed tissue slices were then infiltrated with JB-4
methacrylate resin using a freshly catalysed monomer for 12 h at 4°C. Infiltrated tissue was then embedded by placing it in a mould in which there was a solution of monomer A and plasticizer B that had been mixed in a ratio of 25 mL to 1 mL. Embedding (polymerization) was allowed to occur at room temperature.

The semi-thin sections (2 μm) were cut using glass 'Ralph' knives on a Bright microtome and were then floated from a water bath (room temperature) onto a glass microscope slide. Staining with one of four histochemical stains followed: Toluidine Blue, Giemsa, Massons Trichome and Periodic-acid Schiff (PAS). The first three stains are good general stains for differentiating histological detail, whereas the PAS stain is used for mucoid and glycoprotein.

(b) Uptake of Nickel in Renal Slices

All radioactive working solutions were made up just prior to use. Before a run was initiated, 95% O₂/5% CO₂ (v/v) was bubbled through these solutions for 20 min at room temperature. In addition, approximately 400 mL of HEPES buffer solution was placed on an ice bath and 95% O₂/5% CO₂ (v/v) was bubbled through it for 20 min. During the subsequent incubation, the atmosphere was not enriched in oxygen, although such an atmosphere was maintained above the incubation medium in one experiment.

The rats were sacrificed by cervical dislocation and the kidneys were excised. Subsequently, the capsule was removed
## Table 4.3

<table>
<thead>
<tr>
<th>Description</th>
<th>Source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrikon T-2055</td>
<td>Kontron</td>
<td>Ultracentrifuge</td>
</tr>
<tr>
<td>Tissue Culture Multi-well plates</td>
<td>Flow Laboratories,</td>
<td>24-Flat bottom wells (1.7x 1.6 cm), approx. 3.6 mL; used for incubation of renal tissue slices</td>
</tr>
<tr>
<td>LKB Multirack</td>
<td>LKB</td>
<td>Fraction collector for a Sephadex G-75 column</td>
</tr>
<tr>
<td>LKB Uvicord 2</td>
<td>LKB</td>
<td>Measurement of elution profile at A-254 nm</td>
</tr>
<tr>
<td>LKB Vairoperpex Pump</td>
<td>LKB</td>
<td>Pump for G-75 column</td>
</tr>
<tr>
<td>MSE Gradient</td>
<td>MSE</td>
<td>Fractionation of a Percoll gradient nickel in serum.</td>
</tr>
<tr>
<td>Prepspin 50</td>
<td>MSE</td>
<td>Ultracentrifuge</td>
</tr>
</tbody>
</table>
and the kidneys were placed in the buffer solution that was immersed in an ice bath. Rasor cuts were made along the lines illustrated in Figure 4.1.

![Figure 4.1. Diagram illustrating the lines along which surgical cuts were made on the excised kidney.](image)

After dissection, the kidney segments BB' DD' and CC' DD' were placed in a Stadie-Riggs microtome with the cuts BB' and CC' face down on hardened filter paper so that slices could be taken off the surfaces DD'. This allowed the uptake of nickel to be measured on a cross section of the rat kidney. Freshly cut renal slices were immediately placed in oxygenated buffer (on an ice bath).

One mL of the appropriate incubation medium was placed in wells of a tissue culture multi-well plate (Table 4.3). A plastic spatula was used to remove two slices from the cold buffer. They were blotted on hardened filter paper and placed in the appropriate well in the multi-well plate, which was then attached to a steel block (to transfer heat and maintain temperature) anchored in a water bath at 25°C. This incubation system was shaken under moderate speed (~40 rev. min⁻¹) on a horizontal oscillating shaker for the appropriate time. After the incubation period, the tissue was removed
with a plastic spatula, blotted on hardened filter paper and weighed to obtain the wet tissue weight. A 0.3 mL aliquot of the post-incubation medium and the pre-incubation medium were placed in scintillation tubes. Next, 4 mL of scintillation fluid (PICO-FLUOR 30) was added to each tube and the radioactivity was measured on a LKB Wallac 1219 RACKBETA. All nickel-containing scintillation cocktails were kept in the dark for ≥ 12 h prior to counting to avoid chemiluminescence. No quenching was observed for the tritium nor the carbon-14 channels. There was also no indication of quenching when internal standards (10-20 µL spike) of tritium (for \( ^3\text{H}-\text{PAH} \)) or \( ^{63}\text{Ni}^{2+} \) or its complexes were added, as judged from an absence of spectral shifts and the good count recovery of spiked samples (including tissue specimens).

The slice to medium ratio (s/m) was calculated from Equation 4.1.

\[
\text{Slice/Medium (s/m) ratio} = \frac{\text{CPM(pre-incubation)} - \text{CPM(post-incubation)}}{\text{CPM(pre-incubation)} \times \text{Wet tissue mass (g)}}
\]

The uptake of \( \text{PAH}^3 \), \( ^{63}\text{Ni}^{2+} \) and its histidine complexes inhibited by probenecid had 20 µL of a 0.04 M solution of this compound added to the appropriate incubation medium. Inhibition was measured by changes in the s/m ratio.

The efflux of \( \text{PAH}^3 \), \( ^{63}\text{Ni(His)}_2 \) and \( ^{63}\text{Ni}^{2+} \) from slices preincubated with the labelled compound for 2 h (as above) was measured for a range of time periods in multiwell culture
plates at 25°C with 1 mL buffer (100mM HEPES, pH 7.4). The tissue was removed from the uptake medium, blotted on hardened filter paper and placed in a new multi-well with fresh buffer as the efflux medium. This procedure of removing, blotting and placing in fresh buffer was repeated after each incubation period (either 15 min, 30 min or 1 h) until a total efflux time of 3 h had elapsed.

(c) Isolation of Glomeruli and Tubules

The following procedure was adapted from that developed in Dr. P. Bach's laboratory at the Robens Institute, University of Surrey.

Tubules and glomeruli are isolated by forcing kidney cortical tissue through a series of stainless steel sieves ranging from 250 μm (to disrupt the tissue) through 150 μm (tubules), 124 μm (some tubules and some large glomeruli) and to 75 μm (glomeruli).

Whole kidneys were removed from ~150-g male Wistar Albino rats and the capsule, papilla and medulla were subsequently removed. The cortices were cut into small pieces and these were broken up gently pressing them through a 250 μm sieve that was on top of a series of progressively smaller sieves (150, 125 and 75 μm). The tissue fragments were washed through the sieves using Tyrode's solution. The tubular fragments and glomeruli were collected from the appropriate sieves and centrifuged for 3 min (300 rpm). The pellets formed were then washed twice with Tyrode's solution
and resuspended in a known volume of HEPES buffer (50 mL for tubules from 10×150 g male Wistar Albino rats which yields a protein concentration between 2 and 3 mg mL\(^{-1}\)). For subsequent incubations, 1.0 mL aliquots of tubule suspension were used. The final incubation was done at 37°C in a tissue-culture incubator.

Incorporation studies were performed on tissue isolated as described in the previous paragraph. Incubation medium consisted of 1 mL of tissue suspensions (in HEPES buffer) with 50 μL aliquots of approximately 20 μCi mL\(^{-1}\) of test chemical (e.g. \(^{63}\)Ni(His)\(_2\), \(^3\)H-proline and \(^3\)H-thymidine) in wells of a multiwell plate maintained in a tissue culture incubator at 37°C.

After the incubation period, the entire sample (1 mL) was removed and added to 3 mL of chilled (ice bath) 6% (v/v) TCA (containing μM quantities of cold incorporation test chemicals) to precipitate the macromolecules.

The samples were then spun down and the supernatant removed. The precipitates were washed twice in cold 6% TCA (see above) and finally resuspended, vortexed and filtered onto 25 mm, glass-fibre filters.

Filters were dried by rinsing with absolute alcohol followed by a drop of diethyl ether (to remove TCA). The dry filters were then placed into the bottom of clean, dry scintillation vials and 200 μL of 0.5 M NaOH added to dissolve the precipitate on the filter. These solutions were allowed to sit for approximately 2 h before 4 mL of Picofluor 30
scintillant was added. Each vial was then shaken thoroughly and left to stand for at least 30 min to ensure low chemiluminescence before counting. Each sample was counted on an LKB Wallac 1219 RACKBETA scintillation counter (a preset $^{63}\text{Ni}^{2+}$ channel or a tritium channel with quench correction).

(d) **Simultaneous Isolation of Basolateral and Brush-Border Membranes**

The following procedure derives from Boumendil-Podevin and Podevin (1983) and is routinely employed by Drs. M. Taylor and M. Dobrota of the Robens Institute (University of Surrey) for the isolation of basolateral and brush-border membranes from rat kidney cortex. This procedure has been used in a number of studies with gold and cadmium.

The rats (approx. 200g) were anesthetized with ethyl ether and incision was made in the neck. An i.v. injection (0.3 mL) was made with $^{63}\text{Ni}^{2+}$ (in HEPES; pH = 7.4) so that each rat was given 6 μg Ni$^{2+}$ kg$^{-1}$ in its jugular vein. All rats were sacrificed 10 min after the injection. The kidneys were removed and placed in a beaker containing ice cold 0.25M sucrose/0.1mM PMSF/2 mM TRIS-HCl (pH = 7.4). The capsule was removed and the cortex was excised from each kidney. The cortices were then placed in about 50 mL of 0.25 M sucrose/0.1 mM PMSF/2 mM TRIS-HCl (pH = 7.4) that had been preweighed. The solution was then weighed so that the mass of the cortex could be obtained by difference. The cortices were cut up roughly with scissors and then
homogenized with 5 strokes on a Potter Elvehjem, type C homogenizer (A. H. Thomas, Philadelphia, Pennsylvania, USA) at reduced speed (1000 rev/min). Enough sucrose solution was added to make the homogenate up to 10 % (w/v). The homogenate was poured into 50 mL screw cap plastic Oak Ridge bottles and centrifuged at 3,700 g·min (2,200 rpm, for 10 min; Temp = 4°C) on a Beckman TJ6 centrifuge (brake off). This pellet was labelled P₁ and was later resuspended in 15-20 mL sucrose. The supernatant was transferred to 8x50 rotor with 50-mL Oak Ridge bottles and spun at 137,700 g·min(13,500 rpm, for 15 min) with the brake off on an HS-18 centrifuge (MSE, Scientific Instruments, Ltd.). The supernatant was carefully removed so that the pellet was not disturbed (this supernatant was kept and labelled S₂). The fluffy top of the pellet was carefully removed and labelled P₃. The bottom part of the pellet (i.e. non-fluffy portion) was labelled P₂. The P₃ pellet was rehomogenized in 10 to 12 mL of 0.25 M sucrose (without PMSF) using the Potter by hand with about 4 to 5 strokes (on an ice bath).

Subsequently, 100% Percoll was added to the P₃ suspension to give a final concentration of approximately 12 % (v/v). After mixing, this self-orienting gradient was placed in the 12x35 ml angle rotor (type TFT 50.38) and centrifuged at 40,000 g for 35 min in a Centricon ultracentrifuge (Kontron, Zurich, Switzerland). The resulting gradient was separated and collected into fractions with the aid of an MSE gradient extraction unit (Table 4.3).
This resulted in three major bands ranging from low to high density: basolateral membrane fraction, brush-border membrane fraction and the mitochondrial fraction. The presence of these membranes on the Percoll gradient was located by enzyme assays. The Na\(^+/\)K\(^+\)-ATPase activity was used to identify the basolateral fraction and the brush-border fraction was enriched in alkaline phosphatase. The location of \(^{63}\)Ni was identified by counting on the LKB RACKBETA as described in the previous section.

The Na\(^+/\)K\(^+\)-ATPase assay is based on that of Forbush (1983) where the Na\(^+/\)K\(^+\)-ATPase activity is measured in the presence and absence of ouabain. The assay mixture (500 \(\mu\)L; one set with ouabain and a second set without ouabain) and sample (100 \(\mu\)L) were added to a disposable test-tube. This solution was incubated (in a water bath) for 17 min at 37\(^\circ\)C. Solution A (yellow) was added and kept on an ice bath for 10 min, then 1.5 mL of solution B was added and the mixture incubated for 10 min at 37\(^\circ\)C. The absorbance of both sets of solutions (with and without ouabain) were read at 705 nm on a Perkin Elmer Lambda-5 UV/VIS spectrophotometer. The activity was measured by the difference in absorbance between the ouabain-present and ouabain-absent solutions using a standard \(\text{KH}_2\text{PO}_4\) curve.

The alkaline-phosphatase enzyme assay mixture contained:

- 0.8 mL of buffer + activator
- 0.1 mL of sample
- 0.1 mL of substrate
This solution was incubated for 20 min at 37°C in a water bath and the absorbance was measured on a Perkin-Elmer, Lambda-5 UV/VIS spectrophotometer at 405 nm. A standard p-nitrophenol curve was prepared. The alkaline phosphatase activity was plotted against the appropriate fraction to identify the brush-border membranes.

(e) Intravenous Injection Experiments

The method used for tissue homogenisation and fractionation had been adapted from that of Boumendil-Podevin and Podevin (1983) and Andersen et al. (1987) by Dr. M. Dobrota of the Robens Institute (University of Surrey). Male Wistar Albino rats (~250g) were anesthetized with i.p. injection of Sagatal and an incision was made in the neck region. An i.v. injection (0.3 mL/200 g) was made with $[^{63}\text{Ni}](\text{His})_2$ (in HEPES, pH 7.4) so that each rat was given $6 \mu g \text{Ni}^2+ \text{kg}^{-1}$ in its jugular vein. All rats were allowed to recover except for the rats for the 10-min time point. After recovery, the rats were permitted free access to Spratts Lab Diet No. 1 and water. A blood sample was taken from the vena cava after the rats were given an i.p. injection of Sagatal or exposed to ether. The kidneys were excised, the capsule removed and placed in HEPES buffer (pH = 7.4). Approximately 4-mm cross section of tissue was taken from the centre of the kidneys and placed in 10% (v/v) formalin for the autoradiography study. The remainder of the kidney was placed in a 100 mL beaker that contained $\approx 50$ mL of HEPES.
buffer (preweighed). This beaker was weighed again to obtain the tissue mass by difference. The kidneys were cut up with scissors and then homogenized with 5 strokes on a Potter Elvehjem (type C) at a reduced speed of 1000 rpm.

Enough HEPES buffer was added to make the homogenate up to 10% (w/v). The homogenate was poured into 50-mL screw-cap Oak Ridge plastic centrifuge bottles and spun at 1,115 g·min (1,600 rpm for 3 min) on a Beckman TJ6 (brake on). This pellet was labelled N, the nuclear fraction. The supernatant was spun at 27,530 g·min (10,000 rpm for 3 min) on an HS18 centrifuge (MSE, Scientific Instruments Ltd., Crawley, U.K.) with the brake on. The pellet formed was labelled the ML fraction (mitochondria/lysosomes). The supernatant was spun at 137,700 g·min (13,500 rpm for 15 min) on an HS18 with the brake off. The supernatant was carefully (but quickly) poured off without disturbing the top fluffy layer of the pellet. The supernatant was kept and labelled S₁. The fluffy layer was carefully resuspended in HEPES buffer by pouring medium in and gently swirling so that the pellet below this fluffy coat was not disturbed. The resuspended fluffy coat was labelled P₃ and contained luminal and basolateral membrane vesicles of the proximal tubule. The supernatant S₁ was combined with the pellet below the fluffy coat, resuspended and spun at 7,950,000 g·min (40,000 rpm for 60 min) in the Centricon centrifuge. The pellet was labelled MIC (microsomes) and the supernatant was the cytosol.
The cytosol was passed over a G-75 column using a LKB Multirack fraction collector and the elution profile was measured with a LKB Uvicord 2 at 254 nm (Table 4.3). Sixty 2-mL fractions were collected for each sample. The column was standardized using the following standards: blue dextran (MM = 2,000,000), ribonuclease (MM = 14,000), Cd-thionein (MM = 7,000), insulin (MM = 5,500), vitamin B-12 (MM = 1,400) and adenosine monophosphate (5'-AMP) (MM = 365).

All the pellets formed were resuspended in HEPES buffer; the total volume was recorded and the $^{63}$Ni radioactivity of the resulting suspensions was measured on the LKB RACKBETA as described previously. The total protein of each fraction was measured by the Bio-Rad method described in Chapter 3.

The mass of the other tissues investigated (liver, testes, lung, heart, spleen, brain, intestine and thigh muscle) were found by difference (weighing was in HEPES buffer). The individual tissues were vigorously homogenised on an Ultra-Turrex' (Janke, Kunkel, Staufen, West Germany) tissue homogeniser in HEPES buffer (pH = 7.4). The volume was adjusted so that a 10% (w/v) suspension was obtained. These suspensions were measured for the presence of $^{63}$Ni using the LKB RACKBETA as described previously in Section B ii d.

The blood collected was allowed to clot ($\approx$ 2 h), spun down ($\approx$ 1000 rpm) and the serum removed. The radioactivity of the serum was measured using a 25 µL aliquot in 4 mL of Pico-fluor 30.
(f) **Incubation with Subcellular Fractions**

The pellets (N, ML, P₃, and MIC) were resuspended in HEPES (pH = 7.4) and vigorously homogenised on a Turrex Tissue homogeniser (full speed for 1 min). They were then spun down at 40,000 rpm on a Centricon centrifuge for 1 h. This regime was repeated twice more. Finally, the appropriate pellets were marked N, ML, P₃ and MIC and were treated as if they were tissue slices. These pellets were divided into two groups, one of which was resuspended in HEPES (pH 7.4) solution that contained $^{63}$Ni²⁺ (200 µg L⁻¹) and the other was resuspended in HEPES (pH 7.4) that contained $^{63}$Ni(His)$_2$ (200 µg Ni L⁻¹). They were incubated at 25°C for 2h, after which they were spun down on a Centricon centrifuge for 40,000 rpm for 1h (7,950,000 g·min). The pellets were then washed with HEPES buffer and spun down as before. Subsequently, the pellets were resuspended in HEPES and the volume recorded. The radioactivity of the resulting suspensions was measured on the LKB RACKBETA as described in SectionB ii d. The total protein of each fraction was measured by the Bio-Rad method described in Chapter 3.

(g) **Autoradiography**

This process involves the generation of an image on photographic emulsion by the decay of radioactive substances. It is based upon the same principles as photography. In autoradiography the emulsion is not affected by light photons, but registers the radiation emitted by radioactive
decay of atoms. The silver-halide crystals, mostly silver bromide, struck by ionizing radiations form an invisible image which is transformed into a visible image when the film is developed. Autoradiography is said to have high resolution when the visible image in the emulsion and the radioactive source in the sample are closely aligned; the maximum displacement being in the order of a few microns or less (Baserga and Malamud, 1969).

The sections of tissues cut from the centre of the kidney as described in Section (e) were fixed in chilled (4°C) formalin calcium fixative for 24 to 48 h: (4% (v/v) formaldehyde (BDH), 1% (w/v) calcium chloride (BDH); Bancroft and Stevens, 1982). These sections were then washed in 0.1 M phosphate buffer (pH = 7.2) for four 30-min periods on a rotatory mixer. Fresh buffer was used for each washing. Tissue was dehydrated using increasing concentrations of aqueous ethanol at 4°C as follows:

- 50% (v/v) ethanol 0.5 h
- 70% (v/v) ethanol 0.5 h
- 100% (v/v) ethanol (anhydrous) 2x1.0 h

These dehydrated kidney sections were then infiltrated with JB-4 methacrylate resin using a freshly catalysed monomersolution A for 12 h at 4°C. Infiltrated tissue was then embedded by placing it in a mould in which there was a solution of monomer A and plasticizer B that had been mixed in a ratio of 25 mL to 1 mL. Embedding (polymerization) was allowed to occur at room temperature in a vacuum dessicator.
under vacuum or nitrogen atmosphere for 2 to 3 h.

Semi-thin sections (≈1 to 2 μm) were cut using glass 'Ralph' knives on a Bright 5030/WD/MR-CV ultramicrotome and were then floated from a water bath (room temperature) onto a gelatin coated glass microscope slide. They were then allowed to dry onto the slide by being placed onto a hot plate at 60°C.

These semi-thin sections were dip-coated with Ilford K-5 emulsion following the method of Williams (1977) which is summarized below.

The following steps were done in a darkroom with a red safelight. The molten emulsion was diluted and then tested by dipping a clean slide into the emulsion to determine whether it gave an even layer that was free from bubbles. If there were bubbles present the mixture was allowed to stand for a few minutes and then the test was repeated. Two slides were placed back-to-back with the tissue sections on the outside and the combination was dipped into the melted emulsion for ≈2 s; the backs were carefully wiped and allowed to gel. These slides were placed in plastic light-proof slide boxes (≈ 10 to 12 slides per box) which were then placed in a drying chamber containing dried silica gel overnight. The slide boxes were closed, sealed with pressure-sensitive black tape and wrapped in two black photographic bags. The packages were marked with an appropriate code number and date. These packages were stored at -20°C for 3 to 6 weeks exposure after which they were
removed and developed.

The emulsion was developed and fixed without interruption at room temperature in a darkroom (red safelight only). The emulsion-coated slides to be developed were removed from the light-tight box and placed in a tray and developed for 5 min in D19 (Kodak) developer with occasional agitation. They were then rinsed for 5 min in distilled water and subsequently fixed for 8 min in 30% (w/v) sodium thiosulphate fixer with occasional agitation. This step was followed with a 10-min rinse with running cold tap water. The slides were then allowed to air dry.

Staining was carried out without interruption at room temperature. After air-drying, the slides were placed in staining dishes and stained with Toluidine Blue (for 1 min) as described previously. The presence of nickel was identified by the dark spot images of the developed silver grains and quantified by a Quantimet 920 image analysis system (Cambridge Instruments). This can be used for counting, measuring, comparing and classifying images. The input to the Quantimet was through a ZEISS Universal optical microscope (47-79-01-9901; Carl Zeiss Oberkochen, West Germany) employing the 40x objective. These images were processed by QUIPS, a Cambridge instruments software package. This method converts the field of view of the microscope into picture points (630x784 pixels) in each field of view. The operator sets a level above which the signal from a picture point (i.e. the light intensity at any picture point)
becomes recognised by the computer circuitry of the system. This allows the system to count all the picture points brighter than a given intensity; the points brighter than the preset level can also be recombined into areas of brightness, and the number and size of these areas can be assessed. If the slide is viewed under low magnification so that individual silver grains are not resolved, it is possible to use the Quantimet 920 as a densitometer.

Initial measurements made on the Quantimet 920 involved its use as a densitometer to count all the grains in specific regions of the kidney (e.g., outer cortex, inner cortex and medulla). The QUIPS program was reprogrammed so that the area of the mask (area of interest) and the occupied area (grains) were automatically counted and the results were expressed as the area of grains/area of mask (gr/μ²). Before any measurements were made, the system was standardized using one of the 1.5-h slides. One area next to the papilla was easily located because of a heavy black deposit which was located in the lower left hand of the monitor frame. Five areas on the monitor screen were counted and the settings were accepted if the results were in the range of 0.0360 to 0.0378 gr/μ² using the 40x objective and a projection lens of x1.3. If the results were outside this preselected range then the sensitivity was adjusted.

The S1, S2, and S3 segments of the proximal tubules, the distal tubule and collecting ducts were measured by masking the area to be investigated (e.g. a glomerulus) and then
having the Quantiment count the number of grains in the masked area, calculating the area enclosed by the mask. The results are in turn expressed as $gr/\mu^2$. When $\geq 20$ of these masks were evaluated on one area (e.g. glomerulus) for one time point, then the program was instructed to terminate and the average results were printed out. These mean values ($gr/\mu^2$) were corrected to a standard of 0.035 $gr/\mu^2$ and the control value of 0.00974 $gr/\mu^2$ was subtracted. The nickel levels in each segment of the nephron were expressed as $gr/\mu^2$.

(h) Cation Column Chromatography

Solutions of Ni(His)$_2$ and Ni(His)$_1$ were passed over a cation exchange resin to identify differences in formal charge between these complexes. Solutions of $1 \times 10^{-3}$ M Ni(His)$_{2.05}$ were prepared in DDW at pH values of 5.0 (Ni(His)$_1$) and 8.0 (Ni(His)$_2$), as described in Section B (i) d, except that no $^{63}$Ni or HEPES buffer was used. In this work the slight excess of histidine ($1.0 \times 10^{-4}$ M) was used to buffer these solutions. The eluting solution consisted of $5.0 \times 10^{-5}$ M histidine made up in DDW and adjusted to the appropriate pH.

Bio-Rad analytical-grade cation-exchange resin was used in this work. A 1x10 cm column was packed with 1.53 g of resin and washed with 30 mL of DDW and left overnight. The column was then washed with 1 M NaCl. One mL of pH 8 eluting solution (with 4 drops of bromothymol blue indicator;
Materials and Methods, Chapter 2) was placed on the column and was observed to come off the column after eluting with 1 mL of eluent. The Ni(His)$_2$ and Ni(His)$_1$ solutions turned red when tested with 3 drops of 1% (w/v) dimethylglyoxime (DMG) in 95% (v/v) ethanol and 3 drops of 1% (v/v) NH$_3$, while the eluent did not turn red. The 1 mL of 1x10$^{-3}$ M Ni(His)$_2$ was observed (by DMG/NH$_3$) to come off in the first 2 mL of eluent. However, the Ni(His)$_1$ (pH = 5) did not come off the column until strontium nitrate (0.5 M) was used to displace the nickel-histidine complex.

C RESULTS

(i) NiCl$_2$ INJECTION STUDY

(a) Post Injection Status

After i.p. injection, both the rats administered 3 mg Ni kg$^{-1}$ (intermediate dose) and 6 mg Ni kg$^{-1}$ (high dose) appeared to be extremely relaxed and lethargic. The ears became red with dilated vessels. After 15 to 20 min., the 3 mg Ni kg$^{-1}$ group were beginning to show signs of recovery; while it took 2 1/2 h for the high-dosed animals to begin recovery. At a low dose (1.5 mg Ni kg$^{-1}$) the animals did not display any of these effects.

(b) Solid and Liquid Balance

The non-histological effects of i.p. injections of NiCl$_2$
on male Wistar Albino rats are illustrated in Figure 4.2. There appears to be an inverse dose-response relationship between amounts of powdered diet eaten by the rats during the first 24-h and amount of nickel injected. All of the dosed rats in this category showed a significant drop in diet (p<0.005) when compared to the control group. However there was no significant difference (p>0.05) between the low and intermediate doses while there was a difference between low and high dose (p<0.05). The intermediate and high dosed rats exhibited no significant difference in food intake (p>0.05). The faecal output appears to drop in the first 24-h for all animals injected, but not according to dose; only the low- and high-dosed rats were significantly different from the controls (p<0.005). The decrease in consumption of powdered diet is concurrent with a drop in faecal output.

In the second 24-h period, the amount eaten increased somewhat for all dosed animals and approached the control level with just a small decrease in food intake for the nickel-exposed rats (all not statistically significant, p>0.1). This increase in food intake is also reflected in the faecal output for the second 24-h period; it has increased so that there was no significant difference between the controls and the Ni^{2+}-dosed rats (p>0.05).
Figure 4.2: Dietary and excretion parameters of male Wistar Albino rats housed in metabolic cages measured before and after i.p. injections of NaCl.

a) No statistically significant difference between controls of the 24-h and 48-h data sets (p>0.01).
b) Statistically significant difference between parameter and the appropriate control (p<0.005).
c) Statistically significant difference between parameter and the appropriate control (p<0.05) or between the 24-h and 48-h data sets.
d) Statistically significant difference between parameter and the appropriate control (p<0.01).
The amount of urine produced in the first 24-h by the low-dose group was statistically smaller (p<0.05) than for the controls. This observation coincides with the lower water intake, which was statistically different (p<0.05) from the controls. In the second 24-h collection, the amount of urine produced for the low-dose group is close to the control rats (not significant p>0.1) while the water intake is still low (p<0.01). This apparent discrepancy is resolved when the urine output for both periods is compared. The urine outputs of the low-dosed rats for both time periods are not statistically different (p>0.05), which agrees with the same overall water intake for both time periods (not significant, p>0.1). Therefore both the 24-h and 48-h periods are characterized by low water intake and urine output. For the intermediate and high doses, the amount of urine produced in the first 24-h period is higher than the controls, although it reaches statistical significance only for the high dose (p<0.005). There was no statistical difference between the urine output of the low and intermediate doses (p>0.1), while there was a difference between the low- and high- dosed rats (p<0.005). The water intake for the second 24-h period is only significantly greater than controls (p<0.01) for the high-dosed group, while for both the intermediate and high groups the average urine output is higher than control with only the high dose reaching significance (p<0.005). There appears to be a lag between high urine output of the high-dosed rats and increasing water intake, since it is not
until the second 24-h period that we see an increase in water intake (p<0.01) over controls. This illustrates an increased trend to larger urine volumes for the high-dosed rats, while the low-dosed rats tend to have a depressed urine output. However, both the urine output in the first collection and the increase in water intake in the second appear to be dose related. The urine outputs in the second observation period are lower than in the first for the intermediate and high injections, with a slight indication of a dose response.

(c) Histology

The tissue for the low dose (1.5 mg Ni kg⁻¹) pulled away from the resin block, making it impossible to cut semi-thin sections. The results described in this section are limited to the high (6 mg Ni kg⁻¹) and intermediate (3 mg Ni kg⁻¹) dose groups.

There were no characteristic morphological tissue changes observed in the kidneys, such as a lack of platelets in the capillaries, cellular disruption and nuclear changes in the interstitium and collecting duct epithelia of the papilla. This indicates that there was no renal papillary necrosis (RPN). In the 6 mg Ni kg⁻¹ group, a large number of proximal tubules exhibited a significant number of lysosomal granules. Cortical morphological alterations were evident in the size and shape of the glomeruli. Figures 4.3a and b show control tissue stained with Giemsa with normal appearing glomeruli. The capillary spaces are evident, and are defined
Figure 4.3. Photomicrographs of kidney tissue from rats 48-h after i.p. injections of NiCl$_2$. 

A Control Giemsa-stained tissue with normal appearing glomeruli. 100X.

B Control Giemsa-stained tissue with normal appearing glomeruli. 400X.
C Intermediate-dosed (3 mg Ni kg\(^{-1}\)) tissue with increased Giemsa staining of the basement membrane of the glomeruli and a reduction in the volume of Bowman's space. 100X.

D Intermediate-dosed (3 mg Ni kg\(^{-1}\)) tissue with increased Giemsa staining of the basement membrane of the glomerulus and a reduction in the volume of the Bowman's space. 400X.
E High-dosed (6 mg Ni kg\(^{-1}\)) Giemsa-stained tissue with majority of the glomeruli within this section solid and no evidence of Bowman's space. 100X.

F High-dosed (6 mg Ni kg\(^{-1}\)) Giemsa-stained tissue with a solid glomerulus and no evidence of Bowman's space. 400X.
G High-dosed (6 mg Ni kg\(^{-1}\)) Giemsa-stained tissue with a solid glomerulus and proximal tubule profiles with a considerable number of protein uptake granules. 250X.

H High-dosed (6 mg Ni kg\(^{-1}\)) Giemsa-stained tissue illustrating an example of a badly necrosed glomerulus with total loss of structural integrity and characteristic shape. 400X.
I Control Giemsa-stained tissue with two examples of glomeruli with normal patent capillaries and Bowman's space. 400X.

J Control Giemsa-stained tissue illustrating the podocyte/capillary membrane region of the glomerulus that appear to be most affected by nickel. 1000X.
by podocytes and by Bowman's (capsular) space around the glomerulus between the inner or visceral layer (glomerular epithelium) and the outer or parietal layer (Fig. 4.3a). Under higher power (Fig. 4.3b), details of the glomerulus are shown to be normal histologically. There are wide-open (patent) capsular spaces and a well-defined Bowman's area around the glomerulus.

For the intermediate-dosed rats, the photomicrograph under low magnification (Fig. 4.3c) shows an increase in staining of the basement membrane of the glomerulus, with a resultant reduction in the number of visible glomerular capillaries and a reduction in the volume of Bowman's space (compare to Fig. 4.3a). High magnification (Fig. 4.3d) reveals further details of the changes in the glomerulus when compared with control tissue (Fig. 4.3b). There is evidence of increased staining in the basement membrane and a decrease in the number of visible capillaries and a shrinking of the Bowman's space.

Under low magnification, the tissue from the high-dosed rats shown in Figure 4.3e illustrates that the majority of the glomeruli within this section are solid with no evidence of the Bowman's space (c.f., to Fig. 4.3a and c). The podocytes and capillaries merge together. There is also an increase in interstitial staining extending out from affected glomeruli between the proximal tubules. At 400X magnification when Figure 4.3f is compared to Figure 4.3b, an increase in the thickness and/or volume of the basement
membrane of the glomerular podocytes is evident. In fact, there is no Bowman's space in evidence at all. A mitotic body is present below the glomerulus in the distal tubule. Under a medium magnification of 250X (Fig. 4.3g), tissue from the 6 mg kg\(^{-1}\) animals shows a "solid" glomerulus, although the proximal tubule profiles also have a considerable number of protein uptake granules which might be lysosomal in nature. Figure 4.3h shows an example of a drastically-changed glomerulus with total loss of structural integrity and characteristic shape.

The next two photomicrographs again show control tissue. Figure 4.3i illustrates at 400X two glomeruli with "normal" patent capillaries and Bowman's space. The change that occurs in nickel-treated rats is evident when Figure 4.3i (control) is compared to Figures 4.3g and h (nickel-treated) which show changes in basement-membrane and interstitial staining. Under very high magnification (1000X), Figure 4.3j illustrates the podocyte and capillary membrane regions of the glomerulus that are most affected by nickel. The increase in staining, both in thickness and intensity, suggests increased deposition of glycosaminoglycans (proteoglycans free of protein) within the cell membrane matrix.

Histopathological examination of the rat kidney also revealed alterations restricted to scattered proximal tubules but occurring along the entire length of the tubules altered. The changes included: dilated tubules, loss of
brush border, flattened epithelia, and some regenerative activity.

(ii) UPTAKE OF NICKEL IN RENAL SLICES

The uptake curve of p-aminohippuric acid (PAH) by kidney slices from male Wistar Albino rats in Tyrodes buffer shows an increase in the slice to medium ratio (s/m) of PAH with time (Fig. 4.4). There is also a decrease in the s/m for slices treated with the anion transport inhibitor probenecid (8x10^-4 M). A similar uptake curve was observed for Ni^{2+} in HEPES buffer (Figure 4.5). The results from the uptake of nickel as its histidine complexes by isolated rat renal slices were subject to large variations. In an attempt to compare this data, it was normalized by the slice to medium ratio (s/m) of various incubation components such as PAH and Ni^{2+} (Table 4.4). Studies in the absence of an oxygen-enriched atmosphere above the incubation medium resulted in a lower slice/medium (s/m) ratios for Ni(His)\(_2\) and Ni(His)\(^+\) relative to Ni^{2+} (p > 0.01 and p < 0.01, respectively).

Probenecid (8x10^-4 M) inhibited the uptake of PAH in all cases. This reduction was on average 30 ± 4% (four runs in triplicate) and reached statistical significance at p=0.01. The uptake of PAH in dead tissue (ethanol, boiling and 2,4-dinitrophenol) resulted in a much lower s/m ratio than when it was inhibited by probenecid. The average reduction observed in three runs (triplicate) was 62 ± 9%. and was
Figure 4.4. The accumulation of PAH in renal tissue slices, measured by the slice/medium (s/m) ratio over a 4-h incubation period; each point represents the mean ± SD of three replicates except where noted; ○, 0.25 M PAH; △, 0.25 M PAH inhibited by 8x10⁻⁴ M probenecid; PAH accumulation in the presence of probenecid is significantly lowered: p<0.05 (point a) and p<0.01 (point b).
Figure 4.5. The accumulation of 100 μg L⁻¹ Ni²⁺ in renal tissue slices, measured by the slice/medium (s/m) ratio over a 4-h incubation period. Each point represents the mean ± SD of three replicates.
Table 4.4
SLICE/MEDIUM (s/m) RATIO
NORMALIZED UPTAKE DATA$^{a,b}$

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Live Tissue</th>
<th>Live Tissue + Probenecid (3x10$^{-4}$ M)</th>
<th>Ethanol (n = 1)</th>
<th>Dead Tissue Boiling (n = 1)</th>
<th>Dinitrophenol (n = 1)</th>
<th>Dead Tissue Average</th>
<th>Normalization</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-aminohippuric acid (PAH)</td>
<td>1.00 ± 0.00</td>
<td>0.72 ± 0.12 (n = 4)</td>
<td>0.36</td>
<td>0.36</td>
<td>0.48</td>
<td>0.38 ± 0.09 (n = 3)</td>
<td>Normalized to uptake of PAH live tissue</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>3.28 ± 0.79 (n = 8)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ni(His)$_2$</td>
<td>2.95 ± 0.78 (n = 7)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ni(His)$_1^{+}$</td>
<td>1.68 ± 0.42 (n = 5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>1.00 ± 0.00</td>
<td>1.08 ± 0.37 (n = 4)</td>
<td>1.64</td>
<td>2.12</td>
<td>0.66</td>
<td>1.47 ± 0.74 (n = 3)</td>
<td>Normalized to uptake of Ni$^{2+}$ by live tissue</td>
</tr>
<tr>
<td>Ni(His)$_2$</td>
<td>0.90 ± 0.13 (n = 7)</td>
<td>1.09 ± 0.35 (n = 4)</td>
<td>1.36</td>
<td>2.19</td>
<td>—</td>
<td>1.78 ± 0.59 (n = 2)</td>
<td>—</td>
</tr>
<tr>
<td>Ni(His)$_1^{+}$</td>
<td>0.51 ± 0.22 (n = 5)</td>
<td>0.68 ± 0.35 (n = 4)</td>
<td>1.59</td>
<td>1.46</td>
<td>0.36</td>
<td>1.14 ± 0.67 (n = 3)</td>
<td>—</td>
</tr>
<tr>
<td>Ni$^{2+}$ + 10x Ca$^{2+}$</td>
<td>0.87 ± 0.34 (n = 4)</td>
<td>0.98 ± 0.36</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ni(His)$_1^{+}$</td>
<td>1.00 ± 0.00</td>
<td>1.30 ± 0.14 (n = 3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ni(His)$_2$</td>
<td>1.90 ± 0.77 (n = 5)</td>
<td>2.45 ± 0.46 (n = 3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ni(His)$_2$</td>
<td>1.00 ± 0.00</td>
<td>1.25 ± 0.40 (n = 4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$^{a}$ These experiments were conducted in the absence of an oxygen-enriched atmosphere above the incubation medium. As explained in the text, pre-experiment purging with 95% O$_2$/5% CO$_2$ (v/v) for 20 min occurred.

$^{b}$ Number of experiments with triplicate samples designated by n.

$^{c}$ Statistically significant: p<0.05.
different from the probenecid inhibited uptake (p<0.02).

The picture is not as clear for the inhibition of uptake of Ni\(^{2+}\) or its complexes (Table 4.4). For Ni\(^{2+}\) and the Ni\(^{2+}\)/Ca\(^{2+}\) combination, the probenecid-treated slices have approximately the same s/m as untreated samples (p > 0.05). For the nickel histidine complexes there appears to be an increase in the mean s/m value with probenecid that is not significant (p > 0.05). Relative to Ni\(^{2+}\), there appears to be a nonsignificant decrease in nickel uptake for Ni(His)\(_2\) and Ni\(^{2+}\)/Ca\(^{2+}\) (p > 0.05) compared to a large decrease for Ni(His)\(_1\) (p < 0.01). Tissue that had been previously treated with ethanol or by boiling (i.e. dead tissue) had a larger nickel mean uptake for Ni\(^{2+}\), Ni(His)\(_2\) and Ni(His)\(_1^+\) than nontreated (live) tissue. Only Ni(His)\(_2\) uptake by boiled tissue slices was significantly different from Ni\(^{2+}\) (live tissue) (p < 0.01), the remainder approached significance with p< 0.05. Tissue treated with DNP appears to have a reduced mean nickel uptake for both Ni\(^{2+}\) (not significant p > 0.05) and Ni(His)\(_2\) (significant p < 0.01) when compared to Ni\(^{2+}\) s/m for live slices.

For the one study on the uptake of nickel and PAH while the incubation medium was continually bathed with 95% O\(_2\)/5% CO\(_2\) (v/v) atmosphere, there was a significant increase (p < 0.01) in the uptake of PAH compared to air-exposed slices, but a nonsignificant (p > 0.01) change occurred in the s/m for Ni\(^{2+}\) and Ni(His)\(_2\) (Fig. 4.6).

Figure 4.7 illustrates the efflux of PAH from live
Figure 4.6. The accumulation of $^{63}\text{Ni}^{2+}$, $^{63}\text{Ni(His)}_{2}$ and $^3\text{H-PAH}$ in renal tissue slices, measured by the slice/medium (s/m) over a 2-h incubation period. Each histogram represents the mean ± SD of three replicates. The uptake without 95% O$_2$/5% CO$_2$ (v/v) atmosphere is described in the text. The uptake with 95% O$_2$/5% CO$_2$ (v/v) represents one run where the slices were treated as described in the text except that the incubation medium was kept under an atmosphere of 95% O$_2$/5% CO$_2$ (v/v) during the incubation period.

$^a$Not statistically significant (p>0.05).

$^b$Statistically significant (p<0.01).
Figure 4.7. The total efflux of PAH and $^{63}\text{Ni}^{2+}$ from tissue expressed as a percent of that accumulated during a 2-h incubation. The PAH efflux from ethanol-treated tissue had the same profile and values as live tissue. The efflux of $^{63}\text{Ni(His)}_2$ from ethanol treated slices resembled that of $^{63}\text{Ni}^{2+}$ in both its profile and amount.
tissue and nickel from live and dead tissues after it had been preincubated with PAH$^3$ or $^{63}$Ni$^{2+}$ for 2 h. There is a rapid increase in the amount of nickel released from the $^{63}$Ni$^{2+}$-preincubated tissue slices during the first h. After 1 h, a residual slow release of nickel remained, reaching a maximum recovery in 3 h of $37 \pm 1\%$ compared to $30 \pm 1\%$ at 1 h. The profile for the efflux of $^{63}$Ni$^{2+}$ from ethanol-treated tissue was very different from the live tissue as illustrated in Figure 4.7. The largest release still occurs in the first h, but the % efflux is much lower for the ethanol-treated slices. The efflux of $^{63}$Ni(His)$_2$ from ethanol-treated slices resembled that of $^{63}$Ni$^{2+}$ in both its profile and amount (data not shown). The release profile of PAH from live tissue was similar to that of Ni$^{2+}$, but with a much higher recovery at all time periods (Figure 4.7). The PAH efflux from ethanol treated tissue had the same profile and values as live tissue.

The release curves for PAH, Ni$^{2+}$ and Ni(His)$_2$ were found to follow first-order kinetics (Table 4.5). The half-life for the release of PAH from both live and ethanol-treated tissue was relatively short (live tissue $17 \pm 2$ min and dead tissue $16 \pm 3$ min). By contrast, the release of Ni$^{2+}$ from live tissue had a much shorter half-life than from dead tissue. The half-life for the release of Ni(His)$_2$ from dead tissue ($33 \pm 3$ h) was not significantly different from that for the release of Ni$^{2+}$ ($37 \pm 2$ h). Release of Ni$^{2+}$ by live tissue took place more rapidly ($t_{1/2} = 8.9 \pm 0.2$ h).
Table 4.5

FIRST-ORDER KINETIC ANALYSIS OF PAH AND NICKEL
IN VITRO EFFLUX FROM RAT RENAL SLICES\textsuperscript{a}

<table>
<thead>
<tr>
<th>Substance Released</th>
<th>$k$\textsuperscript{b} (h\textsuperscript{-1})</th>
<th>$t_{1/2}$ (h)</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH (Live Tissue)</td>
<td>2.4 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>0.98-0.99</td>
</tr>
<tr>
<td>PAH (Dead Tissue Ethanol)</td>
<td>2.5 ± 0.4</td>
<td>0.27 ± 0.04</td>
<td>0.90-0.95</td>
</tr>
<tr>
<td>Ni\textsuperscript{2+} (Live Tissue)</td>
<td>0.078 ± 0.002</td>
<td>8.9 ± 0.21</td>
<td>0.90-0.93</td>
</tr>
<tr>
<td>Ni\textsuperscript{2+} (Dead Tissue Ethanol)</td>
<td>0.019 ± 0.001</td>
<td>36.6 ± 2.0</td>
<td>0.97-0.98</td>
</tr>
<tr>
<td>Ni(HIS)\textsubscript{2} (Dead Tissue Ethanol)</td>
<td>0.021 ± 0.002</td>
<td>32.6 ± 2.6</td>
<td>0.98-0.99</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The efflux of PAH and \textsuperscript{63}Ni\textsuperscript{2+} from tissue illustrated in Figure 4.7 expressed in terms of first-order kinetic parameters.

\textsuperscript{b} At 25°C.
(iii) STUDIES WITH ISOLATED PROXIMAL TUBULES

PAH uptake by proximal tubule fragments was shown to be inhibited by 200 μM probenecid. The incorporation of 200 and 400 μg L⁻¹ ⁶³Ni as ⁶³Ni(His)₂ into proximal tubule fragments was shown to have a time-dependent change but not to be concentration dependent. The incorporation of thymidine, proline and histidine into rat proximal tubule fragments was studied in the presence of the complex Ni(His)₂. The incorporation of histidine was severely inhibited (> 90 %), whereas proline incorporation was lowered to a much smaller amount (15 % max). Similarly, thymidine uptake was reduced (10%) by 50 μg L⁻¹ Ni, with a subsequent rise to 100% of controls at 200 μg L⁻¹ Ni. UV/VIS spectral analysis suggested that the formation of a Ni²⁺-proline complex occurred under the experimental conditions (characterized by an absorption band at 640 nm), but this was not observed for thymidine.

(iv) INTRAVENOUS INJECTION EXPERIMENTS

The simultaneous isolation of the basolateral and brush-border membranes from the P₃ pellet of 200-g male Wistar-Albino rat kidney cortex 10 min after intravenous injection of 6 μg ⁶³Ni/kg (as ⁶³Ni(His)₂) into the jugular vein showed almost equal amounts of nickel associated with both membranes. Nickel was also found to be associated with the P₃ pellet at 1.5h, 3h and 24h post injection.
The 24-h time course after i.v. injections of 6 μg $^{63}\text{Ni}$ kg$^{-1}$ (as $^{63}\text{Ni}(\text{His})_2$) is summarized in Table 4.6a and Figure 4.8a. Nickel is present in the kidney and liver 10 min after the injection. The level in the kidney built up to a maximum 1 1/2-h after injection then decreased rapidly reaching similar levels in all fractions after 24 h. There is a steady decrease in the percent of injected dose in the plasma and the liver, which is consistent with the rapid renal excretion of Ni$^{2+}$ ($t_{1/2} \approx 24\text{h}$).

The molecular-mass profile of the kidney-cell cytosol fractionated by Sephadex G-75 column consistently produced two peaks: one a high relative molecular-mass (≈ 111,000) fraction and the other of ≈ 480 (Fig. 4.9 a-d). The low molecular-mass cytosol fraction (Fig. 4.9d) coincides exactly with the peak of authentic $^{63}\text{Ni}(\text{His})_2$. This was confirmed after calibration with the standard 5’-AMP (MM = 365) which came off in the fraction just below nickel. There was no large peak around fraction 17 where albumin is expected. However, the 1.5- and 3-h time points (Figs. 4.9 b and c) do not show as dramatic a drop after fraction 15 as the 10-min and 24-h time points. Over the initial 3-h period, there are increases in the nuclear fraction and the mitochondria/lysosomes fraction. All the rest of the fractions remain relatively constant or show a decrease in Ni content (see cytosol and brushborder/basolateral fractions). The ratio of low/high molecular-mass ratio for $^{63}\text{Ni}$ in cytosol follows the same profile as the percent retention in
Table 4.6
DISTRIBUTION OF $^{65}$Ni DETERMINED BY SCINTILLATION SPECTROSCOPY ON SUBCELLULAR FRACTIONS AFTER IV INJECTION OF $^{65}$Ni(His)$_2$ OR AFTER IN VITRO INCUBATION OF SUBCELLULAR FRACTIONS WITH $^{65}$Ni$^{2+}$ OR $^{65}$Ni(His)$_2$.

(A) IV INJECTION OF $^{65}$Ni(His)$_2$ (6 μg Ni kg$^{-1}$)

<table>
<thead>
<tr>
<th>Kidney Sample Substance</th>
<th>10 minutes</th>
<th>1.5 h</th>
<th>3 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G$^{65}$Ni CPW (μg)</td>
<td>Total Protein (mg)</td>
<td>CPW/mg (protein)</td>
<td>G$^{65}$Ni CPW (μg)</td>
</tr>
<tr>
<td>Homogenate</td>
<td>3931553</td>
<td>213</td>
<td>18458</td>
<td>5695113</td>
</tr>
<tr>
<td>Brushborder/ Bandolateral</td>
<td>120542</td>
<td>3.02</td>
<td>41961</td>
<td>320816</td>
</tr>
<tr>
<td>Gynosol</td>
<td>2221605</td>
<td>92.8</td>
<td>23941</td>
<td>3204600</td>
</tr>
<tr>
<td>Mitochondria/ Lysosomes</td>
<td>742317</td>
<td>55.6</td>
<td>13351</td>
<td>1435004</td>
</tr>
<tr>
<td>Nuclear</td>
<td>30850</td>
<td>27.7</td>
<td>9546</td>
<td>976868</td>
</tr>
<tr>
<td>Microsomes</td>
<td>424618</td>
<td>15.8</td>
<td>25875</td>
<td>707459</td>
</tr>
<tr>
<td>%</td>
<td>98.6</td>
<td>98.6</td>
<td>113.9</td>
<td>103.2</td>
</tr>
<tr>
<td>% Injected dose G$^{65}$Ni</td>
<td>6.67 ± 0.44$^a$</td>
<td>9.74</td>
<td>7.60</td>
<td>1.25 ± 0.21$^{ab}$</td>
</tr>
<tr>
<td>Sera CPW/μL</td>
<td>65065 ± 65012</td>
<td>315153</td>
<td>20651</td>
<td>0.36 ± 0.11$^{ab}$</td>
</tr>
<tr>
<td>% Injected dose G$^{65}$Ni</td>
<td>10.4 ± 1.9$^{ab}$</td>
<td>4.93</td>
<td>229240</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>129471 ± 154730</td>
<td>1157885</td>
<td>1252478</td>
<td>118961 ± 87877$^{ac}$</td>
</tr>
<tr>
<td>% Injected dose G$^{65}$Ni</td>
<td>2.50 ± 0.73$^b$</td>
<td>1.35</td>
<td>1.46</td>
<td>0.27 ± 0.31$^b$</td>
</tr>
</tbody>
</table>

(B) INCUBATED FRACTIONS (200 μg Ni L$^{-1}$)

<table>
<thead>
<tr>
<th>Kidney Sample Substance</th>
<th>10 minutes</th>
<th>1.5 h</th>
<th>3 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G$^{65}$Ni CPW (μg)</td>
<td>Total Protein (mg)</td>
<td>CPW/mg (protein)</td>
<td>G$^{65}$Ni CPW (μg)</td>
</tr>
<tr>
<td>Homogenate</td>
<td>3931553</td>
<td>213</td>
<td>18458</td>
<td>5695113</td>
</tr>
<tr>
<td>Brushborder/ Bandolateral</td>
<td>120542</td>
<td>3.02</td>
<td>41961</td>
<td>320816</td>
</tr>
<tr>
<td>Gynosol</td>
<td>2221605</td>
<td>92.8</td>
<td>23941</td>
<td>3204600</td>
</tr>
<tr>
<td>Mitochondria/ Lysosomes</td>
<td>742317</td>
<td>55.6</td>
<td>13351</td>
<td>1435004</td>
</tr>
<tr>
<td>Nuclear</td>
<td>30850</td>
<td>27.7</td>
<td>9546</td>
<td>976868</td>
</tr>
<tr>
<td>Microsomes</td>
<td>424618</td>
<td>15.8</td>
<td>25875</td>
<td>707459</td>
</tr>
<tr>
<td>%</td>
<td>98.6</td>
<td>98.6</td>
<td>113.9</td>
<td>103.2</td>
</tr>
<tr>
<td>% Injected dose G$^{65}$Ni</td>
<td>6.67 ± 0.44$^a$</td>
<td>9.74</td>
<td>7.60</td>
<td>1.25 ± 0.21$^{ab}$</td>
</tr>
<tr>
<td>Sera CPW/μL</td>
<td>65065 ± 65012</td>
<td>315153</td>
<td>20651</td>
<td>0.36 ± 0.11$^{ab}$</td>
</tr>
<tr>
<td>% Injected dose G$^{65}$Ni</td>
<td>10.4 ± 1.9$^{ab}$</td>
<td>4.93</td>
<td>229240</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>129471 ± 154730</td>
<td>1157885</td>
<td>1252478</td>
<td>118961 ± 87877$^{ac}$</td>
</tr>
<tr>
<td>% Injected dose G$^{65}$Ni</td>
<td>2.50 ± 0.73$^b$</td>
<td>1.35</td>
<td>1.46</td>
<td>0.27 ± 0.31$^b$</td>
</tr>
</tbody>
</table>

---

a The mean ± SD of two studies (2 rats per study).

b The volume of whole blood was estimated to be 10 mL and serum volume 7.5 mL for a 250 g rat.

c Median 1449298 CPW; Range 68163 to 220433 CPW.
Table 4.7

CYTOSOL PROFILE

Molecular-Mass Profile of Kidney Cell Cytosol Determined by Sephadex G-75 Column Chromatography

<table>
<thead>
<tr>
<th>Time (h) After i.v. Injection</th>
<th>Relative $^{63}$Ni Radioactivity$^a$ of Peak Maximally Located in Fractions:</th>
<th>Percent of Nickel in Low-Molecular-Mass Component</th>
<th>Ratio$^a$ of $^{63}$Ni Radioactivity in Cytosol Fractions $34/15^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{15b}$</td>
<td>$^{34b}$</td>
<td></td>
</tr>
<tr>
<td>0.17</td>
<td>1.0</td>
<td>5.0</td>
<td>83</td>
</tr>
<tr>
<td>1.5</td>
<td>1.2</td>
<td>7.3</td>
<td>86</td>
</tr>
<tr>
<td>3.0</td>
<td>1.1</td>
<td>7.1</td>
<td>87</td>
</tr>
<tr>
<td>24</td>
<td>0.22</td>
<td>0.56</td>
<td>72</td>
</tr>
</tbody>
</table>

$^a$ Measured by the area under the curves of the peaks located maximally at fractions 15 and 34 (evaluated by a Hughes-Owens polar planimeter).

$^b$ High-molecular-mass component occurs maximally in fraction 15.

Low-molecular-mass component occurs maximally in fraction 34.
Figure 4.8. (A) Time course of $^{63}$Ni in subcellular fractions after i.v. injection of $^{63}$Ni(His)$_2$ (6 $\mu$g Ni kg$^{-1}$).

(B) Distribution of $^{63}$Ni in subcellular fractions incubated for 2-h (25°C) with $^{63}$Ni$^{2+}$ or $^{63}$Ni(His)$_2$ (200 $\mu$g Ni L$^{-1}$).
Figure 4.9. G-75 Molecular-mass profiles of kidney-cell cytosol and authentic $^{63}\text{Ni(His)}_2$.

(A-D) Molecular-mass profiles of the kidney-cell cytosol that was passed through a G-75 column.

(E) Profile of the $^{63}\text{Ni(His)}_2$ complex on the G-75 column.

Standards: A 2,000,000 (blue dextran); B 14,000 (ribonuclease); C 7,000 (Cd-thionein); D 5000 (insulin); E 1,400 (vitamin B-12); F 365 (adenosine monophosphate).

(A) 10 MIN POST INJECTION

![Graph showing molecular-mass profiles](image-url)
(B) 1.5 h POST INJECTION

(C) 3 h POST INJECTION
(D) 24 h POST INJECTION

(D) 24 h POST INJECTION

(CYTOSOL)

(FRACTION No.)

(63Ni) CYTOSOL

(FRACTION No.)

(E) 63Ni(HIS)2

(FRACTION No.)

(63Ni(HIS)2)
the kidney of the injected dose (c.f. data in Tables 4.6 and 4.7). Thus as $^{63}$Ni accumulates in the kidney, the low/high ratio of components in the cytosol increases. Subsequently, as the percent retention of injected dose decreases (3 h and 24 h), the low/high ratio also decreases. The $^{63}$Ni/mg(protein) ratio indicates that all kidney fractions follow the same trend as the percent retention in the kidney of the injected dose.

Table 4.8 illustrates the distribution of injected dose of $^{63}$Ni(His)$_2$ in rat tissues and body fluids. At 3 min after injection, 2.45% of the label is present in the bladder urine; after 10 min it has increased to 13.4% and decreased to 0.31% after 24 h. The 24-h urine contained 88% of the injected dose illustrating the rapid clearance of Ni(His)$_2$. The tissue with the largest percent of injected dose for both the 10-min and 24-h time points is the kidney, with the brain being the lowest. Of all the tissues investigated at the 24-h time point, 93.4% of the injected dose is accounted for. This percent does not include the intestine or muscle. When this data is reported in terms of ng nickel g$^{-1}$ tissue (Table 4.9), it is evident that the same accumulation order is present as in Table 4.8 with the kidney having the highest nickel content and the brain the lowest. The excised brains of both rats investigated at 24-h time point portrayed a subarachnoid haemorrhage. The significance of this observation is unclear at this time.

The autoradiography data for kidney tissue corresponding
### Table 4.8
PERCENT OF INJECTED DOSE IN TISSUES AND BODY FLUIDS
FOLLOWING A 6 μg Ni kg⁻¹ IV INJECTION OF ⁶⁵Ni(His)₂

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Percent Injected Dose</th>
<th>Percent Reduction 10 min → 24-h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.8 ± 8.6</td>
<td>10.4 ± 1.9</td>
</tr>
<tr>
<td>Urine (Bladder)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.45</td>
<td>13.3</td>
</tr>
<tr>
<td>B</td>
<td>13.4 ±0.1</td>
<td>0.43</td>
</tr>
<tr>
<td>Urine (24-h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>6.67 ± 0.41</td>
</tr>
<tr>
<td>Liver</td>
<td>2.50 ± 0.73</td>
<td>1.27 ± 0.21</td>
</tr>
<tr>
<td>Testes</td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>0.74</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td>Thigh Muscle</td>
<td></td>
<td>1.92</td>
</tr>
<tr>
<td>Faeces</td>
<td></td>
<td>3.04 ± 2.65</td>
</tr>
</tbody>
</table>

---

**a**Volume of serum for a 250 g rat was taken to be 7.5 mL.

**b**Bladder was empty.

**c**Expressed as ng Ni g⁻¹ tissue.
Table 4.9

TISSUE CONCENTRATION OF $^{63}\text{Ni}$ IN THE RAT 10 min AND 24-h FOLLOWING A 6 $\mu\text{g Ni kg}^{-1}$ IV INJECTION OF $^{63}\text{Ni(His)}_2$

<table>
<thead>
<tr>
<th>Tissue</th>
<th>10 min (ng Ni g$^{-1}$ tissue)</th>
<th>24-h (ng Ni g$^{-1}$ tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>66</td>
<td>12.6</td>
</tr>
<tr>
<td>Lung</td>
<td>8.3</td>
<td>0.81</td>
</tr>
<tr>
<td>Liver</td>
<td>7.5</td>
<td>0.45</td>
</tr>
<tr>
<td>Heart</td>
<td>6.8</td>
<td>0.40</td>
</tr>
<tr>
<td>Intestine</td>
<td>4.4</td>
<td>0.34</td>
</tr>
<tr>
<td>(Small)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>3.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Testes</td>
<td>2.4</td>
<td>0.31</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.9</td>
<td>0.90</td>
</tr>
<tr>
<td>(thigh)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.39</td>
<td>0.11</td>
</tr>
</tbody>
</table>
to the time-course study is in agreement with the subcellular fractionation study. Figure 4.10 illustrates typical grains observed and masking used in the autoradiographic studies of various areas of the kidney. The amount of nickel in the cortex for the four time periods in both studies is almost identical (compare data in Table 4.10). At 10 min, nickel is present in the S3 segment of the proximal tubule, while relatively little is present in the other portions of the nephron investigated (Fig. 4.11). With time (1.5 and 3h), it is also present in the glomerulus and in the other segments of the proximal tubule, the distal convoluted tubule and the collecting duct. After 24 h, nickel appears to be present only in the S3 segment of the proximal tubule.

Histopathological examination of the kidneys after the 6 μg Ni/kg dose showed no evidence of a toxic response in any segments of the nephron.

The incubation of $^{63}$Ni and $^{63}$Ni(His)$_2$ for 2 h with subcellular fractions indicates that they both have a high affinity for the mitochondria/lysosome fraction. More $^{63}$Ni(His)$_2$ is associated with both the nuclear and microsomal fractions than $^{63}$Ni (Fig. 4.8b). Both the results for the incubated subcellular fractions and the iv injection study show a large affinity for the mitochondria/lysosome fractions (Table 4.13).

(v) CATION COLUMN CHROMATOGRAPHY

The Ni(His)$_2$ (pH = 8) complex did not have high affinity
Figure 4.10. Autoradiography of kidney sections, after $^{63}\text{Ni(His)}_2$ i.v. injection, illustrating structure and grains developed. The regions outlined represent examples of the masking of specific nephron segments identified and selected for counting on the Quantimet 920 image analyzer.

(A) Outer Cortex X570.
(S1 segment of the proximal convoluted tubule)
(B) Inner Cortex X570.

(C) Inner Cortex/Medulla Junction X570.
Figure 4.11. Time-course distribution of nickel along the nephron identified by $^{63}\text{Ni}$ autoradiography and quantified by image analysis of grains using the Quantimet 920 analyzer. "Grains/$\mu^2$" represents the ratio (total area of grains)/(total area of nephron segment) selected for 30-40 samplings of each specific segment.
<table>
<thead>
<tr>
<th>Anatomical Region</th>
<th>10 minutes</th>
<th>1.5 h</th>
<th>2 h</th>
<th>3 h</th>
<th>24 h</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer Cortex</td>
<td>5777</td>
<td>11905</td>
<td>17333</td>
<td>12330</td>
<td>46.8</td>
<td>5368</td>
</tr>
<tr>
<td>Inner Cortex</td>
<td>17096</td>
<td>10996</td>
<td>13353</td>
<td>9060</td>
<td>31.1</td>
<td>9317</td>
</tr>
<tr>
<td>Medulla</td>
<td>15381</td>
<td>10996</td>
<td>13353</td>
<td>9000</td>
<td>31.1</td>
<td>9317</td>
</tr>
<tr>
<td>Total</td>
<td>5777</td>
<td>11905</td>
<td>17333</td>
<td>12330</td>
<td>46.8</td>
<td>5368</td>
</tr>
</tbody>
</table>

- Data from Table 4.10

- Counts = 0/20 frames
- Total area = 18,000 μ²
- Control counts = 4567; Control area = 18,000 μ².
for the cation column. This indicates that this complex has either a very low positive or a negative charge. By contrast Ni(His)\(_1\) (pH = 5) complex had extremely high affinity for the cation column as indicated by the need to use strontium nitrate solution to remove this complex. This indicated that the Ni(His)\(_1\) complex is positively charged.

**DISCUSSION**

(i) **NiCl\(_2\)** **INJECTION STUDY**

(a) **Post-Injection Status**

Both the high (6 mg kg\(^{-1}\)) and intermediate (3 mg kg\(^{-1}\)) i.p. doses resulted in the rats becoming extremely relaxed and lethargic with dilated blood vessels in their ears about \(\pm 5\) to 8 min post injection. Although the main thrust of this chapter is to examine the renal toxicity of nickel, these observations suggest a rapid, shortlived (15 min - 2.5 h) pharmacological effect the implication will be briefly considered. Nickel has been associated with cardiotoxicity in two major ways. Injection of Ni\(^{2+}\) has been shown to induce *in vivo* coronary vasoconstriction in dog heart and in perfused isolated hearts (Rubanyi *et al.*, 1981). Furthermore, hypernickelemia has been observed in 50 to 75% of patients with acute myocardial infarction or unstable angina pectoris (Leach *et al.*, 1985). As noted by Nieboer *et al.* (1987a) the mechanisms and sources of nickel released
into the serum for the above cardiac cases is not established. It is obvious that the direct action of Ni\textsuperscript{2+}, such as in the dog studies, may alter normal haemodynamic function. Another possibility is that the smooth muscle of the blood vessels could respond in a similar manner to that of the isolated uterine strips described by Rubanyi and Balogh (1982) were low concentrations (10\textsuperscript{-7} to 10\textsuperscript{-5} M) of NiCl\textsubscript{2} increased the basal tone of uterine strips isolated from 20-day-pregnant Wistar rats, while high concentrations (10\textsuperscript{-4} to 10\textsuperscript{-3} M) of NiCl\textsubscript{2} depressed spontaneous contractions and decreased basal tone. Large concentrations of Ni\textsuperscript{2+} in the blood might thus result in depressed "spontaneous" contractions causing the dilation of the blood vessels resulting in the redness observed in the ears. If this dilation of blood vessels occurs throughout the vascular system, then it could result in at least a short-term lowering of blood pressure. Lethargy and weakness are common complaints of patients who suffer from chronic hypotension (Engelman and Braunwald, 1977), which agree with the observed reactions of the rats in the present study.

The transient nature of these post-injection observations is consistent with the nickel levels in the serum being rapidly lowered with concurrent rapid urinary excretion (Table 4.8; 3- and 10-min time points). This rapid removal of nickel from the serum would result in the levels of nickel being lowered, allowing the cardiovascular system and blood pressure to normalise and recovery after 15 min to
The above observations may be due to the interaction of \( \text{Ni}^{2+} \) and \( \text{Ca}^{2+} \) ions. As already indicated, there are a variety of agonistic/antagonistic interactions that \( \text{Ni}^{2+} \) can have with \( \text{Ca}^{2+} \) (see Table 1.1). This illustrates that the mode of action causing the observed effects may be complex. Additional \textit{in vivo} and \textit{in vitro} studies are required to substantiate and quantify in a systematic manner the way in which these stimulatory and inhibitory effects are consistent with laboratory behavioral observations.

(b) Relationship between Physiological Function and Structural Changes Induced by Nickel

The most obvious change in the liquid balance is that the volume of urine produced is higher than the controls for intermediate and high doses \((p<0.05)\), while the volume of water consumed is only slightly increased in the second 24-h period. By contrast, the low-dosed rats have a urine volume lower than controls with a corresponding drop in the volume of water consumed \((\text{not significant } p>0.1)\). As illustrated in the histological study, nickel appears to have a major effect on the glomeruli of the nephron for which a dose response decrease in the Bowman's space was observed. Although it is known that fixation of kidney tissue with formalin may cause glomerular swelling \(\text{(personal communication, Dr. C. Powell, Robens Institute)}\), the effect observed in this study was dose-dependent and was not observed in control tissues.
Consequently, it is concluded that the observed changes in the glomerulus were primarily due to nickel and not to the type of fixation. The discussion that follows will first focus on possible changes in the glomeruli in relation to diuresis and proteinuria before considering alternate interpretations.

Although it was not possible to obtain histological sections of the low-dosed rats, it seems reasonable that the effects of nickel would have been less, namely larger Bowman's space than the intermediate dose. This is substantiated by the fact that there was no observable change in the Bowman's space in the 6 μg kg⁻¹ i.v. injection (autoradiography) study described in Section C (iv) of this Chapter. It is well established that there is a dose-response relationship between total protein appearing in rat urine and injection of Ni²⁺ (Gitlitz et al., 1975 and Sunderman et al., 1976b). Gitlitz et al. (1975) found a dose response between proteinuria and single i.p. injections of NiCl₂ between 2 and 5 mg Ni²⁺ kg⁻¹ (Table 4.11). Therefore, there appears to be a similar dose-response effect between the dose of Ni²⁺ and the amount of protein excreted and the decrease in Bowman's space.

For each nephron there is a feedback mechanism operating
Table 4.11
EFFECT OF PARENTERAL NiCl₂ UPON EXCRETION OF TOTAL PROTEIN AND AMINO ACIDS

<table>
<thead>
<tr>
<th>Measured Parameter</th>
<th>Percent Change of Total Protein and Amino Acids in Rat Urine a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0 mg Ni kg⁻¹ as NiCl₂ Day 1</td>
</tr>
<tr>
<td></td>
<td>5.0 mg Ni kg⁻¹ as NiCl₂ Day 1</td>
</tr>
<tr>
<td></td>
<td>5.0 mg Ni kg⁻¹ as NiCl₂ Day 2</td>
</tr>
<tr>
<td>Total Protein</td>
<td>26</td>
</tr>
<tr>
<td>Neutral α-Amino Acids</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>0</td>
</tr>
<tr>
<td>Alanine</td>
<td>0</td>
</tr>
<tr>
<td>Valine</td>
<td>35</td>
</tr>
<tr>
<td>Leucine</td>
<td>50</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>29</td>
</tr>
<tr>
<td>Serine</td>
<td>9</td>
</tr>
<tr>
<td>Threonine</td>
<td>2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>-10</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>21</td>
</tr>
<tr>
<td>Methionine</td>
<td>-10</td>
</tr>
<tr>
<td>Cystine</td>
<td>5</td>
</tr>
<tr>
<td>Acidic α-Amino Acids and Amides</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0</td>
</tr>
<tr>
<td>Asparagine</td>
<td>-6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>18</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0</td>
</tr>
<tr>
<td>Basic α-Amino Acids</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>120</td>
</tr>
<tr>
<td>Arginine</td>
<td>12</td>
</tr>
<tr>
<td>Lysine</td>
<td>14.5</td>
</tr>
<tr>
<td>Ornithine</td>
<td>5</td>
</tr>
<tr>
<td>Substituted and Miscellaneous Amino Acids</td>
<td></td>
</tr>
<tr>
<td>β-Alanine</td>
<td>-5</td>
</tr>
<tr>
<td>γ-Aminobutyric</td>
<td>11</td>
</tr>
<tr>
<td>1-Methylhistidine</td>
<td>-33</td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>31</td>
</tr>
<tr>
<td>Sarcosine (N-methylglycine)</td>
<td>11</td>
</tr>
<tr>
<td>Taurine</td>
<td>-21</td>
</tr>
</tbody>
</table>

a The percent change, based on 24-h urinary excretion rates (mg/kg/d, protein: μmol/kg/d, amino acids) was calculated from the data reported by Gitlitz et al. 1975.
between the macula densa segment of the distal tubule and the afferent arteriole of the corresponding glomerulus (Bell and Navar, 1982). The latter component participates in the regulation of the glomerular filtration rate through the macula densa cells responding to flow-related alterations in the tubular fluid. By affecting changes in the glomerular filtration rate, they help control fluid flow along the nephron and thus may have an effect on the volume of urine produced. Bell and Navar (1982) found, by retrograde microperfusion studies in rats, that "cytoplasmic calcium within the receptor cells may participate in the transmission of feedback signals to the contractile cells". If low doses of Ni$^{2+}$ are able to stimulate the feedback mechanism to the afferent arteriole, then there will be a reduction in the glomerular filtration rate which, if dramatic enough, would result in a measurable reduction in the volume of urine produced.

If high doses of Ni$^{2+}$ have the opposite effect on the macula densa to low doses (i.e. similar to that observed by Rubanyi and Balogh (1982) for uterine contraction), then the feedback mechanism between the macula densa and the afferent arteriole would be reduced or may not respond normally to tubular flow fluctuations. This could result in a larger flow of blood through the glomerulus and in a higher glomerular capillary blood pressure, forcing effective filtration pressure and glomerular filtration rates to increase. The result would be an increase in the urine
volume. If this change in pressure were large enough it would cause a swelling of the surrounding tissue (i.e., edema) and perhaps a reduction in the Bowman's space.

As background for a discussion of tissue swelling in relation to proteinuria a brief description of the filtration barrier is presented. The glomerular filtration barrier in the glomerulus is composed of "thin endothelial cells, with large open pores, the hydrated gel of the basement membrane with its dense network of long fibrillar particles of proteoglycans and narrow slits between the pediciles of the podocytes, which are bridged by the slit diaphragm. These structures are covered by strongly negatively charged cell coats on both the vascular side and the urinary side of the filter. Additionally, negative charges are present within the layers of the basement membrane, especially in both laminae rarae" (Kriz and Kaissling, 1985). The filter has been shown to select macromolecules by their charge, size and configuration under normal flow conditions (Kriz and Kaissling, 1985). If the observed swelling causes a change in filtration characteristics (size and configuration) and a reduction in the charge of the basement membrane by interaction with Ni²⁺ as noted by Templeton (1987a, b and c) in in vitro studies with isolated basement membranes, then there will also be an increase in the amount of high-molecular-mass proteins being filtered. If the tubular maximum for their reabsorption is exceeded, then proteinuria would result. Thus structural changes observed in the
histological study can explain both the increased volume of urine and the increased proteinuria observed by Sunderman and Horak (1981). Section (iv)a of this discussion includes an explanation of the decrease in Bowman's space caused by glomerular damage.

The above discussion has been centered on the swelling of the glomerular tuft. However, it should be noted that there could also be a change in the tissue surrounding the Bowman's capsule. For instance, if there was a swelling in the proximal or distal tubules and their surrounding tissue due to interaction with Ni$^{2+}$, then this could result in a decrease in the Bowman's space surrounding the glomerular tuft without a large change in the tuft tissue. If this postulated change in the proximal tubules occurs, then it is also possible that there is concomitant interference with the reabsorption of protein which in turn would lead to proteinuria.

It is also feasible to get diuresis by alterations in function along the nephron that are not dependent on the swelling of the glomeruli. For instance, in the proximal convoluted tubule it is known that Na$^{+}$ reabsorption is coupled to the reabsorption of glucose, amino acids and some organic anions (e.g. lactate and citrate)(Jones, 1985). Water is known to pass through the proximal tubulular epithelium by osmosis so rapidly that the osmolar concentration of solutes on the interstitial side is almost the same as the intratubular side. Therefore if there is an
interruption in Na\(^+\) or amino acid reabsorption, then it would cause less water to be reabsorbed resulting in diuresis. Although there is no direct evidence that Ni\(^{2+}\) interferes with Na\(^+\) reabsorption in the proximal tubule, it is known that it causes amino aciduria (Gitlitz et al., 1975; Sunderman et al., 1976b; and Mathur and Tandon, 1981). Two of these studies (Gitlitz et al., 1975; Mathur and Tandon, 1981) indicate that there is a concurrent increase in concentration of most amino acids measured in the plasma. Thus it seems reasonable to speculate that nickel interferes with amino acid metabolism which may alter the reabsorption processes in the proximal tubule in a manner that produces amino aciduria and diuresis.

Proteinuria can also be caused by defects in the proximal tubule via leakage from damaged cells. This is presumably the cause of increased excretion of N-acetyl-\(\beta\)-D-glucosaminidase (NAG) found in rat urine by Sunderman and Horak (1981) on the second day after i.p. injection of NiCl\(_2\) (6 mg Ni kg\(^{-1}\)). NAG is an enzyme that is associated with lysosomes and hence would presumably require cellular damage to cause large amounts of it to appear in the urine. This is consistent with the observed damage such as dilated tubules, loss of brush border and flattened epithelia.

The above discussion illustrates that the mode of action of Ni\(^{2+}\) in the kidney may be very complicated and that further experimental studies are required before the validity
of the various theories presented above can be tested.

(ii) UPTAKE OF NICKEL IN RENAL SLICES

(a) Comments on Experimental Procedures

A brief review of the literature has shown that there is no "ideal" composition of incubation media to be employed in studies with tissue slices. The final composition of the HEPES incubation medium used here was based on the Tyrodes buffer solution which had been altered to maximize PAH accumulation (Cross and Taggart, 1950; Foulkes and Miller; 1959; Chung et al., 1970). The concentration of HEPES buffer used was 100mM as recommended by Perrin and Dempsey (1974).

The concentration of PAH used in various studies has ranged from 67 µM (Chung et al., 1970) to 0.25 mM (Ganote et al., 1975). The concentration selected for the present work was that recommended by Ganote et al., (1975). Probenecid (1x10⁻⁴ M) is known to inhibit the uptake of PAH in renal dog slices (Berndt, 1976). Daley-Yates and McBrien (1982) have shown by isolated perfused rat kidney studies that probenecid (0.3 and 3.0 mM) increased the clearance of cisplatin (cis-dichlorodiammine platinum(II)) to approximately twice the GFR (i.e., inulin clearance). This indicated that probenecid inhibited the reabsorption of cisplatin. Because of this action of probenecid on a metal complex, it was chosen to investigate the transport mechanism of Ni^{2+} and its histidine complexes.
The incubation time for all the nickel uptake studies was chosen to be 2 h because this allowed adequate time for uptake and inhibition of PAH (see Fig 4.4) and the attainment of nearly the maximum s/m ratio for the uptake of Ni²⁺ (see Fig. 4.5). This incubation time is at the upper end of the values (90 to 120 min) usually reported in the literature (Berndt, 1987). All solutions were incubated at 25°C, since this temperature seemed to provide better tissue survival than 37°C. This discrepancy may result from cellular anoxia due to the combination of increased oxygen consumption at the higher temperature and a lower oxygen tension (Cross and Taggart, 1950). The oxygenation of the incubation medium is usually done at 100% O₂ or 95% O₂/5% CO₂ (Berndt, 1987). Because of the possible interaction of CO₂ as CO₂⁻ or HCO⁻³ with Ni²⁺ (or its complexes), it was decided to oxygenate all media only prior to incubation; just one run was done under an atmosphere of 95% O₂/5% CO₂. The adopted protocol also resulted in a minimum change in the pH of the buffer, ensuring that the species distribution of Ni(II)-histidine complexes did not change significantly during incubation. No runs were carried out with 95% O₂/5% CO₂ being bubbled constantly through the incubation medium.

Many of the published uptake studies involving renal tissue slices have examined the cortex of the kidney only. The tissue sampling procedure adopted in the current work (see Materials and Methods) assured that uptake was investigated for a cross-section of the kidney rather than a
single component. Thus renal slices of rats were used to study uptake (i.e., total uptake by the cortex, medulla, proximal convoluted tubule and distal convoluted tubule) in a manner that closely resembled that of in vivo studies.

(b) Interpretation of Results

Functional integrity of anion-transport processes in kidney slices was confirmed by the observed 30% inhibition of PAH uptake by probenecid (Table 4.4). All the s/m ratios for the uptake of PAH in this study were appreciably larger than unity. This has been interpreted to indicate active transport (a s/m value of $\leq 1.0$ is usually interpreted to mean that the compound in the tissue got there by passive means). Similarly, the s/m ratios of $\text{Ni}^{2+}$, $\text{Ni}^{2+} (\text{His})_2$ and $\text{Ni}^{2+} (\text{His})_2^+$ in live tissue exceeded unity considerably. In fact, they were all substantially above the s/m value for PAH (Table 4.4). However for the uptake of nickel and its complexes in dead tissue (ethanol-treated and boiling), the s/m was also above that found for live tissue. With slices incubated in medium poisoned with dinitrophenol (DNP), the uptake of nickel and its complexes was much lower than the corresponding live tissue (Table 4.4). The classical interpretation of the DNP results is that they indicate an active component to the uptake of nickel or its complexes, which probably depends on the uncoupling of oxidative phosphorylation (White et al., 1973). This inhibition causes ATP formation to be reduced or abolished on which active ion
transport and also the tubular secretory mechanisms depend. This is consistent with the PAH results in this study for boiled ethanol- and DNP-treated medium being lower than those for the uptake of PAH inhibited by probenecid (Table 4.4).

The high s/m of Ni$^{2+}$, Ni(His)$_2$ and Ni(His)$_1$ for both live and dead renal slices appear to require another interpretation. Nieboer et al. (1984c) and Menon and Nieboer (1986) have investigated the uptake of Ni$^{2+}$ by human erythrocytes and peripheral mononuclear leukocytes (mostly lymphocytes) and cultured cells. The presence of lipophilic ligands such as DDC and APDC enhanced the cellular association of Ni$^{2+}$, while more polar ligands such as L-histidine, D-penicillamine (D-PEN) and ethylenediaminetetra-acetic acid (disodium salt) (EDTA) prevented uptake. Their observations were interpreted in terms of the "Equilibrium" model of metal-ion uptake by cells originally proposed by Williams (1981). In this model, no active membrane processes are required for metal-ion uptake under steady-state conditions (i.e., at fixed pH, redox potential, intracellular and extracellular ligand concentrations). "The distribution of metal ions among the various compartments of a cell is then determined by thermodynamic parameters such as the pK$_a$ values of ligands, the binding constants of the metal-ligand complexes, their solubilities in aqueous and lipid phases (i.e., distribution coefficients), and the effective ligand concentrations in the various compartments" (Nieboer et al., 1987a). This
perspective suggests that the uptake of Ni$^{2+}$ is most probably passive.

The uptake of Ni$^{2+}$ by boiled, ethanol- or DNP-treated tissues corresponds to nonsteady-state conditions, because internal pH, redox potentials and intracellular ligand concentrations are not maintained and cell membranes are disrupted. The intracellular proteins and enzymes will be denatured and perhaps organelles are changed by the above treatments. This may be expected to make available many more binding sites than normally found in living tissue. This phenomenon is known for metal-ion uptake in plant tissues (Boileau et al., 1985). As shown in the intravenous time-course study, there is rapid accumulation of nickel in the kidney (Tables 4.6 and 4.8; Figure 4.8) where in the cytosol it is probably present as the Ni(His)$_2$ complex. Therefore, it is not surprising that Ni$^{2+}$, Ni(His)$_2^+$ and Ni(His)$_1^{+}$ are able to cross cell membranes in live and dead renal-slices. The large s/m ratio for the dead tissue is consistent with the postulate of additional extracellular and intracellular sites to which nickel can bind. Similarly in live tissue, accumulation of nickel with high s/m ratio can be interpreted to reflect compartmentalization under steady-state conditions and not due to active transport. This hypothesis is supported by the efflux of PAH, Ni$^{2+}$ and Ni(His)$_2$ from live and ethanol-treated tissues. The PAH has a very short half-life when compared to Ni$^{2+}$ and Ni(His)$_2$, which implies that there is a small amount of intracellular
binding for PAH (live and ethanol-treated). Effective intracellular binding for Ni\(^{2+}\) and Ni(His)\(_2\) would result in much slower release from both live and ethanol-treated tissues. The distribution of nickel for the in vitro incubated subcellular fractions and in vivo i.v. injection study (Table 4.12a and b; Figure 4.8a and b) in a sense illustrate the "Equilibrium" model. The higher percent of nickel associated with the mitochondrial/lysosomes (M/L) and nuclear (N) fractions in vitro is an indication of the type and number of binding sites available intracellularly when uptake is not restricted by cellular membranes. The passive nature of cellular uptake of Ni\(^{2+}\) has been illustrated in a number of systems. As noted by Nieboer et al. (1987a), the temperature-dependence of Ni\(^{2+}\) accumulation by Chinese hamster ovary cells (CHO) is indicative of a passive (diffusion) process because the estimate of the temperature coefficient \(Q_{10}\) is near 1.0. Passive uptake has also been demonstrated by in situ perfusion of rat jejunum by Foulkes and McMullen (1986). There was a biphasic uptake of Ni\(^{2+}\), with the second step (uptake from mucosa) being passive in nature with ion flow occurring in both directions.

Low s/m values observed in DNP-treated renal slices such as for PAH and Ni\(^{2+}\) is usually interpreted as an indication of active transport. A similar effect has also been found in the accumulation of Ni\(^{2+}\) into cultured pneumocytes, which was reduced by metabolic inhibitors (\(\text{NaN}_3\), NaCN, NaAsO\(_2\)) after incubation at 37°C in Tyrodes (pH = 7.4) medium (Saito and
Table 4.12

DISTRIBUTION OF $^{63}$Ni DETERMINED BY SCINTILLATION SPECTROMETRY ON SUBCELLULAR FRACTIONS AFTER IV INJECTION OR in vitro INCUBATION OF SUBCELLULAR FRACTIONS

<table>
<thead>
<tr>
<th>Fraction</th>
<th>A: iv Injection (6 µg Ni kg$^{-1}$) as $^{63}$Ni(His)$_2$</th>
<th>B: Incubated Fractions (200 µg L$^{-1}$ $^{63}$Ni(His)$_2$ and $^{63}$Ni$^{2+}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>1.5 h</td>
</tr>
<tr>
<td>$P_3$</td>
<td>4901</td>
<td>77839</td>
</tr>
<tr>
<td>Mitochondria/</td>
<td>13351</td>
<td>37692</td>
</tr>
<tr>
<td>Lysosomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear</td>
<td>9546</td>
<td>26873</td>
</tr>
<tr>
<td>Microsomes</td>
<td>26875</td>
<td>58333</td>
</tr>
</tbody>
</table>

$^a$CPM mg$^{-1}$ protein in each fraction.
Menzel, 1986). However, treatment of cells as above would result in loss of the steady-state "equilibrium" condition which could result in a decrease in the uptake of Ni$^{2+}$ and in preferential intracellular compartmentalization. For example, cytoplasmic ligands may be lost to the incubation medium where they can complex with Ni$^{2+}$.

The change in s/m for renal slices incubated with and without a 95% O$_2$/5% CO$_2$ (v/v) atmosphere (Fig. 4.6) is statistically significant (p<0.01) for PAH, while for Ni$^{2+}$ and Ni(His)$_2$ there was very little dependence. This illustrates again that nickel and PAH have very different transport mechanisms in the nephron. PAH is very dependent on O$_2$ whereas Ni$^{2+}$ and Ni(His)$_2$ are not. O$_2$-independence points to passive uptake for the latter.

(iii) UPTAKE OF NICKEL WITH ISOLATED PROXIMAL TUBULES

The uptake of PAH in proximal tubule fragments is consistent with the renal-tissue slice work in that it is inhibited by probenecid in both instances. This observation indicates that the tissue fragment procedure results in viable tissue and provides a useful tool to probe site-specific absorption along the nephron. For example, it facilitates the investigation of effects that may occur only in a specific segment (e.g., S3) of the proximal convoluted tubule, which would be masked when a cross-sectional slice is used.

As pointed out by Davies (1973), there are upper limits to both passive and active transport across cell membranes.
If both the 200 $\mu g \ L^{-1}$ and 400 $\mu g \ L^{-1}$ Ni(His)$_2$ are above this upper limit for reabsorption of Ni(His)$_2$ in the proximal tubule fragments, then a time-dependent but not a concentration-dependent uptake would be expected. Further work in this area should be done with a wider range of nickel concentrations with particular attention to concentrations below 200 $\mu g \ L^{-1}$ to ascertain if the values chosen in this study were above the saturation level.

Histidine incorporation into proximal tubule fragments appears to be severely inhibited by Ni(His)$_2$ concentrations from 50 to 200 $\mu g \ L^{-1}$. If this occurs in vivo, it would result in large amounts of histidine appearing in the urine. This is exactly what has occurred in rat studies by Gitlitz et al. (1975) and Horak and Sunderman (1980). Gitlitz et al. (1975) investigated the excretion of total protein, neutral, acidic and basic $\alpha$-amino acids after a single i.p. injection of NiCl$_2$ in dosages from 2 to 5 mg Ni kg$^{-1}$. When the data in the Gitlitz et al. (1975) study is recalculated to express the percent change (Table 4.11), then it is obvious that histidine is the only amino acid for which the excretion increases in the first day, followed by a decrease on Day 2. This amino aciduria is accompanied by a mean decrease in the histidine present in the plasma. Since the half-life of Ni$^{2+}$ in rats is approximately 24 h, and if its excretion is closely associated with the decrease in the reabsorption of histidine, then it is expected that histidine excretion would increase in the first 24-h period, as observed. This would be followed by a decrease in urinary histidine levels in the
second 24-h period when the levels of Ni$^{2+}$ being excreted drop, which is again seen. Interestingly, Horak and Sunderman (1980) have investigated the excretion of total protein and amino acids after inhalation of Ni(CO)$_4$ by rats. Again histidine is the only amino acid for which excretion increases during the first day post-exposure and decreases on the second day.

As summarized by Silbernagl (1985), there are six methods by which amino aciduria can occur. Only a number of these are relevant to the present argument. High levels of one amino acid may inhibit the reabsorption of other amino acids, possibly by competition or blocking the uptake site. The proximal tubule study indicates that the Ni(His)$_2$ complex inhibits the uptake of histidine. This may be due to the complex competing with, or blocking, the uptake site of histidine prompting its increased excretion. However, it is possible that there was ligand exchange in the incubation medium of $^3$H-His with the His in the Ni(His)$_2$ complex which would alter its uptake. Investigation of the cytosol for the presence of Ni($^3$H-His)$_2$ would have been useful in establishing if ligand exchange occurred. Alternatively, reduction of the Na$^+$ gradient could be responsible for the hyperexcretion of amino acids, glucose and phosphate and other compounds that are dependent on Na$^+$ co-transport. Thus if Ni$^{2+}$ is able to interfere with Na$^+$ reabsorption in the proximal tubule, possibly by ATP-ase inhibition or disruption of the brushborder membrane to cause leakiness of Na$^+$, then it would result in amino aciduria. As already indicated, the
excretion pattern of all amino acids is not the same following injection of NiCl₂. Histidine appears to be the only amino acid whose excretion pattern is in phase with Ni²⁺ excretion. Interestingly, the excretion is initially accompanied by elevated serum histidine levels compared to controls at 1 h post i.p. injection, being at the control level or below subsequently. Ni²⁺ appears to have a variety of effects on other amino acids. Valine has serum concentrations below controls for the entire 48-h period post i.p. injection of NiCl₂, while its excretion has increased 1050% on day one and 1700% on day two (Gitlitz et al., 1975). The serum concentration of aspartic acid remains relatively constant throughout the entire 48-h period with no change in the urine on both days following the NiCl₂ injection. Glutamine had serum concentrations below controls for the entire 48-h period, while its excretion increased 277% on day one and 423% on day two. It appears that Ni²⁺ has vastly different effects on amino acids and suggests that it acts by different inhibitory mechanisms of reabsorption to produce the reported amino aciduria patterns.

The present proximal-tubule fragment study also suggests that Ni(His)₂ inhibits the uptake of proline. However, the above-mentioned in vivo studies did not investigate the excretion pattern of this amino acid. Therefore it is not possible to comment on whether the observed in vitro phenomenon produces a change in the excretion pattern of this amino acid. The proximal tubule fragment work suggests that this technique can be used to investigate the different
mechanisms underlying the differential effect that Ni\(^{2+}\) or Ni(His)\(_2\) have on the uptake of amino acids in the proximal tubule.

In the present work with proximal tubules, the uptake of thymidine in proximal tubular fragments was not significantly inhibited by the presence of Ni(His)\(_2\). Hui and Sunderman (1980) have shown that in vivo incorporation of thymidine into liver and kidney DNA of rats was inhibited by i.v. injection of Ni(CO)\(_4\), while i.m. injection of NiCl\(_2\) did not significantly affect thymidine uptake into liver DNA, but did inhibit thymidine uptake into kidney DNA. These differential responses are likely related to the lipophilic character of Ni(CO)\(_4\) and organ-dependent metabolism of this compound.

(iv) **RENAL HANDLING OF IV ADMINISTERED Ni(His)\(_2\)**

(a) **Association of Nickel with Soluble Intracellular Components**

The cytosol from all experimental time points after i.v. injection of \(^{63}\)Ni(His)\(_2\) separated into high- (HMM) and low-molecular-mass (LMM) nickel-containing fractions on a Sepadex G-75 column (Fig. 4.9). The percent of nickel in the ultrafiltrable LMM fraction was between 72 and 87 percent, depending on the time after i.v. injection of Ni(His)\(_2\) (Table 4.7). In the past, there have been several studies that have obtained the same results with various renal subcellular studies of rats and mice (Sunderman et al., 1981; Sunderman et al., 1983; Herlant-Peers et al., 1983; Abdulwajid and
Sarkar, 1983). Although up to 80% of the nickel in the renal cytosol was in the low-molecular-mass form, the emphasis in the mentioned studies was directed to characterizing the macromolecular forms. They were carried out between 1 and 24 h after i.p, i.m. or i.v. injections of $^{63}\text{NiCl}_2$ or $^{63}\text{Ni(CO)}_4$. It appears therefore, that injection of the Ni(His)$_2^+$ complex does not induce a drastic change in the distribution pattern of nickel between HMM and LMM fractions of renal cytosol.

As mentioned in Chapter 1, nickel is transported in the serum via both HMM and LMM carriers. The HMM ligand is human albumin, and possibly a nickel metalloprotein which is believed to be an alpha-2-macroglobulin, also called nickeloplasmin (Sunderman, 1977; Nomoto, 1980; Scott and Bradwell, 1983; Nieboer et al. 1987a). The LMM component is believed to be a nickel(II)-amino acid complex by in vitro $^{63}\text{Ni}$-addition experiments (Lucassen and Sarkar, 1979). Oskarsson and Tjälve (1979a) in a study with mice found that most of the nickel was associated with the HMM component albumin and the remainder with a LMM component. After i.v. injection of NiCl$_2$ into the mice, the serum was separated according to molecular mass by passing it through a G-75 column. It is interesting to note that the LMM component found in the cytosol of both the lung and kidney cells had approximately the same elution pattern as the serum profile. These cytosol results are similar to the G-75 elution profiles (Fig. 4.9) of the renal cytosol found after i.v. injection of Ni(His)$_2^+$ and of pure Ni(His)$_2^+$ in the current
study. In Figure 4.9 it can be seen that the HMM component began eluting after fraction 10 which is approximately where the standard blue dextran (MM = 2,000,000) eluted, and stopped eluting at approximately fraction 21 which is where the standard Cd-thionein (MM = 7,000) appeared on the column. The LMM component had almost the same elution profile as the standard 5'-AMP (MM = 365) and $^{63}$Ni(His)$_2$. Interestingly, a recent computer simulation by Cole et al. (1985) predicts that plasma LMM complexes are (metal % in parentheses): Ni(His)$_2^0$ (50.6%), Ni(CYS)(His)$^-$ (23.9%), Ni(CYS)$_2^-$ (11.3%) and Ni(His)$_1^+$ (4.4%). Thus it seems reasonable to postulate that the LMM component found in the serum and cytosol of the current and Oskarsson and Tjalve studies was a Ni(II) amino acid complex, such as Ni(His)$_2$.

The renal cytosol studies by Sunderman et al. (1983) 1 h after i.m. injection of $^{63}$NiCl$_2$ separated into six fractions on using high-performance size-exclusion chromatography with TSK-GEL SW-2000 and SW-3000 columns. The largest component (fraction F) contained approximately 80% of the cytosolic $^{63}$Ni eluted near the permeation volume on the SW-2000 column and beyond the total permeation volume on the SW-3000. This made it impossible to make a reliable estimate of its MM, although from the elution pattern its MM can be predicted to be below 7,000 to 10,000. The remaining 20% divided into 5 components with molecular masses between ~10,000 and 168,000. In a previous study, 0.5 h to 1 h after i.v. injection of $^{63}$NiCl$_2$ Sunderman et al. (1981) found essentially the same MMs for the 5 HMM components, but found ~70% of the renal
cytosol associated with a LMM component of <2,000. The current work is consistent with the high-molecular-mass component being between approximately 7,000 and 168,000.

The renal cytosol does not have a large shift in the ratio of nickel associated with the LMM and HMM components until the 24-h time point where the percent of nickel in the LMM component has been lowered from a maximum of 87 to 72 (Table 4.7). This can be interpreted as either corresponding to an increase in the HMM fraction (compartmentalization) or a decrease in the LMM fraction (cellular clearance). The increase in HMM components may represent the association of nickel with cytosolic proteins such as those found by Sunderman et al. (1983). If transport across the cellular membrane of LMM cytosolic component such as Ni(His)$_2$, or Ni$^{2+}$ is by diffusion, nickel could be rapidly released across the epithelial-cell membrane to the tubular lumen as the nickel levels in the serum are lowered.

As pointed out by Abdulwajid and Sarkar (1983), many proteins with a similar MM can elute together under the peaks found in their own work and that of Sunderman et al. (1981, 1983). This depends on the nature of the proteins and may result in a change in the apparent MMs that have been cited. Recently, Templeton and Sarkar (1985) investigated the composition of the LMM nickel component of renal homogenate after centrifugation at 105,000g for 60 min (cytosol). In this fraction, they found two major components; one is consistent with a MM of 4000, the second was found in a region rich in carbohydrate. They also investigated bovine
kidney homogenates incubated with excess NiCl₂ (containing ⁶³Ni), which showed the same cytosolic G-75 and LMM pattern as the in vivo study. It is interesting that the UV/visible absorption spectra of this LMM component collected off a G-75 column has spectral characteristics very close to that obtained for the Ni(His)₂ complex at pH + 7.5 (λ_max = 626 nm; a₆₂₆ = 2.9 cm⁻¹ M⁻¹) prepared in the present study. As Templeton and Sarkar (1985) lowered the pH to 4.0, the appearance of the Ni(H₂O)⁶⁺²⁺ spectrum was observed. Similarly when the pH of the Ni(His)₂ complex was lowered to 5.5 (present work), spectrum assumed the characteristic features of the Ni(H₂O)⁶⁺²⁺ species. It is interesting to note that the G-75 profile of ⁶³Ni(His)₂ was the same as the LMM-species of rat cytosol. As already indicated (Section (iv) a; of this discussion), there are more components in the LMM fraction of plasma than just ⁶³Ni(His)₂. The G-75 column would not distinguish between such species because of similar MMs.

The levels of nickel injected into rats in both the above studies (Abdulwajid and Sarkar, 1983; Templeton and Sarkar, 1985) were ±20 times that used in the present fractionation/autoradiography work and that by Oskarsson and Tjalve (1979b). No abnormality was found in the glomerulus for the latter conditions. Since the HMM nickel complexes described by Sunderman and Sarkar have not been identified in the serum, they may result from the toxic interaction with tissue during glomerular filtration. The bulk of nickel was found to be associated with oligosaccharide fractions. The source of these oligosaccharides may be from damage to
cellular membranes such as the capillary endothelium, epithelial cell foot process or the basement membrane. If such damage occurs, it may also cause a change in the cell membrane permeability allowing accumulation of fluid inside these tissues resulting in a swelling of the glomerulus and a decrease in the Bowman's space. This model potentially provides an explanation of the delay in the glomerular damage noted in the fusion of the foot processes 48 h post injection and the observation that at 96 h after injection there was no evidence of this change (Gitlitz et al., 1975). The delay may be due to the slow buildup of fluid in the tissue that reaches a maximum \( \approx 48 \) h post-injection, after which the cell membrane is presumably repaired (or regenerated) with a slow concomitant removal of the fluid which is complete after 96 h (see Chapter 5 for a further discussion).

(b) Mechanisms of Uptake

From the present time-course study and in vitro work (Sections (ii) and (iii)), it is impossible to conclude the exact mechanism by which nickel is absorbed from the tubular fluid into the cytosol. Among the possibilities are the transfer across the cell membrane as the Ni(His)\(_2\) complex, partial dissociation into and transport of the Ni(His)\(_1\) complex, or complete dissociation to Ni\(^{2+}\) before crossing the cell membrane. The cation-exchange chromatography work reported in Section C (v) is consistent with the expectation of a neutral charge for Ni(His)\(_2\) and +1 for Ni(His)\(_1\). Figure 4.12 summarizes the different possible processes based on
Figure 4.12. "Equilibrium" model for the transport of nickel across a biological membrane. Processes 1-6 denote various modes of transporting ligands, metal ions such as Ni\(^{2+}\) or their complexes across biological membranes: 1, cation diffusion, ion exchange or ion channel; 2, ligand diffusion or exchange; 3, partitioning via neutral, lipid-soluble protonated ligand; 4, partitioning via a neutral lipid-soluble metal complex; 5, exchange by extraction; and 6, receptor-mediated transfer. Process 7 represents the diffusion of Ni(His)\(_2\) across the membrane. Symbols: M, represents Ni\(^{2+}\) or Ni(His)\(^+\); L, ligand; HL is a neutral protonated ligand molecule; ML is a neutral metal-ligand complex; and R denotes a receptor. Charges on L, HL, and ML and M/ligand stoichiometry are omitted for convenience. Under "steady-state" conditions the processes summarized in this figure do not require additional energy input and thus may be considered as "passive". Adapted from Menon (1985).
the "Equilibrium" model for the transport of nickel across a biological membrane. The diffusion of Ni(His)$_2$ across the cell membrane (Fig. 4.12; process 7) is probably less effective than more lipid-soluble complexes such as Ni(DDC)$_2$. Since the cell membrane is lipophilic in character, lipid-soluble metal complexes are taken up more readily than hydrophilic (polar) complexes such as those of amino acids. Interestingly, administration of DDC to mice 10 min prior to $^{63}$NiCl$_2$ dosing increased the amount of nickel associated with the lung, liver, and brain (Oskarsson and Tjälve, 1980). By contrast, when DL-penicillamine (which forms a polar complex with Ni$^{2+}$) was administered similarly, tissue levels of nickel were lowered in all tissues including the kidney.

Based on the in vivo and in vitro work in this thesis, it is impossible to conclude if the ultrafiltrable Ni(His)$_2$ complex outside the renal cells in the tubular fluid retains the same histidine ligand when taken up into renal cytosol (i.e., diffusion across the membrane). Putting it another way, to what extent do the processes depicted in Fig 4.12 take place? Dual labelling might be the way to explore this. For example if $^{63}$Ni($^{14}$C-His)$_2$ were injected, then it would be possible to trace both the nickel and the histidine entities. This would permit an assessment to establish if the Ni(His)$_2$ complex isolated from the renal cytosol has the same radioactivity ratio ($^{14}$C/$^{63}$Ni) as the serum ultrafiltrate. Such an experiment should, in principle, indicate if the Ni(His)$_2$ complex has crossed the renal-cell membrane by diffusion without change or if ligand exchange is involved.
and may give information as to how Ni(His)\textsubscript{2} inhibits L-histidine uptake in the nephron.

Cellular uptake by phagocytosis has been illustrated for particulates of crystalline (relatively insoluble) nickel compounds (e.g., Ni\textsubscript{3}S\textsubscript{2}; Costa and Mollenhauer, 1980; Nieboer et al., 1987a, b). Pinocytosis might be expected for dissolved nickel compounds. Fluid-phase endocytosis is indeed an important uptake process in the kidney (Moffat, 1982). Subsequent to pinocytic uptake, subcellular redistribution would be expected to occur after release of Ni\textsuperscript{2+} or Ni(II)-complexes (e.g., Ni(His)	extsuperscript{+}) from acidified (pH \(\approx 5\)) fused lysosomal/endosomal structures.

(c) Tissue Distribution of Nickel

The distribution of \(^{63}\text{Ni}\) in various tissues tested after i.v. injection of Ni(His)\textsubscript{2} is given in Tables 4.8 and 4.9. This is essentially the same distribution found in a number of reports in the literature, prior to 1979, that describe the tissue accumulation of divalent nickel following i.p., i.v. or s.c. injection of nickel salts (EPA, 1986). Although there appear to be differences in the distribution of \(^{63}\text{Ni}\) which are dependent on the type of animal (mouse, rat, guinea pig, rabbit) and protocol used, the EPA (1986) review came to the following conclusions. The highest accumulation of nickel is "in the kidney, endocrine glands, lung and liver. Relatively little nickel is lodged in neural tissue, which is consistent with the observed low neurotoxic potential of divalent nickel salts. Similarly, there is relatively slight
uptake into bone, consistent with other evidence that nickel is rather rapidly and extensively cleared from organisms, with little retention in soft or mineral tissue" (EPA, 1986). Oskarsson and Tjalve (1980) found the same order as in Table 4.9 for mice tissues 4 h after i.v. injection of 7 \( \mu g \) \( ^{63}\text{Ni}^{2+} \) kg\(^{-1}\). Sunderman et al. (1976b) also found the same order as in Table 4.8 for rat tissue 6 h after i.p. injection of 5.9 mg kg\(^{-1}\) of \( ^{63}\text{NiCl}_2 \). From this short review, it appears that i.v. injection of the \( ^{63}\text{Ni(His)}_2 \) complex does not drastically alter the distribution of nickel in tissues observed following injection of \( ^{63}\text{NiCl}_2 \). This is probably due to a fast in vivo conversion of the injected \( \text{Ni}^{2+} \) to the \( \text{Ni(His)}_2 \) complex.

(d) Distribution of Ni in Different Regions of the Nephron

Figure 4.10 illustrates the change in distribution of nickel along the nephron at various time points after i.v. injection of \( \text{Ni(His)}_2 \) (6 \( \mu g \) Ni kg\(^{-1}\)). Over the 24-h period, only the S3 segment of the proximal tubule appears to have a large retention of nickel. Most importantly, the S3 segment also shows the highest proportion after 10 min and all the subsequent time points, suggesting that the uptake in this region is very rapid and that Ni is most strongly bound and retained. Interestingly, uptake by the S3 segment has also been related to the nephrotoxic action of cisplatin (Daley-Yates and McBrien, 1982). The nickel associated with all other segments of the nephron reached a maximum and then decreased over the 24-h period. For example, there is an
increase in the accumulation of nickel in the glomerulus at the 1.5-h time point after which it rapidly decreases. This accumulation is probably due to Ni\(^{2+}\) binding to the glomerular-basement membrane (Templeton, 1987a; Templeton, 1987b). It should be noted that the glomeruli in the time-course study were normal, with no indication of a decrease in the Bowman's space. This is due to the much lower levels (\(\sim 250 - 1000\) fold) of nickel used in the i.v. study than in the histological study.

It is interesting to speculate why there is rapid accumulation and large retention of Ni(His)\(_2\) in only the S3 segment of the nephron. In the rat, the brush border microvilli of the S3 segment are the longest and most densely packed compared to the S1 and S2 segments of the proximal tubule (Kriz and Kaissling, 1985). Therefore, the high surface area associated with the S3 microvilli would allow the largest and quickest movement of nickel across the cell membrane from the lumen to renal cytosol.

(e) Serum and Intracellular Distribution of Ni

The subcellular fractions in the in vivo time-course experiments following i.v. injection of Ni(His)\(_2\) (Fig. 4.8a and Table 4.6a) correspond to the same tissue as used in the autoradiography study (Fig. 4.10). There was a continuous decrease in the percent of injected dose found in the serum and liver. The nickel content peaked at 1.5 h in the kidney and its subcellular fractions. This profile illustrates the rapid accumulation of nickel in the kidney and subcellular
fractions followed by rapid clearance. It appears that nickel is removed less rapidly from both the mitochondria/lysosome (M/L) and nuclear fractions (see Fig. 4.8a). This is also illustrated when the percent loss of the maximum content at 1.5 h is calculated at the 24-h time point using the data in Table 4.6a. The average reduction for P₃, cytosol and microsomes was 92.9 ± 1.3 % (based on both cpm and cpm/mg protein), while for the M/L fraction it was 79.1 ± 0.2 %; for the nuclear fraction it was 83.8 ± 1.8 %. Therefore, it appears that nickel is preferably compartmentallized intracellularly within the nuclear and M/L subcellular fractions. In the absence of direct histological evidence of endocytosis mediating nickel uptake, the observed retention in the M/L fraction can only be taken as circumstantial evidence of this pathway. Since nickel(II)-phosphate complexes are of relatively low solubility, localization within lysosomes as insoluble phosphates may occur by analogy to the trivalent metal ions (Al³⁺, Ga³⁺, In³⁺) (Galle, 1983).

(f) Relationship Between Distribution and Toxicity

In Chapter 1, it was pointed out that the major postulate of nickel carcinogenesis and other toxic effects of nickel is that the Ni²⁺ ion is the ultimate putative agent (Sunderman, 1984b). However, as pointed out by Nieboer et al. (1986) consideration must be given to a number of factors that may mediate or modify the nickel-ion hypothesis. Among
these are: the concepts of the bioavailability of Ni$^{2+}$, mode of entry and delivery of Ni$^{2+}$ or Ni(II)-complexes to cells and the nucleus of the cell, the biological residence time of nickel and its compounds and the balance between the intracellular compartmentalization of Ni$^{2+}$ and its extracellular transport/excretion. The species that enters renal cells from the tubular fluid is likely to be Ni$^{2+}$ or a LMM complex such as Ni(His)$_2$, which are able to pass quickly into the renal cytosol (e.g., 6.7 % of injected dose in 10 min). The only indication of compartmentalization within the cell is the slower release from both the M/L and nuclear fractions. However, only 1.25 % of the injected dose is present in the kidney 24 h after injection. This illustrates that the biological residence time of nickel within the kidney is short in comparison to that of more insoluble compounds in the lung which have been associated with cancer development. This observation illustrates the relevance of a balance between intracellular compartmentalization of Ni$^{2+}$ and its transport/excretion favours excretion. The long biological-residence time associated with deposits of nickel-containing particulates in the lung presumably provides a vehicle for continuous availability of the Ni$^{2+}$ ion to cells and their nuclei over a long period of time. However, such particulate nickel compounds do not reach the kidney, only the Ni$^{2+}$ ion or its complexes do. Therefore, insoluble nickel compounds that workers are exposed to impact on the kidney in a manner similar to soluble nickel compounds
which is characterized by rapid subcellular clearance. This perspective implies that the incidence of renal cancer in humans exposed to insoluble nickel compounds will be low (see Chapter 5 for a further discussion). Indeed, renal cancers are not associated with industrial exposure to nickel compounds.

At this point it is interesting to compare the subcellular action of Ni\(^{2+}\) to Cd\(^{2+}\), a known nephrotoxin. Cd\(^{2+}\) is largely excreted in the tubular fluid as its metallothionein complex (CdMT). The CdMT complex is reabsorbed, which is believed to be decomposed within the lysosomes with release of Cd\(^{2+}\) (Nieboer and Sanford, 1985 and references therein). Perturbation of the homeostasis of Zn and possibly Cu may also be involved since metallothionein not induced by cadmium contains both Zn and Cu. There is evidence to suggest that the Cd\(^{2+}\) ion impairs cell function by interfering with the function of lysosomes and the nucleus, resulting ultimately in tubular loss of LMM protein and suppression of amino acid transport (Fowler, 1983; Foulkes, 1983). In Table 4.13 a comparison is made of subcellular fractions found in the present study and after a single i.v. injection of \(^{109}\)CdMt into rats (Squibb et al., 1979). From this it is apparent that there is more rapid association of cadmium with the nuclear and M/L fractions than for nickel. After 0.5 h, there appears to be a transfer of cadmium from the M/L fraction to the cytosol, which is consistent with the toxic action of Cd\(^{2+}\). This transfer is
Table 4.13

RENAL SUBCELLULAR FRACTIONATION STUDIES

$^{63}\text{Ni(His)}_2$ (iv injection; 6 $\mu$g Ni kg$^{-1}$)$^a$

Percent of Total $^{63}\text{Ni}$ Present in Kidney.

<table>
<thead>
<tr>
<th>Subcellular Fraction</th>
<th>10 min</th>
<th>1.5 h</th>
<th>3 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_3$</td>
<td>3.2</td>
<td>5.3</td>
<td>4.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Cytosol</td>
<td>56.5</td>
<td>56.8</td>
<td>48.0</td>
<td>32.9</td>
</tr>
<tr>
<td>Mitochondrial/Lysosomes</td>
<td>18.9</td>
<td>25.6</td>
<td>28.3</td>
<td>48.3</td>
</tr>
<tr>
<td>Nuclear</td>
<td>9.2</td>
<td>11.9</td>
<td>11.9</td>
<td>18.7</td>
</tr>
<tr>
<td>Microsomes</td>
<td>10.8</td>
<td>13.8</td>
<td>9.8</td>
<td>10.2</td>
</tr>
</tbody>
</table>

$^{109}\text{CdMT}$ (iv injection; 0.17 mg Cd kg$^{-1}$)$^b$

Percent of Total $^{109}\text{Cd}$ Present in Kidney.

<table>
<thead>
<tr>
<th>Subcellular Fraction</th>
<th>0.5 h</th>
<th>3 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>25.6</td>
<td>61.8</td>
<td>70.4</td>
</tr>
<tr>
<td>Mitochondria/Lysosomes</td>
<td>38.6</td>
<td>6.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Nuclear</td>
<td>33.2</td>
<td>30.2</td>
<td>22.4</td>
</tr>
<tr>
<td>(plus large cytoplasmic bodies)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsomes</td>
<td>2.5</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>(plus small mitochondria and lysosomes)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Data taken from present study.

$^b$Data taken from Squibb et al. 1979.
not seen for Ni\(^{2+}\). The comparison of these studies indicates a different compartmentalization profile, lower bioaccumulation and shorter cellular or subcellular residence time of nickel in comparison to cadmium. Therefore it appears that these properties of nickel account for its relatively low nephrotoxicity.

It is interesting that even at the 3-min time point, \(\cong 2.5\%\) of the injected ⁶³Ni\(^{2+}\) dose occurs in the bladder urine of rat (Table 4.8), while at 10 min it is up to 13\%. It is unlikely that biliary nickel content would contribute to the excretion of nickel in rats at these time points because it has been shown to be less than 0.5\% of the injected dose over a 24-h period (Marzouk and Sunderman, 1985). In the current work, it was possible to account for 93\% of the injected dose 24 h post-injection (without including muscle or intestine). This illustrates that it is unlikely that there are other pools in the body with large retention of nickel. The two tissues (brain and thigh muscle) with the lowest nickel accumulation were most reluctant to release Ni\(^{2+}\) during the 24-h post-injection observation period (column 5, Table 4.8), but did not induce any serious tissue damage. Previous animal studies reviewed above have established that the brain is not a primary target organ for inorganic forms of nickel, which is in direct contrast to the lipophilic compound Ni(CO)\(_4\) that does impact severely on the central nervous system in man and animals.

The work reported in this chapter illustrates that high
doses of NiCl₂ result in toxic responses in all segments of the rat nephron. By contrast for low doses of Ni(His)₂, the initial accumulation of ⁶³Ni is followed by rapid subcellular clearance, with the rat renal tissue having no histological evidence of nephrotoxicity in any segment of the nephron. These findings will be discussed further in Chapter 5 within the context of the results reported in Chapter 3 for the survey of electrolytic nickel-refinery workers.
A RENAL CLEARANCE OF NICKEL(II) AND IMPLICATIONS FOR BIOLOGICAL MONITORING

The amount of serum nickel(II) associated with the low-molecular-mass (ultrafiltrable) fraction ($\alpha_{NI}$) in six electrolytic nickel workers was found to be $0.24 \pm 0.06$ (Chapter 3). Evaluation of this parameter allowed nickel clearances and fractional clearances to be calculated. Clearly nickel is reabsorbed in the human kidney. The average renal clearance of nickel (volume of serum from which available nickel is removed per min) was estimated from the slope of Fig. 3.3 to be $42 \text{ mL min}^{-1}$ compared to $120 \text{ mL min}^{-1}$ for creatinine. From this it was possible to estimate that about 65% of the nickel in the glomerular filtrate is reabsorbed in the nephron. The shape of the fractional clearances curves closely resembled that of urea whose tubular reabsorption occurs by passive diffusion. The renal excretion of the metabolite creatinine is the only known endogenous substance for which the clearance approximates the glomerular filtration rate (Duarte et al., 1980; Epstein, 1977). This means that creatinine is not reabsorbed in the nephron (in fact it is thought to be slightly secreted). Total solutes assessed as $\rho$-1 in the urine depend to a large extent on the amount of substances such as $\text{Na}^+$, amino acids and urates that are reabsorbed in the nephron. From the current work it can be seen that the excretion pattern of nickel(II) resembles that of total solutes more closely than
of creatinine. This indicates that normalization of spot voids employing specific gravity ($\rho$) rather than creatinine, allows flow-dependent variations in urinary nickel to be compensated.

The close relationship between total solutes and nickel excretion is clearly illustrated in Figures 2.31, 2.33, 2.34 and 2.35. When the creatinine normalization of 24-h nickel was examined in detail, two curves were found when $U_{Ni}/U_{\text{creat}}$ was plotted against $U_{Ni}$ (Fig. 2.31). For the same data set, $U_{Ni}/U_{\rho-1}$ versus $U_{Ni}$ yielded a single straight-line relationship (Fig. 2.34). However, when $U_{Ni}/U_{\text{creat}}$ was plotted against the excretion rate ($E_{Ni} = U_{Ni}V$) a straight line was observed (Fig. 2.33). These relationships demonstrate the presence of a residual, uncompensated flow-rate dependence for creatinine adjustment of urinary nickel concentrations, which is not present for solute correction. Thus specific gravity normalization is more appropriate than creatinine adjustment.

In Chapter 2, a rationale was developed for the volume correction of nickel in spot-urine voids. This approach can be used to generate protocols for other substances. It was based on Equation 2.1, which was first proposed by Araki (1980) and centered on the evaluation of the power coefficient $b_i$ of the urine flow rate. Evaluation of $b_i$ for any analyte of interest (e.g., nickel) and $b_{\text{ref}}$ for a reference analyte (e.g., creatinine or total solutes) is a prerequisite. If the analyte of interest (i) and reference
metabolite (ref) have the same mathematical dependence on urine flow rate, then \( b_i = b_{\text{ref}} \) (e.g., \( b_{\text{Ni}} = b_{\rho-1} \)). This equality forms the basis for selecting the most appropriate reference substance. The evaluation of the reported \( b_{\text{Ni}} \) values relied on a large variation in the natural flow rate (at least \( \Delta V > 1 \text{ mL min}^{-1} \), although \( \Delta V > 2 \text{ mL min}^{-1} \) was preferable) and an adequate number of timed urinary voids (e.g. \( n \geq 4 \)). Also, an even distribution of \( V \) values throughout the range studied was mandatory. These criteria resulted in a rejection of the data for a number of donors in this study. To circumvent these limitations, water-loading or restriction is advisable. Such practice would allow the above requirements for the evaluation of \( b_i \) values to be fulfilled more readily.

The curves in Figures 2.25 to 2.30 were interpreted as belonging to a family of theoretical curves depicted in Figure 2.38. A generalized form of Equation 2.6 namely Equation 2.25 which incorporates the mathematical function describing this family of curves, was employed in urinary analyte normalization.

\[
\frac{U_1}{U_2} = \frac{U_1^0}{U_2^0} \sqrt{\Delta b} \pm SD \quad \text{(2.25)}
\]

where, \( \Delta b = b_2 - b_1 \) and \( b_1 \) refers to the analyte being normalized and \( b_2 \) to the reference analyte. Further modification of Equation 2.25 was based on the 95% confidence
limits of $\overline{\Delta b}$ (i.e., the confidence interval $\overline{\Delta b} \pm t(\text{SD}/\sqrt{n})$) for the normalization of nickel by total solutes taking into account the size ($n$) of the volunteer group. This provided a rationale for the establishment of boundary values for urinary specific gravity and creatinine (Tables 2.11 and 2.12) as determined by a preselected confidence level of $\Delta b$ and an acceptable error in $\sqrt{\Delta b}$ (the uncompensated flow factor). For example, for a group of 20 volunteers, 95% of the time the error in the residual flow factor $\sqrt{\Delta b}$ will not exceed $\pm 10\%$ if $\rho$ falls in the range 1.010 to 1.039. This approach permits an investigator of nickel-exposed individuals to measure the specific gravity of a urine sample on a work site with a hand-held refractometer. If $\rho$ falls within the range predetermined by the acceptable error and confidence limits, the specimen can be accepted for further processing (e.g., nickel analysis). When the measured $\rho$ is outside this range, then the specimen should be rejected and another obtained (providing the dip stick levels of other metabolic and nephrotoxic indicators such as glucose or protein are acceptable). For individuals or small groups, the use of the standard score for $\Delta b$ and a percentile table permits the recommendation that if the specific gravity of a urine specimen is between 1.015 and 1.026 then 95% of the time the deviation of $\sqrt{\Delta b}$ from unity would not exceed 30%. The use of a 24-h specimen would reduce the rejection rate based on this criterion.

As illustrated in the above discussion, the approach to
normalization summarized in Equation 2.25 is general and can be employed for any analyte (metallic or organic). For example, this is important when a worker is exposed to metals such as lead, for which it is necessary to evaluate urinary levels of biochemical metabolites such as coproporphyrin and δ-aminolevulinic acid in the urine (Lauwerys, 1983). Both of these compounds have had their $b_i$ values evaluated by Araki (1980). This concept of flow-rate correction of spot urine samples is also applicable to exogenous exposure to organic compounds such as trichloroethylene. Major portions of the latter are metabolized to trichloroethanol and trichloroacetic acid, which are excreted in the urine (Lauwerys, 1983). In this case, the $b_i$ coefficients for these metabolites of trichloroethylene would have to be measured and the appropriate concentration boundaries selected relative to a suitable reference analyte before an appropriate rationale for biological monitoring can be established. As illustrated, biological monitoring utilizing spot urine specimens can be based on sound scientific principles and thus the choice of protocol should not be left to the arbitrary discretion of an individual investigator.

In Chapter 3, it was shown that the evaluation of the clearance and fractional clearance of nickel was important in establishing that nickel is reabsorbed in the nephron. However, the concept of clearance may have toxicological application for well-established nephrotoxins such as
cadmium. It could serve as a reliable indicator of renal damage. Cadmium is known to be reabsorbed in the proximal tubules as the metallothionein complex, where it is catabolised within lysosomes (Nieboer and Sanford, 1985 and references therein) and the released Cd$^{2+}$ is thought to attach to de novo synthesized metallothionein in the kidney (Lauwerys, 1983). The current hypothesis is that saturation of this renal scavenging process releases free Cd$^{2+}$ allowing its toxic action to occur. This critical event could result in a dramatic change in the clearance of cadmium. Before there is renal dysfunction, the CdMt complex is being reabsorbed in the kidney and thus has a clearance less than creatinine. When early dysfunction occurs, cadmium may be expected to be released from proximal-tubule cells into the tubular fluid, which would dramatically increase the cadmium clearance. An investigation of Cd clearances as an early indicator of renal damage is currently being conducted at McMaster University.

It is interesting to note that in the past some investigators have recommended the rejection of very dilute urine samples (specific gravity < 1.010 or creatinine concentration < 0.3 g L$^{-1}$; Lauwerys, 1983) and $\rho < 1.012$ (Bernacki et al., 1978). They advise that analyses should be repeated on a more concentrated specimen if the specific gravity is less than these values. Using specific gravity adjustment of urinary nickel spot voids (Chapter 2D (v)) for 20 workers exposed to nickel, the upper and lower limit for
acceptable specific gravity measurements would be 1.010 and 
1.039 for 95% confidence level in $\Delta b$ and $\sqrt{\Delta b} = 1.0 \pm 0.1$. 
This would have resulted in 15% of the specimens in the 
electrolytic nickel refinery study (Chapter 2) being 
rejected, all of which were below 1.010 and none above 1.039. 
Thus the recommendation by Lauwerys (1983) appears to be a 
reasonable first approximation to the problem for selection 
of spot voids for nickel analysis. However, as pointed out 
in Chapter 2D (v), as the group size gets smaller or $\Delta b$ 
larger (Fig. 2.38), then the range of acceptable specific 
gravity values becomes narrower. The choice of the lower 
specific gravity limit of 1.010 and no upper limit of 
Lauwerys (1983) represent boundary values that are widely 
separated and would result in accepting urine specimens that 
should be rejected.

The value of solute normalization was illustrated by 
calculating $U_{Ni}^o$ (estimated from Eqn. 2.6 or $U_{Ni}$ adjusted to a 
mean $\rho$ value during the 24-h observation period). $U_{Ni}^o$ values 
generally increased during the shift with a subsequent 
decrease in the post-shift period (Figs. 3.6 - 3.10) and also 
correlated with job category (Table 3.11). However, it would 
be more appropriate to establish a relationship between 
breathing zone levels of nickel measured by personal sampling 
and $U_{Ni}^o$ levels throughout a 24-h period. The observed 
variation in $U_{Ni}^o$ during the 24-h collection period (Figs. 3.6 
- 3.10) might reflect some diurnal variation in the excretion 
pattern of nickel for electrolytic refinery workers.
Circadian rhythms involving the kidney have long been recognized. For example, the low urine flow at night is an classical observation and is of obvious practical convenience. Disturbances of the usual urinary rhythms underly some cases of bed-wetting (Mills, 1976). It is also known that sodium and potassium excretion undergo a circadian change (Elliott et al., 1972 and Mills, 1976). A person's inherent rhythms can be changed by the intake of coffee and tea that influence the excretion of water, sodium and chloride. Alterations may also derive from time shifts such as those found in transatlantic flights (Elliott et al., 1972). In the present work, the systematic increase of $U_{Ni}$ during the 24-h time period including an 8-h shift is consistent with exposure to soluble nickel and not due to a circadian rhythm. It may be possible for nickel excretion to follow a circadian rhythm in nonexposed healthy adults, which would of course be masked by exposure during a work shift. Because working on the afternoon and midnight shifts involves a time shift and a change in body rhythms, a circadian component might be included in the design of future biological monitoring programs.

**B ABSORPTION AND TOXIC EFFECTS OF NICKEL IN RAT KIDNEY**

The renal slice work, the isolated proximal-tubule fragments experiments and i.v. time-course studies all illustrate that nickel can be absorbed in the rat kidney, likely by the S3 segment of the proximal tubule. Nickel was
also found to be reabsorbed in the kidneys of electrolytic refinery workers (Chapter 3). It appears therefore that nickel is absorbed from the glomerular filtrate through the brushborder membrane into the epithelial cells of the proximal tubule, eventually passing through the basolateral membrane to the blood capillary. Indeed, nickel was associated in almost equal amounts with the basolateral and brush-border membranes isolated from rat kidney after i.v. injection of $^{63}\text{Ni(His)}_2$ (Section 4C (iv)). Such reabsorption of $^{63}\text{Ni}$ would afford an opportunity for some intracellular accumulation of nickel such as in the M/L and nuclear fractions. All the animal work reported in this thesis is most consistent with a passive absorption process (e.g., fluid phase endocytosis), although some contribution from active components can not be ruled out. The largely passive nature of uptake of nickel in the rat studies agrees with the urea-like reabsorption of nickel in man described in Chapter 3F (iii). As pointed out in Chapter 1C (i), homeostatic control of a substance by controlled reabsorption in the kidney is partial evidence of its essentiality. Further evidence of homeostatic regulation are the narrow ranges of nickel concentration observed in human body fluids of non-exposed individuals (Chapter 1C (ii) and Nieboer et al., 1987a).

In the histological study, high (6 mg Ni kg$^{-1}$) and intermediate doses (3 mg Ni kg$^{-1}$) of nickel appear to have a major effect on the glomeruli of rats. A dose response
decrease in the Bowman's space was observed. However, in the low i.v. injections (6 μg Ni kg⁻¹) employed in the subcellular fractionation study there was no change in the Bowman's space observed. A number of mechanisms have been proposed to explain the observed changes in the glomerulus and the associated nephrotoxic observations of proteinuria, amino aciduria and polyuria. They are: (i) enlargement of the glomerular capillaries due to an increase in renal capillary pressure and filtration rate with associated edema; (ii) interaction of Ni²⁺ with the negative charge located on the filtration barrier; (iii) formation of immune complexes in the glomerular basement membrane (GBM) and (iv), cellular or tissue damage with decreased reabsorption of amino acids and proteins in the proximal convoluted tubule (possibly the S3 segment). The common explanations for proteinuria are the reduction in surface charge and the formation of immune complexes in/on the GBM (Hook and Hewitt, 1986). However, the available data does not permit exclusion of the other explanations. It may be that the four mechanisms described are not mutually exclusive and all contribute to the observed effects.

The time-course rat study indicates that there is quick clearance of nickel of the injected label (6 μg ⁶³Ni kg⁻¹) from all subcellular fractions, with only a slight indication of retention in the M/L and nuclear fractions. If the rat kidney handles high-dose injections similarly, which result in nephrotoxicity characterized by increased total proteinurea,
amino aciduria and N-acetyl-β-D-glucosaminidase (NAG), then such effects might be expected to be transient. There are two studies that support this hypothesis. The first demonstrates a dramatic rise in urinary total protein and 18 of 20 amino acids the first day after i.p. injection of 5.9 mg Ni kg⁻¹, which reach maxima on the second day post-injection (Sunderman et al., 1976b). Subsequently, these excretions diminish systematically so that on day 4 they have returned to control values. Histidine follows a slightly different pattern as first pointed out by Gitlitz et al. (1975). It reaches a maximum on the first day after injection, after which it continually decreases until on the Day 3 and 4 there is no statistical difference when compared to control rats. This excretion pattern of histidine very closely resembles that of nickel which has a half-life of about 24 h, suggesting concurrent excretion as the Ni(His)₂ complex. Gitlitz et al. (1975) found that fusion of the foot processes of epithelial cells occurred in renal glomeruli 48 h after i.p. injection of 4 mg Ni²⁺ kg⁻¹. This focal lesion was not seen in the glomeruli from any of the control rats or exposed rats 24 or 96 h after the injection, indicating reversibility. Thus as in the published histological study, the major change occurred in the glomeruli which reached a maximum 48 h post-injection (Chapter 4). Interestingly, the maximum in the urinary excretion of total proteins and most amino acids is also seen on the second day after injection (Sunderman et al., 1976b).
This supports the argument that the severe glomerular damage described in Chapter 4C (i) (a) is a major contributing factor to the excess excretion of total protein.

C  KIDNEY FUNCTION STATUS IN ELECTROLYTIC REFINERY WORKERS

The time-course subcellular-fractionation results permit an understanding of how the human kidney handles exposure to soluble nickel. As depicted in Figure 3.1 and 3.6 to 3.10, there is a significant drop in urinary nickel levels after each work shift. Thus if the human kidney acts in a manner similar to the rat kidney, then after each shift and weekend it is expected that there will be at least partial subcellular clearance. It is common to see a gradual increase in the urinary nickel levels during a work week (Fig. 3.1), although weekends allow restoration of urinary and subcellular levels of nickel closer to background values. One month after being layed off work the urinary nickel levels were 36 to 70% lower than the pre- layoff values and were still well above the concentrations expected for healthy adults (see Chapter 3F (iii) for details). This has been interpreted to indicate that there is a slow release of nickel from pools stored in the body. In the Port Colborne operation, most of the donors also worked, mostly in the past, in operations generating nickel-containing particulates (e.g., in smelting and calcining, anode scrap washing, nickel-anode casting). This constitutes mixed exposure to both aerosols of dissolved nickel (as chloride or sulphate)
and less soluble forms (e.g., nickel sulphides and oxides and nickel metal). The data depicted in Figure 3.7 corresponds to an individual who had such mixed exposure over 25 y of employment, while that in Figure 3.6 is for a younger worker employed for only 4.5 y in the electrolytic refinery. The data suggests the presence of respiratory nickel deposits in the former, which would give a slow continuous release of nickel with a longer half-life than associated with dissolved nickel salts.

The work in this thesis emphasizes the importance of utilizing animal and human studies to obtain a better understanding of the renal handling of a potential nephrotoxin such as Ni\textsuperscript{2+}. This requires that animal studies be designed to simulate human exposure. Experiments with animals readily demonstrate nephrotoxicity due to elevated acute doses (e.g., 6 and 3 mg Ni kg\textsuperscript{-1}). These may have no real counterpart in humans because they are never exposed to correspondingly high single doses of Ni\textsuperscript{2+}. Nevertheless, such doses are sometimes valuable in illustrating an intrinsic response, but in this instance it appears that this animal data cannot be readily extrapolated to man. By contrast, single-dose, non-acute microgram-level animal studies do allow an understanding of the dynamics and mechanisms that may be important in human exposure. This can be illustrated by employing the time-weighted-average (TWA) of 0.1 mg Ni m\textsuperscript{-3} recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1986) for soluble
nickel compounds. Assuming that 20 m$^3$ of air is breathed daily, it is possible to estimate the hypothetical nickel intake during an 8-h shift at the TWA level. Under such conditions, approximately 0.7 mg of nickel is inhaled (assuming 100% deposition). For a typical worker of 83 kg (mean of the electrolytic workers surveyed in Chapters 2 and 3), this would represent a dose equivalent to 8 $\mu$g Ni kg$^{-1}$. This is much less than the range of 2 to 6 mg Ni kg$^{-1}$ that is usually injected into rats. For comparison, the exposure for one year can be approximated by estimating the work year to be 240 days. This would result in an equivalent dose of 2 mg Ni kg$^{-1}$. Thus the amount of a single injection usually administered to rats appears to be equivalent to one year of accumulated exposure by a worker exposed to soluble nickel at the TWA level.

It is interesting to note that there have been two reported cases of proteinuria in man resulting from nickel carbonyl poisoning (Sunderman, 1977). This type of poisoning in humans is characterized by a sharp increase in urinary nickel levels following exposure (see Fig. 2.1). This probably results in a large and quick release of Ni$^{2+}$ into the body from the lungs so that it simulates the acute animal injections (2 - 6 mg Ni kg$^{-1}$) which are associated with proteinuria and amino aciduria in rats.

Throughout the in vitro and animal work, all incubations and injections were designed to simulate the uptake and distribution of low-molecular-mass (ultrafiltrable)
components of nickel ($\text{Ni(His)}_2$ and $\text{Ni(His)}_1$). The *in vitro* work of Chapter 4 demonstrated the uptake of $^{63}\text{Ni}^{2+}$, $^{63}\text{Ni(His)}_2$ and $^{63}\text{Ni(His)}_1$ in renal slices and $^{63}\text{Ni(His)}_2$ in proximal tubular fragments. The efflux of $\text{Ni}^{2+}$ from live renal slices was much less than PAH indicating that there is more tissue compartmentalization and retention of nickel than PAH. This retention is reflected in the high affinity that $\text{Ni}^{2+}$ and $\text{Ni(His)}_2$ have for the M/L and nuclear incubated fractions (Table 4.6b and Fig. 4.8b) and the retention of nickel in the ML fraction of the kidney after i.v. dosing (Table 4.13). The PAH uptake in renal slices was significantly increased by a 95% $\text{O}_2$/5% $\text{CO}_2$ (v/v) atmosphere, while $\text{Ni}^{2+}$ and $\text{Ni(His)}_2$ was not. All the nickel uptake studies were interpreted to involve a passive mechanism that can be explained in terms of a steady state "Equilibrium" model in which uptake is regulated by thermodynamic parameters such as the $\text{pK}_a$ values of ligands, the binding constants of the nickel-ligand complexes, their solubilities in aqueous and lipid phases, and the effective ligand concentrations in the intracellular and extracellular compartments. However, as already indicated the data presented in this thesis does not eliminate a contribution of active processes. Injection of $\text{Ni(His)}_2$ into the jugular vein of rats demonstrated rapid renal cellular and subcellular uptake on nickel but, did not alter the distribution of nickel in tissues (Tables 4.8 and 4.9) relative to those reported for $\text{NiCl}_2$ (EPA, 1986; Oskarsson and Tjalve, 1980; Sunderman et al., 1976b). As the
serum nickel levels declined, so did the renal cellular and subcellular levels which is consistent with passive diffusion back into the lumen. The G-75 high- and low-molecular-mass elution profiles after injection of Ni(His)$_2$ did not differ much from NiCl$_2$ studies (Table 4.7 and Fig. 4.9). Therefore, to unravel the uptake and excretion processes both the in vitro and in vivo work described in Chapter 4 has been helpful.

In conclusion, it can be seen from the work in this thesis that the incidence of nephrotoxicity found in electrolytic refinery workers was found to be minimal in comparison to the known nephrotoxic metal Cd. This differential response can be explained by the relatively rapid extracellular, cellular and subcellular clearance of nickel in contrast to the very long biological half-life of cadmium (10 - 30 y). Clearly to obtain a consistent picture between animal and human studies, it is necessary to design experiments that pertain to doses and routes of administration that simulate human exposure. In addition, carefully chosen in vitro and in vivo approaches need to be employed in a complementary fashion to obtain a model of renal retention, nephrotoxicity or detoxification of a potential toxic industrial metal such as nickel.
REFERENCES


ACGIH. (1986). Threshold limit values for chemical substances in the work environment adopted by ACGIH with intended changes for 1986-1987. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, USA.


Evans, R., Engel, C., Wheatley, C. and Nielsen, J. (1982). Modification of the sensitivity and repair of potentially lethal damage by diethyldithiocarbamate during and
following exposure of plateau-phase cultures of mammalian cells to radiation and cis-diaminedichloroplatinum (II). Cancer Research, 42, 3074-3078.


