1980

Techniques of Atomic Absorption: Direct Determination of Cadmium in Biological Materials and Metal Speciation by Differential Atomization.

Susan Deborah Weiss

Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation

https://digitalcommons.lsu.edu/gradschool_disstheses/3543

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.
TECHNIQUES OF ATOMIC ABSORPTION:
DIRECT DETERMINATION OF CADMIUM IN BIOLOGICAL MATERIALS
AND
METAL SPECIATION BY DIFFERENTIAL ATOMIZATION

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in
The Department of Chemistry

by
Susan Deborah Weiss
B.S., Mississippi University for Women, 1976

August, 1980
DEDICATION

To my parents, who inspired in me

a will to achieve.
ACKNOWLEDGMENT

The author wishes to express her appreciation to Professor J. W. Robinson, whose support and guidance were invaluable in the pursuit of this research. Thanks are also due to Mr. Les Edelen, Mr. George Sexton, and Mr. Ralph Seab of the Chemistry Department Electronics Shop for their patient assistance in maintaining the equipment used in this research, and to Mr. Richard Hoffman for assistance and advice regarding the illustrations included here. Thanks also are due to Mr. Christian Boussert and Mr. Charlie Burlow for the fabrication of the quartz atomizers used in these studies.

Special thanks go to Ms. Carol Young, who cheerfully typed this manuscript, and to Mr. Don Budd, who assisted in its duplication. The author also thanks Dr. Mary Good and Mr. Jeff Donner of the Materials Science Department for the ESCA analyses of samples.

The author wishes to express appreciation to Ms. Dorothy Lewis and the staff of the laboratory at the LSU Infirmary for assistance in obtaining blood samples. Many thanks are also due to Dr. J. W. Robinson, Dr. P. E. Koenig, and the numerous individuals who donated urine, sweat, hair, and breath samples. Without their willing assistance, this project would not have been possible.
The author acknowledges financial support from the Dr. Charles E. Coates Memorial Fund of the Louisiana State University Foundation in the preparation of this dissertation.

Finally, the author wishes to express her appreciation to her parents and to many friends, especially Mr. Michael Mudrock, who provided moral support, understanding, and encouragement during the difficult times.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xix</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xxiv</td>
</tr>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1. Metal Toxicity</td>
<td>2</td>
</tr>
<tr>
<td>2. Analytical Problems in the Determination of Metals in Biological Samples</td>
<td>4</td>
</tr>
<tr>
<td>3. Methods Available for the Determination of Metals</td>
<td>6</td>
</tr>
<tr>
<td>a. Colorimetric Methods</td>
<td>7</td>
</tr>
<tr>
<td>b. Electrochemical Methods</td>
<td>7</td>
</tr>
<tr>
<td>c. Neutron Activation Analysis</td>
<td>8</td>
</tr>
<tr>
<td>d. Atomic Emission</td>
<td>9</td>
</tr>
<tr>
<td>e. Atomic Fluorescence</td>
<td>10</td>
</tr>
<tr>
<td>f. Atomic Absorption</td>
<td>11</td>
</tr>
<tr>
<td>4. Historical Development of Atomic Absorption Spectroscopy</td>
<td>12</td>
</tr>
<tr>
<td>5. Advantages of the Quartz &quot;T&quot; Atomizer</td>
<td>15</td>
</tr>
<tr>
<td>6. The Importance of Metal Speciation</td>
<td>18</td>
</tr>
<tr>
<td>7. Methods Available for Metal Speciation</td>
<td>20</td>
</tr>
<tr>
<td>8. Summary</td>
<td>21</td>
</tr>
</tbody>
</table>
**PART I: THE DIRECT DETERMINATION OF CADMIUM IN BIOLOGICAL MATERIALS.**

**CHAPTER 1: THE DIRECT DETERMINATION OF CADMIUM IN WHOLE BLOOD.**

**A. INTRODUCTION.**

1. Biological Effects of Cadmium
2. The Exposure of Man to Cadmium
3. Reasons for This Study
4. Difficulties in the Determination of Cadmium in Biological Materials
   a. Problems in Sample Handling
   b. Problems Due to Low Concentration
   c. Problems Due to the Matrix
5. Common Methods for the Determination of Cadmium in Biological Materials
   a. Colorimetric Methods
   b. Atomic Emission Methods
   c. Atomic Absorption Methods
   d. Other Methods
6. Need For an Improved Analytical Procedure

**B. EXPERIMENTAL.**

1. Equipment
   a. Light Source
   b. Chopper
   c. Atomizer
d. Monochromator and Detector .................. 55

2. Procedures for Obtaining Blood Samples .... 55

3. Development of a Technique for the Direct
   Determination of Cadmium in Whole Blood . . . 56
   a. Direct Injection of Whole Blood ............ 57
   b. Direct Injection at a Reduced Flow Rate . 59
   c. Sample Introduction on a Carbon Disk . . . 61
   d. Stop-flow Methods .............................. 65
   e. Sample Introduction on a Filter Paper
      Disk .................................................... 67
   f. Effect of Hemolysis on Various
      Techniques ........................................ 70

4. Refinement of the Filter Paper Disk
   Technique ......................................... 71
   a. Cleaning of Filter Paper Disks ............. 71
   b. Sample Introduction Techniques .......... 72
   c. Blank and Background Measurements ...... 73
   d. Calibration Procedures ...................... 73

C. RESULTS ............................................. 76
   1. Absorption Traces Obtained in the Analysis of
      Whole Blood ................................. 76
   2. Blank and Background Measurements ......... 76
   3. Average Concentration of Cadmium in Whole
      Blood ............................................ 78

D. DISCUSSION ..................................................................................... 81

1. Concentrations of Cadmium Obtained for Whole Blood ................................. 81

2. Strengths and Weaknesses of the Filter Paper Disk Technique ........................................ 84
   a. Background Absorption Due to the Matrix ...................................... 85
   b. Reproducibility of the Absorption Signal ............................................. 87
      i. Differences in blank absorption levels ........................................... 87
      ii. Differences in sample size ...................................................... 88
      iii. Differences in the reaction of the sample with the bed ............. 88
   c. Sensitivity Changes with Time ................................................. 89

E. CONCLUSIONS AND SUMMARY ..................................................... 90

CHAPTER 2: THE DIRECT DETERMINATION OF CADMIUM IN URINE ........................................ 92

A. INTRODUCTION ................................................................................ 92

1. Relationship Between Urine Cadmium Levels and Body Burden ......................... 92
   a. Animal Studies ........................................................................ 92
   b. Human Studies ........................................................................ 93
2. Analytical Problems in the Determination of Cadmium in Urine ........................................ 95


4. Need for an Improved Analytical Procedure ........................................................................ 100

B. EXPERIMENTAL ..................................................................................................................... 101

1. Equipment ......................................................................................................................... 101

2. Procedure .......................................................................................................................... 101
   a. Sampling Techniques ..................................................................................................... 101
   b. Calibration Procedures ................................................................................................. 103

C. RESULTS .................................................................................................................................. 105

1. Concentration Range for Cadmium in the Urine of Unexposed Individuals .......................... 105

2. Variations in the Cadmium Concentration in the Urine of an Individual ............................ 108

3. Precision of the Analytical Method .................................................................................... 111

D. DISCUSSION .......................................................................................................................... 113

1. General Considerations Concerning the Determination of Cadmium in Urine .................. 113
   a. Advantages of the Method ............................................................................................ 113
   b. Matrix Effects .............................................................................................................. 114

2. Concentration Range for Cadmium in the Urine of Unexposed Individuals ...................... 117

3. Variations in the Cadmium Concentration in the Urine of an Individual .......................... 119
Chapter 3: The Direct Determination of Cadmium in Perspiration

A. Introduction

1. Characteristics of Human Perspiration
   a. Sensible and Insensible Sweating
   b. Mechanisms of Sweat Excretion
   c. Composition of Sweat
   d. Average Amount of Perspiration

2. Analytical Problems in the Analysis of Sweat
3. Common Methods for the Analysis of Sweat
4. Reasons for This Study

B. Experimental

1. Equipment
2. Procedure
   a. Sampling Techniques
   b. Calibration Procedures

C. Results

1. Concentration Range for Cadmium in the Perspiration of Unexposed Individuals
2. Variations in the Cadmium Concentration in the Perspiration of an Individual ........... 137
3. Precision of the Analytical Method ............. 141
4. Comparison of the Cadmium Concentrations Found in Sweat and Urine ................. 142
D. DISCUSSION ................................................. 146
   1. General Considerations Concerning the Determination of Cadmium in Perspiration ... 146
   2. Concentration Range for Cadmium in the Perspiration of Unexposed Individuals .... 151
   3. Estimated Daily Excretion of Cadmium Through Perspiration ......................... 152
   4. Variations in the Cadmium Concentration in the Perspiration of an Individual ...... 153
   5. Comparison of the Cadmium Concentrations Found in Sweat and Urine ............. 154
E. CONCLUSIONS AND SUMMARY ..................................... 158
CHAPTER 4: THE DIRECT DETERMINATION OF CADMIUM IN HAIR .................. 161
A. INTRODUCTION .............................................. 161
   1. Common Methods for the Determination of Cadmium in Hair ......................... 162
   2. Analytical Considerations in the Determination of Cadmium in Hair ............... 163
a. Adsorption of Cadmium on the Hair ........................................ 163
b. Variations in the Concentration of Cadmium in Hair ............. 165

B. EXPERIMENTAL ................................................................. 168
1. Equipment ........................................................................ 168
2. Procedure ........................................................................ 168
   a. Sampling Techniques .................................................. 168
   b. Calibration Procedures .............................................. 169

C. RESULTS ............................................................................. 170
1. Variation in Cadmium Concentration Along the Hair Strand ... 170
2. Variation in Cadmium Concentration Between Hair Strands from an Individual .............................................. 172
3. Range of Cadmium Concentration in the Hair of Individuals in a Non-Exposed Population ......................... 177
4. The Effect of Washing Procedures on the Concentration of Cadmium in Hair ................................................. 183
5. The Concentration of Cadmium in Shampoo ...................... 186

D. DISCUSSION ....................................................................... 187
1. Advantages of the Use of the Quartz "T" Atomizer for the Determination of Cadmium in Hair ......................... 187
2. Variation in Cadmium Concentration Along the Hair Strand ................................................................. 188
CHAPTER 6: DIFFERENTIAL ATOMIZATION USING A PLATINUM LOOP-CARBON BED DUAL STAGE ATOMIZER

A. INTRODUCTION ................................................................. 222b

1. Current Methods for Metal Speciation Analysis ...................... 223

2. Coupled Methods for Metal Speciation Using Atomic Absorption Spectroscopy .................. 225

3. Need for an Improved Analytical Technique ......................... 227

4. Atom-Formation Processes in Graphite Furnace Atomic Absorption .................. 228

5. The Use of Wire Loops in Atomic Absorption Spectroscopy ........ 231

B. EXPERIMENTAL ................................................................. 232

1. Equipment ........................................................................ 232

2. Chemicals ........................................................................ 236

3. Sampling Procedure ....................................................... 237

C. RESULTS ................................................................. 240

1. Lead and Cadmium Inorganic Compounds ......................... 240

2. Effect of EDTA ............................................................. 240

3. Biological Samples ....................................................... 240

4. ESCA Studies ............................................................... 260

D. DISCUSSION ................................................................. 266

1. General Observations Concerning the Platinum Loop Method ........ 266

a. Absorption Traces ...................................................... 266
LIST OF TABLES

1. Approximate Ranges of Values for the Principal Constituents of Human Blood Plasma ................................ 32
2. Comparison of Analytical Techniques for the Determination of Cadmium in Biological Matter .......................... 45
3. Comparison of Data Obtained for Two Blood Samples Using Direct Injection and Stop-Flow Techniques .... 69
4. Comparison of Literature Values for Cadmium in Whole Blood ................................................................................. 82
5. Approximate Composition of 24-Hour Urine in a Normal Adult ................................................................................. 96
6. Values for Concentration of Cadmium Found in the Urine of Unexposed Individuals .............................................. 106
7. Daily Variations in the Concentration of Cadmium in the Urine of Two Individuals ................................................. 109
8. Values Obtained for Repeated Analysis of a Single Urine Sample ................................................................................. 112
9. Comparison of Literature Values for Cadmium in Urine ............................................................................................... 118
10. Approximate Concentration of the Electrolytes in Sweat ............................................................................................. 129
11. Estimated Losses of Trace Metals in Sweat ................................................................................................................. 130
12. Values for Concentration of Cadmium Found in the Sweat of Unexposed Individuals ........................................... 136
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13. Daily Variations in the Concentration of Cadmium in the Sweat of Two Individuals</td>
<td>139</td>
</tr>
<tr>
<td>14. Values Obtained for Repeated Analysis of a Single Sweat Sample</td>
<td>143</td>
</tr>
<tr>
<td>15. Comparison of the Concentration of Cadmium in the Urine and in the Sweat of Unexposed Individuals</td>
<td>144</td>
</tr>
<tr>
<td>16. Comparison of the Concentration of Cadmium in the Urine and in the Sweat of Two Individuals (Repeated Sampling)</td>
<td>147</td>
</tr>
<tr>
<td>17. Comparison of Cadmium Concentration and Slopes of the Calibration Curves for Urine and Sweat Analysis</td>
<td>157</td>
</tr>
<tr>
<td>18. Values Obtained for Analysis of Several Hair Strands from One Individual</td>
<td>176</td>
</tr>
<tr>
<td>19. Concentration of Cadmium in the Hair of Individuals in a Non-Exposed Population</td>
<td>179</td>
</tr>
<tr>
<td>20. Change in Cadmium Concentration per Unit Length of Hair</td>
<td>182</td>
</tr>
<tr>
<td>22. Effect of Washing Procedures on the Concentration of Cadmium in Hair: Detergent vs. Distilled-Deionized Water</td>
<td>185</td>
</tr>
</tbody>
</table>
23. Comparison of Literature Values for Cadmium in Hair ........................................... 197

24. Comparison of the Average Cadmium Concentrations Found in Urine, Sweat, and Hair for Two Individuals and for the Population Sampled .......................... 202

25. Summary of Platinum Loop and Phase Change Data for Some Compounds of Cadmium and Lead ........................................... 274
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Quartz Atomizer Cell</td>
<td>16</td>
</tr>
<tr>
<td>2.</td>
<td>Losses of Cadmium During Drying Stages</td>
<td>35</td>
</tr>
<tr>
<td>3.</td>
<td>Optical Light Path of the Single-Beam Atomic Absorption System</td>
<td>47</td>
</tr>
<tr>
<td>4.</td>
<td>Block Diagram of Atomic Absorption Unit</td>
<td>49</td>
</tr>
<tr>
<td>5.</td>
<td>Demountable Hollow Cathode Lamp</td>
<td>52</td>
</tr>
<tr>
<td>6.</td>
<td>Carbon Bed Atomizer</td>
<td>54</td>
</tr>
<tr>
<td>7.</td>
<td>Typical Absorption Signals for Direct Injection of 5 μl Whole Blood</td>
<td>60</td>
</tr>
<tr>
<td>8.</td>
<td>Typical Absorption Signals for Direct Injection of 5 μl Whole Blood at a Reduced Flow Rate</td>
<td>62</td>
</tr>
<tr>
<td>9.</td>
<td>Typical Absorption Signals for 1 μl Whole Blood Introduced on a Carbon Disk</td>
<td>66</td>
</tr>
<tr>
<td>10.</td>
<td>Typical Absorption Signals for 5 μl Whole Blood Using a Stop-Flow Technique</td>
<td>68</td>
</tr>
<tr>
<td>11.</td>
<td>Example Calibration Curve for Standard Additions to Whole Blood (Analyzed by the Filter Paper Disk Technique)</td>
<td>75</td>
</tr>
<tr>
<td>12.</td>
<td>Typical Absorption Signals for 1 μl Whole Blood Using the Filter Paper Disk Technique</td>
<td>77</td>
</tr>
<tr>
<td>13.</td>
<td>Distribution of Cadmium Concentrations in Whole Blood for Smokers and Nonsmokers</td>
<td>79</td>
</tr>
<tr>
<td>14.</td>
<td>Comparison of Calibration Curves Obtained Using Aqueous Standards and Standard Additions to a</td>
<td>xix</td>
</tr>
<tr>
<td>Topic</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Urine Sample</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>15. Distribution of Cadmium Concentrations in Urine for a Non-Exposed Population</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>16. Distribution of Cadmium Concentrations in Urine of Two Individuals (Repeated Sampling)</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>17. Comparison of Calibration Curves Obtained by Standard Additions to Two Different Urine Samples</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>18. Distribution of Cadmium Concentrations in Sweat for a Non-Exposed Population</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>19. Distribution of Cadmium Concentrations in Sweat of Two Individuals (Repeated Sampling)</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>20. Illustration of Poor Correlation Between Cadmium Concentration in Urine and in Sweat of Individuals Not Exposed to Cadmium</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>21. Concentration of Cadmium in Urine and in Sweat of Subject A as a Function of the Date</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>22. Concentration of Cadmium in Urine and in Sweat of Subject B as a Function of the Date</td>
<td>149</td>
<td></td>
</tr>
<tr>
<td>23. Typical Absorption Signals for 1-cm Segments of Hair</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>24. Change in Cadmium Concentration Along the Length of One Hair Strand</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>25. Change in Cadmium Concentration Along the Length of a Hair Strand - Composite of Analyses of Seven</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Strands from the Same Individual ............ 174
26. Correlation Between Concentration of Cadmium at
End of Hair Strand and Total Length of the
Strand .................................................. 180
27. Distribution of Cadmium Concentration at the Root
of the Hair for Males and Females ............ 184
28. Absorption Traces for 1-Liter and 2-Liter Samples
of Room Air Collected on An Active Carbon Bed . 215
29. Absorption Traces for 1-Liter and 2-Liter Samples
of Expired Air Collected on An Active Carbon Bed . 216
30. Absorption Trace for 2-Liter Sample of Expired Air
Obtained on One Occasion ....................... 218
31. Platinum Loop Atomizer ....................... 234
32. Schematic Diagram of Wire Loop Circuit ........ 235
33. Absorption Traces for 10 ppm CdCl$_2$ Using Platinum
Loop Apparatus .................................... 241
34. Absorption Traces for 10 ppm CdI$_2$ Using Platinum
Loop Apparatus .................................... 242
35. Absorption Traces for 10 ppm Cd(NO$_3$)$_2$ Using
Platinum Loop Apparatus ....................... 243
36. Absorption Traces for 10 ppm CdSO$_4$ Using Platinum
Loop Apparatus .................................... 244
37. Absorption Traces for 10 ppm Cd(C$_2$H$_3$O$_2$)$_2$ Using
Platinum Loop Apparatus ....................... 245
<table>
<thead>
<tr>
<th>Page</th>
<th>Absorption Traces for Saturated CdCO₃ Using Platinum Loop Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>246</td>
</tr>
<tr>
<td>39</td>
<td>Absorption Traces for Saturated CdS Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>40</td>
<td>247</td>
</tr>
<tr>
<td>41</td>
<td>Absorption Traces for Saturated CdO Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>42</td>
<td>248</td>
</tr>
<tr>
<td>43</td>
<td>Absorption Traces for 100 ppm PbCl₂ Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>44</td>
<td>249</td>
</tr>
<tr>
<td>45</td>
<td>Absorption Traces for 100 ppm Pb(NO₃)₂ Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>46</td>
<td>250</td>
</tr>
<tr>
<td>47</td>
<td>Absorption Traces for 100 ppm Pb(C₂H₃O₂)₂ Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>48</td>
<td>251</td>
</tr>
<tr>
<td>49</td>
<td>Absorption Traces for Saturated PbSO₄ Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>50</td>
<td>252</td>
</tr>
<tr>
<td>51</td>
<td>Absorption Traces for Saturated PbCO₃ Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>52</td>
<td>253</td>
</tr>
<tr>
<td>53</td>
<td>Absorption Traces for Saturated PbO Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>54</td>
<td>254</td>
</tr>
<tr>
<td>55</td>
<td>Absorption Traces for Saturated PbO₂ Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>56</td>
<td>255</td>
</tr>
<tr>
<td>57</td>
<td>Absorption Traces for Saturated Pb₃O₄ Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>58</td>
<td>256</td>
</tr>
<tr>
<td>59</td>
<td>Absorption Traces for 10 ppm CdCl₂ + EDTA (1:1/2 Molar Ratio) Using Platinum Loop Apparatus</td>
</tr>
<tr>
<td>60</td>
<td>257</td>
</tr>
</tbody>
</table>

xxii
50. Absorption Traces for 10 ppm CdCl₂ + EDTA (1:4 Molar Ratio) Using Platinum Loop Apparatus . . . 258
51. Absorption Traces for 100 ppm Pb(NO₃)₂ + EDTA (1:1 Molar Ratio) Using Platinum Loop Apparatus . 259
52. Absorption Traces for Cadmium in Whole Blood Using Platinum Loop Apparatus ................. 261
53. Absorption Traces for Lead in Whole Blood Using Platinum Loop Apparatus ............... 262
54. ESCA Spectrum for Platinum Wire Coated with 5M CdCl₂ ........................................ 263
55. ESCA Spectrum for Platinum Loop from Atomizer Apparatus .................................. 264
56. Scale Expansion of ESCA Spectrum for Platinum Loop from Atomizer in Region of Cadmium Emission . 265
57. Estimated Temperature of Platinum Loop as Function of Variac Reading During a Heating Cycle . . . 268
A method was developed for the direct determination of cadmium in biological materials. Determination of metals in biological samples was made difficult by the low concentration, the complex matrix, and the small amount of sample generally available. Current methods for this type of analysis involve either a wet-ashing or a dry-ashing step to break down the organic matrix and concentrate the metals present. These procedures often cause positive and/or negative errors due to contamination of the sample or loss of the metal of interest.

The described method avoided these sources of error by eliminating sample pretreatment steps. The method made use of a unique carbon bed atomizer for electrothermal atomic absorption spectroscopy. A sample introduced onto the heated carbon bed was completely decomposed and atomized before being drawn through the lightpath, where atomic absorption was recorded. Use of the carbon bed atomizer efficiently broke down the organic matrix of biological samples, thereby reducing background absorption and eliminating the need for previous ashing steps. The sensitivity of the method (approximately $10^{-13} \text{ g}$) was sufficient so that pre-concentration of the sample was unnecessary.
Use of the carbon bed atomizer was applied to a study of the cadmium concentrations in whole blood, urine, perspiration, hair, and breath samples collected from a population not occupationally exposed to the metal. Liquid samples were introduced into the atomizer by placing 1 μl on a 6-mm filter paper disk. This technique immobilized the sample and promoted complete degradation of the matrix. Background signals were reduced to approximately 6-8% absorption. Hair samples were analyzed by dropping 1-cm segments onto the surface of the hot carbon bed. Breath samples were collected by pulling the sample through a bed composed of activated carbon.

Data were collected indicating the average cadmium concentration and the range and distribution of cadmium values in blood, urine, sweat, and hair for a non-exposed population. Data also were collected illustrating individual variations in the cadmium concentration of urine, sweat, and hair. Comparisons were made between the cadmium concentrations of two or more of these excretory tissues for individuals. Estimates of the average daily excretion of cadmium through each of these tissues were calculated. The total daily excretion of cadmium from the body was estimated to be 180 μg/day, which was approximately equal to the daily intake. The results indicated that the half-life of cadmium in the
human body is considerably less than 20-40 years, as is reported in the literature.

There has been an increasing demand for analytical techniques which provide not only total metal analysis, but also differentiate between various metal compounds. Such techniques would provide information on the chemical form of a metal in a sample, which has particular significance in toxicological and environmental studies. A technique was developed which used a dual-stage atomizer for atomic absorption spectroscopy to distinguish between different chemical forms of metals. The first stage, consisting of a platinum wire loop, was gradually increased in temperature to vaporize different forms of a metal at their characteristic temperatures. The second stage, which was the carbon bed, was maintained hot to atomize the vaporous metallic species.

Absorption traces were obtained for solutions of various lead and cadmium compounds using the platinum loop apparatus. Traces also were obtained for lead and cadmium in whole blood. Differences were observed in the absorption spectra of the various samples.
GENERAL INTRODUCTION

In the past century, man has experienced a great increase in sophistication in many areas of science and technology. Technological advancements have affected fields from mechanics to medicine and from psychology to physics. Machines now do most of man's physical labor; indeed, computers perform many of his mental calculations. Advances in biology and medicine have increased man's understanding of many diseases as well as of the body's response to infection. In fact, entirely new scientific fields, such as genetic engineering or microprocessor electronics, have developed as man's knowledge and inventiveness have expanded.

Unfortunately, technological advancement has brought with it problems as well as benefits. Man has used nature's resources with little regard for the quantity and quality of these resources. Pollution of the environment has increased, posing dangers to vegetation, animal life, and to man himself. The past several decades have seen the development and manufacture of many new chemicals; these chemicals are used in a wide variety of products, including fertilizers, insecticides, drugs and cosmetics, plastics, textiles, and even foods. As a result, man and his environment are exposed daily to an increasing number of poorly understood and potentially toxic compounds.

Awareness of and concern for this problem have increased
in recent years. The Food and Drug Administration, Occupational Safety and Health Administration, and Environmental Protection Agency have enacted numerous regulations concerning the "safe" or allowable limits for many chemicals. The list of controlled chemicals grows steadily; however, the number of new chemicals increases as well, often at a faster rate.

The satisfactory control of chemicals requires a knowledge and understanding of the chemical itself as well as of its toxic effects. In order to gain this knowledge, it is necessary first to have some means of isolating, identifying, and quantifying the compound of interest. This is the challenge facing the modern-day analytical chemist. The challenge is broad and difficult; not only must the chemist identify and quantify small quantities of these chemicals, but he must do so in a wide variety of sample matrices that are often complex.

Chemical compounds may be broadly divided into three categories: organic, organometallic, and inorganic. The research described in this dissertation did not deal with organic compounds. This research involved metals and metallic compounds.

1. Metal Toxicity

Among the chemical substances that have aroused concern for their toxic effects are many metals and their compounds. Metals have been known and widely-used for
centuries; indeed, the toxicity of some metals and their compounds has been realized from the beginning of their utilization.\(^1\) For example, arsenic is historically the most important poison. It is now known to act by inhibiting sulfhydryl enzymes that are necessary in metabolism. Lead and mercury, also, have long been known as industrial and environmental toxins. Lead can cause liver and kidney damage, paralysis, and nervous system disorders, while mercury is well-known for its inducement of tremors and psychic abnormalities.

In addition to the more widely-known metal toxins, many other common metals have been shown to have adverse effects in man.\(^1\) Chromium can cause dermatitis and cancer; manganese induces muscular disorders. Cadmium has been shown to cause gastrointestinal distress, irritation of the respiratory tract, liver and kidney damage, and possibly hypertension. Vanadium induces anemia and mucous membrane irritation. Even aluminum can cause pulmonary disorders if inhaled. Often the chemical form of the metal will influence its toxic effects.

Metals are widely-used in numerous manufacturing industries. There thus has been a growing concern for the levels of metals and metal compounds in the workplace. Increasingly, attempts are being made to monitor as closely as possible the actual exposure of the worker to these toxins. Frequently this requires cumbersome and incon-
enient equipment for collection of air samples which must be worn or carried by the employee during working hours. Such monitoring practices are often impractical and uncomfortable. In addition, samples collected in this manner measure the metals present in the atmosphere around the worker, but do not take into account the absorption efficiency, retention, or excretion of these chemicals by the human body. Since the toxicity of a metal or compound will ultimately depend on its concentration within the body, an ideal monitoring procedure would directly measure this concentration. There is, therefore, an increasing demand for analytical techniques to determine metals and other toxins in biological material. Few such methods are currently available.

2. Analytical Problems in the Determination of Metals in Biological Samples

Analysis of biological material presents several unique problems for any analytical technique, including the determination of metals. These problems may be generally attributed to three factors.

The first factor which makes the determination of metals in biological material difficult is the fact that the concentration of many elements in such samples is very low. The concentration of most metal toxins in blood, for example, is within the part-per-million (ppm) or even part-per-billion (ppb) range, depending on the element. Because
of this, most analytical procedures require preconcentration steps in order to determine the amount of metal present. Of course, such treatment of the sample includes the risk of contamination from the reagents added. Loss of some of the element of interest also is possible if, for example, extraction procedures or column chromatography is used.

Analysis of biological samples also is made difficult because often only limited sample is available. In the analysis of blood or urine, sample size is sometimes only a few milliliters; tissue samples are often only a few milligrams. The analytical technique used, therefore, must possess sufficient sensitivity to allow accurate analysis on very small amounts of sample.

A third factor which causes difficulties in the analysis of biological material is the complex matrix. Most biological samples contain large amounts of organic material such as proteins, carbohydrates, and lipids that have a wide range of molecular weights. Numerous inorganic compounds also are present, such as NaCl, KCl, NaHCO₃, and CaCl₂. Often these constituents interfere in the analysis for trace metals. Because of this, many analytical procedures require an initial wet-ashing or dry-ashing step in order to decompose the matrix prior to final analysis. These procedures again risk contamination from reagents, or loss, especially of volatile metals during dry-ashing steps.
Due to the many difficulties in the analysis of biological material, few analytical methods have been available to accurately determine metals in such samples. A direct method; that is, one which requires no pretreatment of the sample, rarely has been reported. A direct method is very desirable for several reasons. First, direct methods are faster and much simpler to perform. The elimination of pretreatment steps greatly decreases the complexity of the procedure. At the same time, direct techniques eliminate the many potential sources of contamination and error associated with pretreatments. No reagents are added to the sample; no sample is removed or transferred. Another advantage in a direct method of analysis is that no sample is lost during transfer or due to heating. Direct methods, therefore, most successfully avoid both positive and negative errors associated with pretreatment steps. Unfortunately, the availability of such methods for the determination of metals in biological samples has been extremely limited.

3. Methods Available for the Determination of Metals

There are several analytical techniques for the determination of metals; several have been used in the analysis of biological or environmental samples. The advantages and disadvantages of these techniques are discussed in the following sections.
a. Colorimetric Methods

Many metals can be determined colorimetrically.\textsuperscript{2} The technique is based on the formation of a colored complex between the element of interest and, generally, an organic reagent. Some organic reagents selectively complex metals depending on the pH. By varying the pH, these reagents therefore may be used in the colorimetric determination of several elements. Other reagents are specific for one element. Common colorimetric reagents include diphenylthiocarbazone (dithizone), used for the determination of lead, cadmium, and zinc; salicylaldoxime, for the determination of copper; benzidine, used to determine lead, gold, and manganese; 2,2'-diquinolyl (cuproin), which is specific for Cu\textsuperscript{2+}; dimethylglyoxime, for nickel analysis; and alizarin, for aluminum determinations.

Colorimetric procedures are generally simple and inexpensive. The major disadvantage in such techniques is the lack of sensitivity. Colorimetric techniques generally have a sensitivity of 1-10 ppm, which is insufficient for the determination of most metals in biological material. Colorimetric techniques also require considerable time and skill for accurate analysis; there are many chances for human error.

b. Electrochemical Methods

Polarography and anodic stripping voltammetry have been used to determine several metals in biological
matrices. Although the sensitivity of polarography is only on the order of a ppm, anodic stripping can detect as little as 1 ppb of several metals. It is also possible to determine more than one metal simultaneously. Unfortunately, these techniques are susceptible to serious matrix effects. Samples and standards must be identical; the matrix also must be homogeneous. Ashing and solvation of a biological sample is therefore required. Both polarography and anodic stripping are also subject to interferences; it is necessary that any possible interferences in the sample be known and removed prior to analysis. These techniques detect only ionic metals; elements commonly determined by polarography and anodic stripping include copper, cadmium, lead, iron, and zinc.

c. Neutron Activation Analysis (NAA)

Neutron activation is a very sensitive technique for analysis of those metals which can be activated. No pretreatment of the sample is generally necessary. In the technique, the sample is exposed to neutrons which are absorbed by many elements within the sample. Absorption of an extra neutron generally makes an atom radioactive. The radioactive elements are then identified and quantified based upon their characteristic emissions (usually gamma rays).

There are some disadvantages to neutron activation analysis. Some elements (for example, tin and iron) are
inefficient neutron absorbers and therefore are poorly activated. These elements cannot be easily determined by NAA. Neutron activation also requires a neutron source, generally a nuclear reactor, which may not be readily available. A serious disadvantage is the fact that biological samples often contain many elements (such as sodium) which are very easily activated; intense emission from these elements often overlaps the weaker emission of the trace elements being determined. Because of this, additional separation steps must be employed to isolate the elements of interest before counting the radioactivity due to these elements.

d. Atomic Emission

Atomic emission is a sensitive technique for the simultaneous determination of numerous metals. The method is based on the excitation of the elements in the sample using an electrical discharge or a plasma. The excited atoms then emit characteristic wavelengths of light. Sensitivities of emission techniques range from approximately a ppm, for arc or spark emission, to 10–100 ppb for inductively-coupled plasma emission.

A serious disadvantage of emission techniques is that they are very matrix-dependent. The efficiency of excitation of an element depends on its chemical form in the sample. Samples and standards must therefore be identical, and the matrix must be homogeneous. Also, the sensitivity
of the technique varies depending on the element and its efficiency of excitation. Elements that emit in the visible region, such as the alkali metals, strontium, and manganese, may be easily detected using atomic emission, and detection limits have been reported that are less than 1 ppb. However, the technique is relatively insensitive for other elements such as selenium, mercury, antimony, and gold, which emit at short wavelengths.

Another problem, which is especially severe with plasma emission, is radiation interference due to emission within the source. Correction therefore must be made for high backgrounds. Atomic emission also requires highly skilled analysts and expensive equipment.

e. Atomic Fluorescence

Atomic fluorescence is based on radiation excitation of atoms of a single element using an intense line source such as a laser or an electrodeless discharge lamp.\(^3\) The atoms subsequently re-emit a characteristic wavelength of light which is measured at right angles to the light source. The method is generally quite sensitive (as little as a ppb of several metals can be detected).

The primary problem in atomic fluorescence is scattered light. This can cause a high and variable background for which correction is difficult. Atomic fluorescence possesses all the interferences of both atomic emission and atomic absorption.
Elements for which atomic fluorescence is particularly sensitive include zinc, calcium, and cadmium; arsenic, gold and selenium are detected poorly.

f. Atomic Absorption

Atomic absorption spectroscopy (AAS) has been the most widely-used technique for the determination of metals in biological materials. The technique is based on the absorption of a characteristic wavelength of light by atoms of an element, resulting in their excitation.\(^3\) The amount of light absorbed depends on the concentration of the element. Atoms are produced either in a flame or in a heated carbon atomizer. The sensitivity of the technique is typically about 1 ppm for flame atomizers and 1 ppb or less for carbon atomizers.

Atomic absorption has the advantage of being widely applicable to all metals. It also is relatively free from interferences. Spectral interferences; that is, overlap of the absorption lines of two or more elements, are rare. Interferences due to molecular absorption or emission in the atomizer are accommodated by background correction and modulation. Chemical interferences exist in AAS, but can be minimized by the use of a carbon atomizer in which sample breakdown and atomization is very efficient.

Atomic absorption is considered to be the most accurate method for routine metal determinations. It is a relatively simple technique that, as a rule, does not require highly
skilled analysts. In addition, the equipment necessary is readily available and comparatively inexpensive.

The development of carbon atomizers has extended the use of atomic absorption to many types of samples, including biological material. Such samples can be more efficiently decomposed and atomized using a graphite furnace than is possible using a flame. Because of the many advantages, AAS has been the most widely-used technique for the analysis of biological matter. Unfortunately, published methods often have included elaborate wet-ashing or dry-ashing steps to decompose the organic matrix prior to atomization. These steps not only complicate the analysis, but they introduce genuine risks of contamination of the sample or loss of the metal of interest. In this research, a method has been developed for the direct determination of metals in biological samples using a carbon atomizer for atomic absorption spectroscopy.

The historical development of the technique of AAS is briefly discussed in the next section.

4. Historical Development of Atomic Absorption Spectroscopy

Atomic absorption is a relatively young technique. It was first introduced by Walsh in 1955. The technique is based on the absorption of a characteristic frequency of light (the resonance frequency, ν) by free atoms of the element of interest. The factors affecting the total amount of absorption are expressed in the fundamental equation of atomic
absorption (Equation 1).\textsuperscript{5}

\[ \int_{0}^{\infty} K_{\nu} d\nu = \frac{\pi e^{2}}{mc} Nf \]  

(1)

where:

\( \int_{0}^{\infty} K_{\nu} d\nu \) = total absorption over the absorption band

\( e \) = charge of an electron

\( m \) = mass of an electron

\( c \) = speed of light

\( N \) = total number of atoms that can absorb the frequency of light \( \nu \)

\( f \) = the oscillator strength at frequency \( \nu \).

It can be seen from the above equation that all the factors affecting the amount of absorption are constants except \( N \), the number of free atoms available which can absorb at frequency \( \nu \). Thus, while \( f \) is the absolute limiting factor for the sensitivity of atomic absorption, for a specific element the size of the signal is determined by \( N \), the number of free atoms produced in the atomizer. It follows that the efficiency of the method of producing free atoms during sample analysis will determine the practical sensitivity, and thus the usefulness, of the technique.

Until the late 1960's, virtually all atomizers used in atomic absorption were flames. Flame atomizers yielded a sensitivity\textsuperscript{6} of approximately \( 10^{-7} \) grams, within the part-
per-million range, which was not low enough for analysis of most environmental and biological samples. Theoretical calculations\(^7\) showed that approximately \(10^{-16}\) grams of an element (\(10^7\) atoms) should give 1\% absorption. Obviously more efficiency in atomization was necessary in order to advance the sensitivity of the method. A significant breakthrough in atomic absorption occurred with the development of the carbon atomizer by L'vov\(^8\) in 1961. Graphite atomizers demonstrated much greater sensitivity,\(^6\) on the order of \(10^{-10}\) to \(10^{-12}\) grams of an element for 1\% absorption, but suffered from a lack of precision and accuracy. Due to these problems, it was not until 1969 that widespread interest was awakened in carbon furnace atomic absorption spectroscopy. In that year, two new designs in graphite furnaces were presented at the International Atomic Absorption Conference in Sheffield, England, one by West\(^9\) and the other by Robinson.\(^10\) Another modification was proposed by Massman.\(^11,12\) The West and Massman atomizers were subsequently adapted for use in commercial atomic absorption spectrophotometers.

Commercial graphite furnaces all operate by the passage of a high current through the carbon piece, causing resistance heating which decomposes and atomizes the sample. Unfortunately, if degradation of the sample matrix is incomplete, this causes considerable interference due to molecular absorption. Commercial carbon atomizers especially
suffer from this problem due to the fact that decomposition and atomization take place within the light path. Therefore, a three-step atomization program is generally followed using commercial spectrophotometers. The first step involves evaporation of the solvent at a relatively low temperature (approximately 100°C). This is followed by an ashing step, wherein the sample matrix is decomposed at a medium temperature (300°-500°C). Finally, atomization is accomplished as the atomizer is rapidly heated to a high temperature of between 600° and 2200°C. Absorption is recorded during this last step, always with simultaneous background correction. The precise temperatures used at each stage and the times for which they are applied depends on the sample and on the element being determined. Although this method requires precise programming of both time and temperature cycles, the technique has been developed to a high degree of reproducibility.6

The major, and indeed a very important, drawback to this atomization process is that significant losses of volatile metals occur during the drying and ashing stages, causing low results.5,13

5. Advantages of the Quartz "T" Atomizer

The problem of vaporization losses, along with the inconvenience of a time-temperature program, is avoided in the Robinson carbon bed atomizer,5 illustrated in Figure 1. This is the atomizer design used for the research reported
FIGURE 1
QUARTZ ATOMIZER CELL
(Reference 104; used by permission)
in this dissertation. The atomizer consisted of a quartz "T"-shaped absorption cell. The vertical neck of the "T" was fitted with a quartz inner sleeve packed with carbon pieces; this was the site of sample breakdown and atomization. The lightpath, which was the crosspiece of the "T", was separated from the atomization site and maintained at approximately 900°C by means of resistance heating. The carbon bed was heated to a temperature of 1450° to 1500°C by coupling to a radiofrequency generator. A vacuum pump connected to exit ports from the lightpath maintained a constant air flow through the carbon bed. A sample introduced onto the carbon bed was thus drawn through the bed at a relatively slow rate, allowing complete decomposition and atomization before the sample elements were drawn into the light path.

Oxygen in the air reacts with the carbon bed according to the following equations.\textsuperscript{14}

\begin{equation}
\frac{3}{2}O_2 + 2C \rightarrow CO_2 + CO
\end{equation}

\begin{equation}
CO_2 \xrightarrow{900^\circ C} CO + O
\end{equation}

At temperatures over 900°C, Equation 3 was favored, producing an abundance of CO in the carbon bed. Carbon monoxide, together with oxygen from the air, provided a reducing atmosphere in which organic components were reduced to CO and H\textsubscript{2}, which have only minimum absorbance in
the ultraviolet region. Molecular background absorption was therefore minimized. Metal compounds were reduced to their atomic state very efficiently, as was demonstrated by the fact that sensitivity for most elements was on the order of $10^{-12}$ grams.\(^5\)

It could thus be seen that the Robinson carbon bed atomizer fulfilled two important requirements needed for the direct analysis of trace metals in biological samples. First, the long contact time of the sample with the carbon bed under a reducing atmosphere significantly reduced molecular absorption caused by the complex organic matrix. Secondly, the sensitivity of the technique was sufficiently low to detect metals present at the sub-part-per-billion level.

It was the objective of this research to develop techniques applying these advantages of the carbon bed atomizer to the direct quantitative analysis of heavy metals in several types of biological samples. Part I of this dissertation describes the direct determination of cadmium in whole blood, urine, sweat, hair, and breath. By analyzing these various specimens, an understanding of the cadmium balance in these body fluids and tissues could be gained. In addition, the various means of excretion of cadmium from the body could be demonstrated.

6. The Importance of Metal Speciation

It has become increasingly evident that the
toxicity of a metal is dependent not only on its concentra-
tion but also upon its particular chemical form. The ion
or compound form of a metal in the environment will often
determine its toxic effects in man and animals. It is
known that the chemical form affects both the efficiency
of absorption into the body and the metabolic fate of an
element within the body system. Speciation of metals has
also become important in the study of synergistic effects
of groups of elements.

There are numerous examples of the importance of
chemical form in determining metal toxicity. For example,
hexavalent chromium is more toxic than trivalent chromium.
Nickel carbonyl is known to be more harmful than metallic
nickel or its other compounds. Lead toxicity, also, is
dependent on chemical form. Metallic lead is less toxic
than the carbonate, monoxide, or sulfate; alkyl lead
compounds are still more toxic than these species.

In view of these facts, it is apparent that there is an
increasing need for analytical techniques which provide not
only total metal analysis, but also differentiation between
various metal compounds. Analytical techniques such as
infrared and ultraviolet absorption, nuclear magnetic
resonance, and X-ray spectrometry are available and will
differentiate between metal species. However, these
techniques possess poor sensitivity and would not be suitable
for metal speciation in biological materials. Mass spectro-
metry, although quite sensitive, requires separation of the compounds of interest before analysis due to the large amount of peak overlap for most compounds. Polarography and anodic stripping voltammetry, as previously mentioned, are subject to interferences and matrix effects. These techniques also detect only ionic species. Few methods are currently available which can both separate metallic species and detect trace amounts of these compounds in biological and environmental samples.

7. Methods Available for Metal Speciation

Methods which have evolved for trace metal speciation typically involve a separation step, using a chromatographic technique or sequential chemical extractions, followed by determination of the metal content of each fraction. There are several variations in the particular technique used for each of these purposes, but most techniques have involved coupling a gas or liquid chromatograph to a metal-specific detector. The chromatographic stage separates various metal compounds based on retention time. Techniques such as atomic absorption, atomic fluorescence, or emission spectroscopy are used in place of the conventional chromatographic detector to measure the metals present as they emerge from the column. The most commonly-used technique for this purpose is AAS. Methods have been published which couple AAS to gas chromatography, ion-exchange and other forms of column chromatography,
and high performance liquid chromatography.

Although such coupled techniques for metal speciation have been successful, the use of two techniques caused additional time for analysis, expense in equipment, and margin for error. Some loss or contamination of the sample also was possible during the separation stage of the analysis. The development of a spectroscopic method which could accomplish both separation and detection of metal compounds would offer distinct advantages in speciation studies.

Part II of this dissertation describes the development of an experimental technique which uses AAS to distinguish between different chemical forms of metals. The technique involved a dual-stage atomizer where the first stage was gradually increased in temperature to selectively volatilize various chemical forms of the metal of interest. The second stage was maintained hot (1500°C) to accomplish atomization of the vaporized metallic species. In the apparatus used in the initial studies reported here, the first stage of the atomizer system was simply a loop of platinum wire which was resistance heated. The second stage was the carbon bed atomizer. The apparatus was used to study the selective volatilization of various lead and cadmium salts.

8. Summary

In summary, the following topics will be discussed in this dissertation:
Part I: The Direct Determination of Cadmium in Biological Materials

Chapter 1: The Direct Determination of Cadmium in Whole Blood

Chapter 2: The Direct Determination of Cadmium in Urine

Chapter 3: The Direct Determination of Cadmium in Perspiration

Chapter 4: The Direct Determination of Cadmium in Hair

Chapter 5: The Determination of Cadmium in Breath

Part II: Metal Speciation by Differential Atomization

Chapter 6: Differential Atomization Using a Platinum Loop-Carbon Bed Dual Stage Atomizer
PART I

THE DIRECT DETERMINATION OF CADMIUM

IN BIOLOGICAL MATERIALS
CHAPTER 1

THE DIRECT DETERMINATION OF CADMIUM IN WHOLE BLOOD

A. INTRODUCTION

The metal chosen for investigation in this research was cadmium.

In nature, cadmium is usually found in association with zinc. It is often recovered by sintering zinc ore with sodium chloride and coal, collecting volatile cadmium chloride.\textsuperscript{15} World production of cadmium in 1974 was 19,000 tons.\textsuperscript{16}

Cadmium has several industrial uses. It is used widely in electroplating metals and alloys. Cadmium is a constituent of several types of solders and bearing metals and is also used in such diverse areas as pigments, fungicides, photography, lithography, and process engraving.\textsuperscript{15} It is increasingly important as a stabilizer for polyvinyl chloride.\textsuperscript{17} Cadmium also is commonly used in the manufacture of nickel-cadmium batteries.

The properties of cadmium closely resemble those of zinc, which lies directly above cadmium in the Periodic Table. Cadmium sometimes also behaves similarly to mercury. In compounds such as oxides, fluorides, and carbonates, cadmium resembles calcium, and in fact cadmium can exchange with calcium in some minerals.\textsuperscript{18} It is a volatile element, with a boiling point of only 765°C. Its common oxidation
state is +2. Cadmium forms many complexes; for example, it combines with ligands such as ammonia, cyanide, and chloride and chelating agents such as sodium diethylthiocarbamate and dithizone.\textsuperscript{15}

1. Biological Effects of Cadmium

Many studies have shown that cadmium is a toxin and can have adverse health effects even in very low concentrations.\textsuperscript{19} Cadmium is known to be a cumulative, or additive, toxin. It accumulates in the body, particularly in the liver and kidneys.\textsuperscript{20} The metal accumulates throughout life, there being very little renal cadmium in a newborn baby.\textsuperscript{21} The estimated renal cadmium concentration in an infant is about 50 \( \mu g/g \) of ashed tissue, compared to 2,000 \( \mu g/g \) for an adult.\textsuperscript{22} The average body burden of cadmium in an adult American has been estimated to be about 30 mg, of which approximately 10 mg are in the kidneys and 5 mg are in the liver.\textsuperscript{21} Excretion of the metal was noted to be extremely slow; the half-life was estimated to be between 20 and 40 years.\textsuperscript{19} This excretion period seems to be unusually long and will be examined in this study.

Several detrimental health effects have been linked to cadmium exposure. It has been suggested that ingestion causes gastroenteritis;\textsuperscript{23} inhalation produces severe pulmonary dysfunction\textsuperscript{24} and emphysema.\textsuperscript{25} Renal dysfunction,\textsuperscript{26-28} anemia,\textsuperscript{29,30} and hepatic dysfunction\textsuperscript{25} have also been reported due to exposure to cadmium.
Cadmium has been shown to interfere with carbohydrate metabolism as well as other metabolic processes.\textsuperscript{31,32} It is believed that such toxic effects of cadmium are primarily due to it chemically binding to cysteinyI residues of many enzymes, inhibiting their activity.\textsuperscript{33-36} Cadmium is thought to displace zinc in various enzymes, thus inducing diseases through renal or hepatic zinc deficiencies.\textsuperscript{37-39} Zinc is known to be an essential nutrient and a necessary metal component of at least eighty metabolic enzymes.\textsuperscript{40} The chemical similarities between the two metals apparently contributes to their exchange in body proteins. In view of the fact that the precise metal components of enzymes dictate the conformation required for catalytic activity, such replacement of zinc by cadmium could severely alter this activity.

It has been proposed that exposure to cadmium may be a major factor in the pathogenesis of human essential hypertension.\textsuperscript{33} Studies have shown that renal cadmium concentrations were significantly higher in hypertensive subjects, some showing as much as a 40-fold increase over normal cadmium levels.\textsuperscript{38} Most of the data relating cadmium to hypertension, however, come from animal studies, primarily with rats. It has been reported that rats fed 5 ppm cadmium in their drinking water developed significant hypertension.\textsuperscript{41} Numerous related studies have been carried out, both on rats given cadmium in their diet\textsuperscript{22,42-44} and
The mechanism by which cadmium raises arterial blood pressure is not clearly understood; however, it has been suggested that cadmium accumulation in the kidneys inhibits sodium excretion, thereby disrupting electrolyte and fluid balance in the body. It is also possible that the metal has a direct effect on vascular smooth muscle.

It has been suggested that the ratio of cadmium to zinc in the body may be a more meaningful measure of the effect of cadmium exposure. The exchangeability of the metals indicated that in some cases zinc can prevent certain cadmium-induced effects.

2. The Exposure of Man to Cadmium

Cadmium is widely dispersed in our environment and is present in trace quantities in air, water, and food. One source of cadmium contamination in the environment is industrial emissions. It has been estimated that 4.6 million pounds of cadmium were lost as emissions during production processes in 1968.

The major source of man's accumulation of cadmium was considered to be the diet. The normal levels of cadmium in food are less than 0.05 ppm, although certain foods such as liver, kidney, and shellfish may contain higher concentrations. The daily intake of cadmium due to the diet has been estimated to be about 50 μg.
Water contributes little to the daily cadmium intake. The E.P.A. limit for cadmium in water is 10 ppb, but most drinking water contains less than 1 ppb. Some incidences of higher concentrations in water have been reported in industrial areas.

Normal cadmium concentration in the air is approximately 0.01 to 0.05 μg/m³ of air, and thus ambient air contributes less than 0.1 μg of cadmium to the daily intake. However, smokers may be exposed to significantly greater amounts of cadmium. It has been shown that a cigarette contains approximately 1.0-1.5 μg of cadmium, part of which is inhaled and retained in the lungs. Other studies indicated that cigarette smoke contained the equivalent of 4.5 μg/m³ cadmium. It has been estimated that between 25 and 50 percent of the cadmium inhaled is absorbed by lung tissue and that between 0.1 and 0.2 μg of cadmium could be inhaled by smoking one cigarette. Cigarette smoke thus was a significant source of absorbable cadmium, strongly contributing to its accumulation in man. Indeed, the body burden of cadmium has been found to be higher in smokers than in nonsmokers, and daily excretion of cadmium in the urine has been shown to have a positive correlation with the average number of cigarettes smoked. A 1976 German study found blood cadmium levels of smokers to be 50% greater than that of nonsmokers.
3. Reasons for This Study

There has been a greatly increased interest in the toxic effects of cadmium in the environment. Unfortunately, the methods for determining cadmium in environmental and clinical samples are of doubtful accuracy and precision. Often values for cadmium concentration reported in the literature can not be taken at face value.\(^\text{16}\) Therefore, there is an increasing demand for a simple and accurate method for monitoring cadmium in biological materials.

In any toxicological study, it is also important to choose a sample which is convenient to collect and which reliably indicates the exposure level or body burden of the subject. In the case of cadmium, it is well known that the metal accumulates in the human body, primarily in the liver and kidneys.\(^\text{20}\) Liver and kidney tissue analysis may thus be the most reliable monitor of cadmium body burden. Unfortunately, this is not a desirable procedure for normal human subjects, primarily because of the trauma it causes for the patient.\(^\text{16}\)

The analysis of whole blood for cadmium was initially investigated in this research because it was thought that such analysis could prove to be a convenient method of monitoring cadmium exposure in man. Development of a technique to determine cadmium in the blood directly could also lead to similar methods for analysis of heavy metals in other biological matrices. In light of the data on the
elevated levels of cadmium in cigarette smoke,\textsuperscript{54-57} it was also felt to be of interest to determine whether the concentration of cadmium in the blood of smokers was higher than that of non-smokers.

4. Difficulties in the Determination of Cadmium in Biological Materials

Little work has been published concerning cadmium analysis in blood, and of those methods found in the literature, none has described a direct determination. "Direct determination" has been used here to describe a method in which no sample pretreatment is required. The absence of direct methods is largely due to the very difficult problems in determining cadmium in biological materials. Difficulties are encountered at every stage of the analysis. This includes initial sample collection, sample storage, standardization, final measurement, and interpretation of the result. These problems apply not only to blood analysis, but also to the analysis of urine, tissue, and other biological materials.

a. Problems in Sample Handling

The first dilemma encountered in the analysis of blood, or other biological materials, is in obtaining and storing the sample. Care must be taken to avoid contamination during sample collection, transfer, or storage. Needles, containers, syringes, and other instruments must be cleaned thoroughly prior to contact with
the sample. Obviously, the presence of any trace cadmium on these items will contaminate the sample; this contamination becomes particularly significant if the metal is initially present in part-per-billion quantities. Cleaning with nitric acid is a common means of decontaminating glass and metal equipment.\textsuperscript{16}

If the sample must be stored, steps must be taken to avoid loss of cadmium due to plating out onto the walls of the container. Blood samples are particularly difficult to store. Agents must be added to prevent coagulation; often Na\textsubscript{2}– or K\textsubscript{2}-EDTA is used for this purpose. Samples should be stored at 4°C, and they must be frozen for long-term storage or transportation over long distances. Storage of standard solutions must also be considered; adsorption of the metal onto the container walls is the most significant problem in this case. Polyethylene, polypropylene, and teflon have been reported to be most suitable for storage of aqueous standards because of their low adsorptive properties.\textsuperscript{62} Solutions should be mixed and stored in containers preequilibrated with solutions of the same concentration.\textsuperscript{18}

Perhaps the most difficult step before analysis of blood or other biological matter is obtaining a reproducible and representative sample for analysis. This has been found to be a particular problem with blood samples due to the high viscosity.
b. Problems Due to Low Concentration

The low concentration of cadmium in the blood, which is on the order of 10 ppb, presents a challenge for most analytical procedures. Using conventional procedures, separation and preconcentration steps are often required prior to analysis. These steps can lead to loss of the metal of interest or to contamination of the sample. These sources of error can generate high results or low results depending on the severity. The net result is that the error involved in the procedure may be greater than the answer obtained, and the data is not only valueless but may be misleading.

c. Problems Due to the Matrix

The variable and dense matrix of blood has caused additional difficulties in analysis. Blood contains a high and variable inorganic content; some of the ions present include sodium, potassium, calcium, magnesium, chloride, phosphate, bicarbonate, and sulfate. The principal cations are sodium and potassium, while chloride is the predominant anion. Blood also contains numerous and diverse organic components, primarily proteins of a wide range of molecular weights, carbohydrates, and lipids. A list of major components of human blood is presented in Table 1. These components can cause irreproducible results when the sample is not completely broken down. High molecular background also can be a
Table 1

APPROXIMATE RANGES OF VALUES FOR THE PRINCIPAL CONSTITUENTS OF HUMAN BLOOD PLASMA

Organic Constituents

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal Range (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>5700-8000</td>
</tr>
<tr>
<td>Urea</td>
<td>20-30</td>
</tr>
<tr>
<td>Glucose</td>
<td>65-90</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>70-105</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>8-17</td>
</tr>
<tr>
<td>Lipids</td>
<td>285-675</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>130-260</td>
</tr>
</tbody>
</table>

Inorganic Constituents

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal Range (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>132-150</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.8-5.4</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.5-5.6</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.6-2.2</td>
</tr>
<tr>
<td>Iron</td>
<td>0.009-0.032</td>
</tr>
<tr>
<td>Copper</td>
<td>0.001-0.002</td>
</tr>
<tr>
<td>Chloride</td>
<td>100-110</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>24-30</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.6-2.7</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0.7-1.5</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.0006-0.001</td>
</tr>
</tbody>
</table>
problem in many analytical procedures if the matrix is not completely destroyed. This limits the sensitivity of the technique as well as its accuracy. The variable matrix of blood also makes it virtually impossible to create standard solutions representative of the sample; this significantly complicates calibration procedures.

As a result of these difficulties with the organic matrix, current methods of blood cadmium analysis include some sample pretreatment to destroy the organic material. The most common treatments are wet ashing or dry ashing.

Wet ashing commonly involves dissolution of the sample in nitric acid together with an oxidizing agent such as sulfuric acid, perchloric acid, or hydrogen peroxide. This treatment breaks down the organic matter. The residue then may be redissolved in a suitable solvent for subsequent analysis. The danger in these procedures, of course, is that contamination of the sample may occur from the reagents added. In fact, the purest acids commercially available contain as much as 1 ppb Cd. Since the concentration of cadmium in the blood is on the order of 10 ppb, a 10% error can occur simply due to the acids used in ashing procedures.

In contrast, dry ashing procedures involve heating the sample to evaporate and burn any organic material prior to actual analysis. There is a genuine danger of
loss of volatile metals (such as cadmium) during the drying and ashing steps of these procedures. In fact, it is known that several cadmium salts have relatively low boiling points; an example of this is Cd(NO$_3$)$_2$·4H$_2$O, which boils at 132°C.$^{66,67}$

Many researchers assumed no loss of cadmium at temperatures below 500°C;$^{68-70}$ however, others have reported losses at temperatures over 300°C using a heated graphite furnace.$^{71,72}$ Studies were carried out using the quartz "T" atomizer employed in this research to investigate possible cadmium losses during evaporation stages.$^{73}$ In these studies a carbon disk impregnated with an aqueous cadmium standard was lowered to within 50 mm of the heated carbon bed. Radiant heat from the atomizer dried the sample, after which the disk was dropped onto the surface of the bed to accomplish atomization. The absorption signal was monitored throughout the procedure. Significant signals were observed during the drying step. Comparison with the background signal (recorded using a hydrogen lamp) confirmed that the losses were indeed cadmium (Figure 2).$^{73}$

Such vaporization losses prior to the atomization stage result in serious negative errors in cadmium determinations by graphite furnace atomic absorption. Conventional graphite furnace techniques essentially accomplish a dry-ashing within the atomizer. A three-step atomization program includes stages for drying and ashing prior to the
This figure shows the cadmium absorption signal obtained during drying of an aqueous cadmium standard over the carbon bed atomizer.
absorption measurement. There is a real danger of loss of volatile cadmium during the preliminary stages of the atomization program.

It has been suggested that losses due to vaporization are often dependent on the particular matrix and that optimum temperature conditions for ashing should be determined for each different matrix. This can be done by monitoring the loss of radioactive $^{109}$Cd added to the sample; unfortunately, such requirements greatly complicate the analysis procedure.

5. Common Methods for the Determination of Cadmium in Biological Materials

Some examples of methods of cadmium analysis found in the literature will be discussed in more detail. As was previously mentioned, several procedures require an initial separation or preconcentration step before actual analysis. This may be accomplished by any one of several methods, the most common of which is extraction into a suitable organic solvent. Typically, trace quantities of cadmium are combined with an organic chelating agent. Commonly used reagents include dithizone, ammonium pyrrolidine dithiocarbamate (APDC), and sodium diethyl-dithiocarbamate (SDDC).

One of the more widely-used separations of this type involves the complexing of cadmium with SDDC at pH 11. Complexation is carried out in a solution containing...
tartarate, ammonium, and cyanide ions. Subsequent extractions often are made into carbon tetrachloride or methyl isobutyl ketone; the latter solvent may be directly aspirated into a flame for elemental analysis.

Other methods of preconcentration may be found in the literature. These include electrodeposition, precipitation as the sulfide (zinc often coprecipitated, however), and ion exchange. The latter two techniques have been commonly used in conjunction with neutron activation analysis or some other nuclear method.

a. Colorimetric Methods

Extraction with dithizone is the basis for the most commonly used spectrophotometric method for cadmium analysis. The method involves extraction of the sample from an aqueous solution containing tartarate, hydroxide, cyanide, and hydroxylamine hydrochloride into chloroform containing dithizone. Cadmium forms a red-colored complex at pH 12. The organic phase is extracted into tartaric acid and an aliquot re-extracted with dithizone solution. Absorbance of the chloroform layer is measured at 518 nm. This method is reasonably specific for cadmium, although other metals such as zinc can be extracted at lower pH values. The reported sensitivity is 1 to 2 ppm. The accuracy of the dithizone method depends on the care and skill of the analyst. Special care must be taken to avoid contamination of the sample from the reagents used.
In addition, there is a danger of loss of some cadmium during the extraction sequence because of less than completely efficient extraction coefficients. The possibility of contamination is multiplied because it is necessary to digest the sample and put it into aqueous solution prior to analysis. Nevertheless, the dithizone method is inexpensive and was the most common procedure for cadmium determination before the advent of atomic absorption methods.

b. Atomic Emission Methods

Atomic emission has been used to determine cadmium using the emission lines at 2288\(^{\AA}\) or 3261\(^{\AA}\).\(^{18}\) An advantage of this method is its multielement capability. Unfortunately, there are several disadvantages associated with the technique. There are difficulties in controlling excitation conditions; pretreatment (by ashing) and the use of an internal standard are necessary. A great disadvantage of the technique is its lack of sensitivity. Detection limits for atomic emission are on the order of a part-per-million.\(^{78}\) Preconcentration is therefore required for most biological samples.

Atomic emission with an inductively-coupled plasma excitation source has become more widely used for determination of trace metals in biological samples. Reported detection limits for cadmium are approximately 2 ppb.\(^{79}\) However, severe problems with radiation interferences make
background corrections imperative. Background interference is frequently high and variable, and it has a direct effect on the resonance emission. Emission methods also require skilled analysts and often expensive equipment.

c. Atomic Absorption Methods

Currently, atomic absorption is by far the most common method for determining trace metals in biological materials. Aspiration as a liquid into a flame is the routine atomization procedure. This method of atomization requires that the sample be digested and dissolved in a suitable solvent. At times preconcentration steps are also necessary. The most common treatment procedures involve ashing with nitric acid, followed by dilution to a known volume and direct aspiration into a flame. Any of the previously discussed methods of ashing or concentration may be used, however.

Several specialized procedures have been developed for the analysis of cadmium in micro-samples of blood. A technique using a tantalum boat has been reported by Hauser. In this method, 0.5 ml of blood was placed in a tantalum boat and dried in a vacuum oven for one hour (60°-62°C, 0.5 atm.). The sample was then ashed at low temperature for 16 hours and finally inserted into a flame for atomization. The absorbance of cadmium was measured at 2288 Å. The reported detection limit for this technique was 0.2 ppb.
A modification of the technique of Hauser, called the Delves cup technique, has been commonly used for blood analysis by flame atomic absorption. This technique was first reported by Delves\textsuperscript{82} and later modified slightly by Ediger and Coleman.\textsuperscript{83} Basically, the technique involved drying a 10-μl sample of blood in a nickel cup at 150°C for one minute. The cup then was mounted near the burner of the spectrometer and pushed to within 15 mm of the center of the flame, where organic material was burned. After combustion, the sample was pushed to the center of the flame and the atomic absorption recorded at 2288Å. The reported detection limit was 0.2 ppb. The Delves cup technique also has been used in conjunction with extraction procedures.\textsuperscript{84}

Obviously, these methods required the rigid control of several variables in order to obtain reproducible results. One of the most important parameters was the time allowed for the combustion of the organic matrix. This time requirement was determined using the 2833Å line of lead.\textsuperscript{85} Blood samples were heated at 425°C for various time periods while the 2288Å line was monitored. The optimum oxidation time was taken as the minimum time required for the absorption at this wavelength to disappear. It was assumed that this initial absorption peak was due to background from the burning organic material. Optimum times for combustion had to be determined for each different sample matrix.
Graphite furnace atomic absorption has been used more frequently in recent years for trace metal determination. It has the important advantage of an increased sensitivity over flame methods. Graphite furnace AAS also requires much smaller samples than other techniques.

All reported methods for cadmium analysis by graphite furnace atomic absorption employ the conventional three-step atomization process, in which programmed heating stages accomplish drying the sample, ashing, and atomization at selected temperatures. Precise control of the atomization program is required in these methods. In addition, correction must be made for high background (molecular) absorption.

Furnace atomic absorption procedures often require pretreatment of the sample by wet-ashing or dilution to decrease the effect of the organic matrix. Some of the remaining background absorption is due to light scattering by smoke from the burning sample, while some is accounted for in the broad-band absorption of molecular species not completely decomposed in the ashing step. Background absorption may be corrected for using any one of several techniques. The most common corrections employ either a deuterium lamp or a non-absorbing line emitted by the hollow cathode light source which is adjacent to the resonance line.
Another consideration in the analysis of blood samples is the preparation of standards. Chemical interferences can occur in AAS due to differences in the efficiency of atomization of different chemical forms of an element. Therefore, it is important that standards be prepared which are representative of the sample to minimize errors due to differences in the matrix. This usually requires the use of standard additions.

Several examples of the use of programmed graphite furnace atomic absorption for determination of cadmium in blood have been reported in the literature. These methods differ in the sample pretreatment or introduction technique as well as in the specific times and temperatures used for the three atomization stages. It cannot be overemphasized that a major source of error inherent in these techniques is the loss of volatile cadmium during the ashing stages.

**d. Other Methods**

Several other methods of cadmium analysis in biological matter deserve mention. Neutron activation analysis (NAA) has been used; the most commonly employed reaction is $^{114}\text{Cd}(n,\gamma)^{115}\text{Cd}$. The $^{115}\text{Cd}$ obtained in this transformation has a half-life of 53.5 hours. Unfortunately, direct determination of cadmium in biological materials is not possible using NAA due to the ease of activation of many other elements with overlapping gamma lines. The most
A troublesome element in this respect is sodium, which very easily forms $^{24}\text{Na}$ (half-life 15 hours). Radiochemical separations are therefore necessary. This technique involves the use of any one of the previously mentioned extraction procedures for separation of the cadmium from the remainder of the activated components in the sample. Activation analysis has the advantage of extreme sensitivity (less than 1 ppb). Several examples can be found in the literature.93-97

Electrochemical methods such as polarography,71 anodic stripping,98,99 and ion-selective electrodes100 have also been reported for cadmium determinations. Both polarography and anodic stripping voltammetry have the distinct disadvantages of long analysis time and extensive sample pretreatment. Ion-selective electrodes lack sufficient sensitivity to determine cadmium at concentrations much less than 1 ppm. Spark-source mass spectrometry is not widely used because of the expense of the equipment and the relatively poor accuracy.16 Atomic fluorescence likewise is not widely used, although it possesses the required sensitivity.101 One of the main problems with this technique is scattered light. An example of the determination of cadmium in blood using atomic fluorescence spectrometry has recently been reported by Michel, et al.102

6. Need for an Improved Analytical Procedure

Each of the aforementioned analytical techniques
has its own advantages and shortcomings. A rough comparison of the more widely used methods is presented in Table 2. The sensitivities given are merely estimated and will vary greatly from laboratory to laboratory depending on the blank level and the criteria used for measurement and expression of a detection limit.\textsuperscript{16} For determination of low cadmium concentrations, only neutron activation, atomic absorption, and electrochemical (anodic stripping) methods have been commonly used. None of the reported methods represents a true direct determination, in which no sample pretreatment or ashing is involved. Such a direct method is very desirable because it eliminates both contamination due to reagents added and losses due to volatilization. The speed and simplicity of analysis also is significantly increased.

The use of the Robinson quartz "T" atomizer made direct analysis of biological samples possible. There were several advantages in the use of this technique as opposed to conventional methods of analysis. First, atomization was a one-step process, eliminating the necessity of precise program control. Secondly, the sample was in contact with the bed for several seconds, allowing a more complete breakdown of the sample matrix without previous ashing steps. The matrix was effectively reduced to CO and H\textsubscript{2}, reducing the molecular background. At the same time, metals were more efficiently atomized, eliminating
Table 2

COMPARISON OF ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF CADMIUM IN BIOLOGICAL MATTER

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sensitivity (ppb)</th>
<th>Accuracy (%)</th>
<th>Sample Preparation</th>
<th>Sample Size</th>
<th>Skill Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dithizone</td>
<td>50</td>
<td>5-10</td>
<td>Ashing, solution</td>
<td>5 ml</td>
<td>High</td>
</tr>
<tr>
<td>Electrochemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC polarography</td>
<td>1,000</td>
<td>5-10</td>
<td>Ashing, solution</td>
<td>1-10 ml</td>
<td>High</td>
</tr>
<tr>
<td>Pulse polarography</td>
<td>10</td>
<td>10</td>
<td>Ashing, solution</td>
<td>1-10 ml</td>
<td>High</td>
</tr>
<tr>
<td>Anodic stripping voltammetry</td>
<td>1</td>
<td>20</td>
<td>Ashing, solution</td>
<td>1-10 ml</td>
<td>Moderate</td>
</tr>
<tr>
<td>Spectroscopy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atomic Emission</td>
<td>50</td>
<td>5</td>
<td>---</td>
<td>---</td>
<td>High</td>
</tr>
<tr>
<td>Atomic Absorption</td>
<td>10</td>
<td>5</td>
<td>Ashing, solution</td>
<td>5 ml</td>
<td>Moderate</td>
</tr>
<tr>
<td>(Flame)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atomic Absorption</td>
<td>1</td>
<td>10</td>
<td>Ashing</td>
<td>5-10 µl</td>
<td>Moderate</td>
</tr>
<tr>
<td>(Furnace)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atomic Fluorescence</td>
<td>1</td>
<td>10</td>
<td>---</td>
<td>---</td>
<td>Moderate</td>
</tr>
<tr>
<td>Neutron Activation</td>
<td>1-10</td>
<td>20</td>
<td>Ashing, solution*</td>
<td>10-100 mg</td>
<td>Moderate</td>
</tr>
<tr>
<td>Mass Spectrometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spark source</td>
<td>10-100</td>
<td>20</td>
<td>---</td>
<td>---</td>
<td>High</td>
</tr>
</tbody>
</table>

* Ashing and dissolution necessary for radiochemical separations.
chemical interferences. Since atomization took place outside of the lightpath, scatter and background absorption caused by smoke or burning organic matter was decreased.

Another advantage of the quartz "T" atomizer was that there was no loss of sample. This was avoided because all the sample elements were drawn through the bed and the lightpath. The absorption signal was constantly monitored. Finally, the more efficient atomization as well as the long lightpath sufficiently increased the sensitivity of the quartz "T" so that preconcentration of the sample was unnecessary.

B. EXPERIMENTAL

1. Equipment

This section describes the components of the single beam atomic absorption spectrometer used in this research. Some components were designed in these laboratories, while others were units taken from commercial equipment. The spectrometer was first assembled and used by a former member of this research group, D. K. Wolcott.73

A diagram of the optical light path can be found in Figure 3. Because this was a single beam system, periodic monitoring of the light intensity and detector response was necessary. Although this requirement was an inconvenience which could have been eliminated by using a double beam system, it was not felt that the increased complexity of
FIGURE 3

OPTICAL LIGHT PATH OF THE SINGLE-BEAM ABSORPTION SYSTEM

(Reference 57; used by permission)

1) Source: Hollow Cathode Lamp
2) Focusing Lens
3) Atomizer
4) Grating Monochromator
5) Photomultiplier Tube (Detector)
a double beam instrument offered sufficient advantage
over the simpler single beam optics.

A list of components follows below. A schematic
diagram of the complete system is presented in Figure 4.

a. Light source: Barnes Glomax Demountable Hollow
Cathode Lamp System

b. Chopper: Jarrel-Ash mechanical, from a Model 82-360
atomic absorption instrument

c. Atomizer: designed and constructed by previous
researchers (Figure 1)

d. Monochromator: Jarrel-Ash Model 82-000, 0.5 meter
Ebert scanning monochromator with variable slits

e. Detector: Jarrell-Ash Model R106 photomultiplier

f. Amplifier: P.A.R. Model 126 lock-in amplifier with
Model 184 photometric preamplifier

g. Recorder: Beckman Model 10005, 10-inch potentiometric
strip-chart recorder

h. Detector power supply: Hewlett-Packard Harrison
Model 6515-A, 0-1600 volt, 0-5 mA

i. Radiofrequency generator: Lepel Model T-5-3-mc-j-b,
5000 watt, 3-10 MHz

j. Cell vacuum pump: Thomas Model 107CA20-1

k. Lamp vacuum pump: Welch Duo- Seal, Model 1404

l. Flowmeters: Matheson Model 7728, Series 602;
Brooks Series 2-65B

m. Potentiometer: Heliopot Corp. Model T-10-A, 10,000 ohm,
FIGURE 4
BLOCK DIAGRAM OF ATOMIC ABSORPTION UNIT
(Reference 104; used by permission)

1. HOLLOW CATHODE LAMP  6. ATOMIZER-CELL  10. POWER SUPPLY
2. CHOPPER  7. FLOWMETER  11. MONOCHROMATOR
3. PLANO-CONVEX LENS  8. AIR PUMP  12. AMPLIFIER
4. SAMPLE INTRODUCTION  9. PHOTOMULTIPLIER  13. POTentiOMETER
5. RF COILS

11

14

13

12

9

10

3

6

5

4

8

2

1

7

3
ten-turn

n. Optics: Amersil Corp. Suprasil Grade fused silica lenses with 180.0 nm transmission cutoff

o. Other equipment:
   i. Eppendorf 5 µl autopipette
   ii. Finnpipette 1-5 µl autopipette
   iii. Hamilton microliter syringe, No. 701, 10 µl, with Chaney adapter
   iv. Whatman 41 ashless filter paper
   v. Union Poco-Graffilters carbon filters, grade XA3
   vi. Ultracarbon Ultra "F" Purity carbon rods
   vii. Spectroscopically pure quartz, General Electric type R-204
   viii. 6 mm paper punch
   ix. Optical pyrometer, Leeds and Northrup Model 8632-C, 750°-3500°C temperature range

The major components of the system are described in more detail below.

a. Light source. A commercially available demountable hollow cathode lamp (Barnes Engineering Company, Stamford, Conn.) was used for part of this research. The advantage of a demountable hollow cathode was the speed and ease with which the cathode could be removed and replaced to change the element being determined. The commercial design was modified and redesigned by Wolcott; subsequently, improvements were made on this design to overcome problems with
air leaks by R. Binder and L. Rhodes. The design which was used for part of the work described in this dissertation is shown schematically in Figure 5. The operating conditions used for cadmium determinations were He filler gas at 8 mm Hg and a current of 30 mA. The analytical line used was the resonance line at 2288 Å.

b. Chopper. The purpose of a chopper was to create a modulated signal from the hollow cathode lamp which was then directed through the light path of the atomizer. The amplifier used was A.C. and so monitored only the interrupted light emitted from the lamp. Any emission of light caused by atoms in the light path returning to the ground state was D.C. and so was not detected. The practice of using a chopper thus eliminated spectral interferences due to emission by components of the sample.

c. Atomizer. The atomizer is illustrated in Figure 1. The absorption cell was constructed of spectroscopically pure quartz in the shape of a "T". Two quartz disks were fused to the ends of the crosspiece, which served as the lightpath of the cell. Two vacuum ports, located approximately one inch from the disks, were connected to a pump which maintained a constant flow through the cell. The lightpath portion of the cell was wound with nichrome wire and several layers of asbestos cord and tape; approximately 6 amps of current was passed through the wire to heat the light path to a temperature of at least 900°C.
FIGURE 5
DEMOUNTABLE HOLLOW CATHODE LAMP
(Reference 103; used by permission)
This elevated temperature was designed to assure that atoms entering the light path remained in the atomic state for absorption to occur.

The vertical stem of the "T" absorption cell was fitted with a quartz inner sleeve. This sleeve was packed with pieces of carbon, approximately 1 cm in length, cut from spectroscopically pure Ultracarbon rods. The bottom of the inner sleeve was slightly tapered to hold a carbon disk approximately 3 mm thick which served to hold the carbon pieces in the quartz tube. Holes were machined into the carbon disk to allow free air flow through the atomizer cell. Use of the inner sleeve had three important advantages. First, it prevented devitrification of the absorption cell itself caused by repeated heating of the carbon bed. Secondly, the inner sleeves could be easily replaced when they became devitrified. Thirdly, the sleeves could be easily removed for refilling with fresh carbon without dismantling the entire absorption cell. A more detailed illustration of the atomizer section of the absorption cell is shown in Figure 6.

The bed was heated by coupling with a radiofrequency (RF) generator and was maintained at 1450°-1500°C. An optical pyrometer was used to monitor the temperature of the carbon bed during use. At temperatures above 1550°C, the quartz became soft and the lifetime of the absorption cell was greatly reduced. The melting point of quartz
FIGURE 6
CARBON BED ATOMIZER
(Reference 73; used by permission)
(1650°C) represented the upper temperature limit at which the atomizer could be operated.

Oxygen in the air drawn over the carbon bed was converted primarily to CO at temperatures greater than 900°C. This provided a reducing atmosphere favorable to the formation of free atoms from a sample introduced onto the bed.

The bed was cleaned before use each day by heating it to 1450°-1500°C and allowing the resonance signal to return to baseline. At this point, it was assumed that no cadmium contamination was present on the surface of the carbon composing the bed.

d. Monochromator and detector. Light passing through the atomizer was focused onto the entrance slit of a monochromator. The monochromator contained a diffraction grating (1120 lines per mm) which separated the wavelength of interest and focused it onto the photomultiplier. The signal was then amplified using a phase-sensitive A.C. amplifier and recorded.

2. Procedures for Obtaining Blood Samples

Blood samples were obtained from the LSU Student Health Service laboratory. Samples were collected from LSU students by Health Service personnel, using cleaned and sterilized needles and glass Vacutainer vials. K₂-EDTA was present in the vials to prevent coagulation. Samples were stored if necessary at 4°C.
It was thought that the presence of EDTA would complex some of the metals present and minimize their plating out onto the walls of the container. EDTA would also decrease chemical interferences by putting much of the cadmium in the EDTA-complexed form.

3. Development of a Technique for the Direct Determination of Cadmium in Whole Blood

This section describes the various techniques which were investigated in the development of a suitable method for directly determining cadmium in whole blood. It was desirable that a method meet the following requirements in order to be an acceptable means of analysis.

a. No sample pretreatment. Any addition of reagents, dilution, separation, or ashing procedures would be a source of contamination errors or loss of volatile cadmium. Therefore, it was considered of primary importance that the method should be for the direct analysis of whole blood; that is, analysis with no prior sample treatment.

b. Low blank level. The absorption due to cadmium in any blank determination should be minimized.

c. Low background (molecular) absorption. The absorption of molecular species; that is, non-specific absorption, must be kept as low as possible.

d. Simple, reproducible sampling. An aliquot of sample for analysis must be easily and accurately measurable.

e. Reproducibility. The absorption for repeated
aliquote of the same sample must be as reproducible as possible. This property depended on many of the conditions of analysis, such as bed temperature, air flow, reproducible sample size, reproducible blank, and condition of the carbon bed. It proved to be the most difficult requirement to fulfill and the source of the most frustration. Reproducibility will be further discussed in later sections.

f. Sensitivity. The method chosen must be sufficiently sensitive to allow direct measurement of cadmium at the ppb level.

Several procedures for the analysis of whole blood for cadmium were attempted, with varying amounts of success. Each of these techniques will be discussed separately.

a. Direct Injection of Whole Blood

One of the first methods of sample introduction attempted was the direct injection of a 5-μl aliquot of whole blood using an Eppendorf micropipette. The sample was injected onto the top of the hot (1450°-1500°C) carbon bed. This was the routine method of injection for aqueous standards; good reproducibility had always been obtained for such solutions. Unfortunately, reproducibility for direct injection of blood was very poor; successive sample aliquots gave widely varying absorption signals. This problem was thought to be due mainly to the viscous nature of whole blood and the
difficulty in completely ejecting all of the sample out of the pipette. At times, if the sample was not injected rapidly, heat from the carbon bed caused the pipette tip to clog or caused some of the blood sample to coagulate on the edge of the pipette tip. Consequently, a consistent amount of sample was not always introduced into the atomizer.

Rapid injection using an Eppendorf pipette required a rather sudden ejection of the sample from the pipette. As a result, many injected samples hit the surface of the carbon bed with force, rather than simply falling onto the surface. This sometimes caused a visible disruption at the surface of the carbon, with carbon dust being dislodged into the air above the bed. After some injections, a visible explosion was seen as the sample burned. These observations indicated that part of the reason for inconsistency in the absorption signals was a differing reaction of the sample at the carbon surface, depending on how and where the sample hit the bed. Explosions at the surface sometimes caused carbon dust to be blown into the light path, scattering some incident radiation. It was also possible that such explosions exposed a new surface on the carbon pieces nearby, containing cadmium contamination not previously cleaned by heating the bed. Thus, the cadmium signal recorded for the sample would be in positive error. Indeed, larger absorption signals were observed when visible explosions
took place at the surface of the carbon bed.

Another equally important problem with the method of direct injection was that the background absorption was very high. Background or molecular absorption was measured by the absorbance of a non-resonance line of the cadmium hollow cathode found at 2266Å. It was felt that this non-absorbing line was close enough to the resonance line at 2288Å to be representative of molecular absorption at the resonance wavelength. Our instrument did not have an automatic background corrector; therefore the background measurement had to be made subsequent to the resonance measurement using a second aliquot of sample.

Figure 7(a) shows a typical resonance absorption signal for 5 µl of whole blood injected directly into the atomizer. Figure 7(b) represents the background absorption at 2266Å for the same sample.

b. Direct Injection at a Reduced Flow Rate

An attempt was made to reduce molecular absorption by decreasing the flow rate of air through the atomizer. The normal flow rate used was approximately 200 cm³/min.; reducing this increased the amount of time the sample was in contact with the carbon bed and thus allowed more time for decomposition and atomization of the blood matrix. The flow rate was decreased first to approximately 70 cm³/min, then further reduced to 10 cm³/min. Reduced flow rate caused absorption signals to be broader
FIGURE 7

Typical absorption signals obtained for the direct injection of 5 μl of whole blood.

(a) Resonance absorption (2288 Å).  (b) Background (2266 Å).
and shorter due to the longer period of time required for all atoms to flow through the lightpath. Background signals were reduced in size, but by approximately the same factor as those recorded on the resonance wavelength. Relative molecular absorption thus was not decreased. Some example spectra may be found in Figure 8.

c. Sample Introduction on a Carbon Disk

The reason for the high background absorption seen when a 5-μl blood sample was injected directly onto the carbon bed was that the matrix apparently was not broken down completely before entering the light path. In order to break down the organic material in the sample more fully, it was necessary to increase the contact area and/or contact time of the sample with the carbon and the reducing atmosphere surrounding the carbon. It was thought that immobilizing the sample on a small carbon disk, which could then be dropped into the atomizer, would accomplish this effect.

Small disks of approximately 6 mm diameter were cut from 47-mm diameter carbon filter disks (Poco-Grafilters, grade XA3) using a lathe. The disks were first cleaned by placing them in the carbon bed of the atomizer and heating the atomizer to 1450°-1500°C until the signal at the cadmium resonance line of 2288 Å returned to baseline. The clean disks were then stored in cleaned polyethylene vials until needed.
Typical absorption signals obtained for direct injection of 5 μl of whole blood at a reduced flow rate (10 cm³/min).

(a) Resonance absorption (2288 Å).  (b) Background (2266 Å).
One-μl samples of blood were placed on the carbon disks using a Hamilton microliter syringe with a Chaney adapter. The adapter increased the consistency with which a 1-μl aliquot could be measured for analysis. The carbon disk was then dropped onto the surface of the carbon bed, where the sample was atomized.

Several problems were encountered with this technique. First, although the cleaned carbon disks were found to show a very low blank after removal from the carbon bed, it was observed that they became contaminated with time when stored. Even when enclosed in a cleaned polyethylene vial, overnight storage sometimes resulted in as much as a 100% increase in blank absorption signal. Disks thus had to be cleaned less than 24 hours before use.

The porosity of the carbon disks was also observed to cause problems in reproducibility if the disks were recovered and reused. Those carbon disks which were porous resulted in broader and smaller absorption signals when used for introduction of a given blood sample. This was thought to be due to the sample soaking into the carbon. During atomization, a longer period of time (perhaps an additional 2-3 seconds) was necessary to vaporize all the sample out of the carbon matrix. This caused a broader signal.

Recycled disks were also observed to contaminate more quickly when stored. It became apparent that repeated
heating by contact with the carbon bed atomizer made the carbon disks active, in which form they adsorbed and more tightly maintained metals to which they were exposed. This led to irreproducible signals when the carbon disks were of varying porosity. To avoid this, it was necessary to use each disk only once. Still, it was observed that aqueous standards gave differently shaped signals from blood samples, indicating that the absorption of the sample into the carbon was matrix-dependent.

A third problem encountered in the use of carbon disks for sample introduction was that the character of the atomizer bed changed as disks accumulated on the surface. The small, thin carbon filters did not couple well with the RF generator; as a result, the surface of the bed where disks were present became cooler than the bulk of the atomizer bed. This changed the atomization efficiency and therefore the sensitivity of the analysis, essentially with each additional sample. Consequently, the absorption signals obtained became smaller and broader as successive samples were analyzed. Reproducibility also decreased.

A fourth, and perhaps the most significant difficulty with the carbon disk method was that the background absorption was not decreased. The signals obtained using the non-resonance line were of the same size as those observed when monitoring the resonance wavelength of 2288Å. One contribution to the background signal could result
from the rather heavy and rigid carbon disk striking the surface of the atomizer bed with sufficient force to dislodge carbon dust. This may cause some variable background absorption due to the physical blockage and scatter of the incident light. Example traces of the absorption at both 2288 Å and 2266 Å using the carbon disk method are illustrated in Figure 9.

d. Stop-flow Methods

A study was conducted to determine whether background absorption could be decreased by stopping the flow through the atomizer during sample introduction. Stopping the flow increased the contact time of the sample with the carbon bed, thereby allowing more complete decomposition of the organic matrix. The procedure involved discontinuing the flow through the atomizer cell before introducing the sample; the flow remained off until a specified delay time had elapsed, after which the flow was resumed and the sample components were drawn into the light path. Delay periods of 10, 20, and 40 seconds were employed in testing the effect of delay time on both background and sample absorption. Direct injection as well as carbon disk introduction techniques were studied.

The stop-flow technique resulted in the greatest reduction in background of any of the methods investigated. The major problem with this procedure was the difficulty in controlling and repeating exactly the conditions for
FIGURE 9

Typical absorption signals obtained for 1 μl of whole blood introduced on a carbon disk.

(a) Resonance absorption (2283 Å).  (b) Background (2266 Å).
each successive sample. The time that the flow is dis-
continued before sample injection, the delay time, the
bed temperature, the flow pattern through the atomizer,
and the sample introduction technique must all be rigidly
controlled and repeated to achieve reproducibility. It
was found to be difficult to achieve this control without
an automated system.

An additional problem with stop-flow methods was that
the signals were characteristically small, broad, and
oddly-shaped. Because of this, it was not possible to
use simple peak height as a measure of the absorption of
the sample; rather, peak area was taken to be proportional
to the concentration of cadmium. This means of data
treatment is somewhat more time-consuming and inconvenient.
An example of absorption spectra recorded using stop-flow
methods can be found in Figure 10.

A comparison of some data obtained on two blood
samples utilizing direct injection and stop-flow tech-
niques is presented in Table 3.

e. Sample Introduction on a Filter Paper Disk

It was felt that some means of immobilizing
the sample for introduction into the atomizer was desirable
in order to reduce the molecular background absorption.
Immobilizing the sample served to increase the time and
area of contact of the sample with the carbon bed, thus
allowing a more complete destruction of the matrix. At
FIGURE 10

Typical absorption signals obtained for 5 µl of whole blood (direct injection) using a stop-flow technique.

(a) Resonance absorption (2283 Å). (b) Background (2266 Å).
### Table 3

COMPARISON OF DATA OBTAINED FOR TWO BLOOD SAMPLES USING DIRECT INJECTION AND STOP-FLOW TECHNIQUES

<table>
<thead>
<tr>
<th>Injection Method</th>
<th>Method of Data Treatment</th>
<th>Resonance Signal (Units)</th>
<th>Background Signal (Units)</th>
<th>% Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μl direct injection flow rate 200 cc/min</td>
<td>peak height</td>
<td>41</td>
<td>44</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46</td>
<td>121</td>
</tr>
<tr>
<td>5 μl direct injection flow rate 70 cc/min</td>
<td>peak area for triangle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>5 μl stop-flow</td>
<td>peak area for triangle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>10 sec delay</td>
<td></td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>5 μl stop-flow</td>
<td>peak area for trapezoid&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>40 sec delay</td>
<td></td>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>18</td>
</tr>
</tbody>
</table>

<sup>a</sup> The second sample listed in each case was that of a smoker; the first sample listed was a nonsmoker.

<sup>b</sup> A = h x (b at h/2) where the base at h/2 was measured in cm.

<sup>c</sup> A = 1/2(a + b)h where a and b were measured in cm.
the same time, any vehicle of introduction must not change the coupling characteristics of the atomizer and must not irreversibly absorb the sample. It was also necessary that any such introduction method yield a low blank level and little or no molecular absorption. The use of filter paper disks was investigated and found to have the best combination of desirable characteristics of any other technique tested. The filter paper method will be discussed in more detail in Section 4.

f. Effect of Hemolysis on Various Techniques

It was thought that one means of reducing molecular absorption would be to hemolyze the red blood cells of the samples prior to analysis. Hemolysis could be accomplished without contact with the sample itself by subjecting the blood to rapid and extreme changes in temperature. The procedure followed involved first freezing the sample in a dry ice-acetone mixture, then putting the frozen sample into a boiling water bath. Two cycles of this treatment were sufficient to destroy all the red blood cell membranes.

Unfortunately, neither an increase in reproducibility nor a decrease in background absorption was observed when the sample was hemolyzed. Hemolyzed and non-hemolyzed samples were analyzed both by direct injection and using carbon disks with no obvious improvement in either reproducibility or background absorption.
4. Refinement of the Filter Paper Disk Technique

a. Cleaning of Filter Paper Disks

Standard Whatman #41 ashless filter paper was cut into small disks of approximately 6 mm diameter using a hand hole-puncher. It was found that when these disks were introduced into the atomizer and the absorption monitored at 2266 Å, no background absorption was detected. The paper was completely burned and no effect on the atomizer bed was observable. Unfortunately, blank filter paper disks showed an absorption of the cadmium resonance line (2288 Å) which was nearly 100%. Obviously, some means of cleaning the filter paper of cadmium contamination was necessary before it could be used to carry a blood sample into the atomizer.

Previous members of this research group had investigated cleaning of filter paper for heavy metals, especially lead, using various leaching agents. D. Wolcott found a 10% solution of nitric acid in deionized water to be an effective leaching agent for lead in filter paper. Successive leaching was recommended for greatest efficiency. R. Garcia experimented with other leaching agents with less success. Notably, EDTA was not found to be effective in leaching lead from filter paper.

Both 10% nitric acid and 0.25 M EDTA were investigated as possible cleaning agents for cadmium contained in the filter paper disks. Those disks leached with HNO₃ were
found to be more effectively cleaned. The procedure which was found to be most successful in reducing cadmium contamination involved successive leaching for two overnight periods using fresh 10% solutions of HNO₃. More frequent changing of the HNO₃ solution seemed to assure a lower blank; however, two overnight soakings were found to reduce the cadmium signal from a blank filter paper disk to an acceptable level. After soaking in the HNO₃ solution, the disks were rinsed in a Buchner funnel with approximately 200 ml of water and partially dried using vacuum suction. They were then allowed to air dry in a clean air environment before use. It was found that placing a filter paper circle over the disks in the Buchner funnel while suction was applied significantly reduced any recontamination of the disks from the air being pulled over them.

b. Sample Introduction Technique

Actual sample introduction using the filter paper disks was essentially the same as that using carbon disks. One μl of whole blood was placed on the disk using a Hamilton microliter syringe and the disk and sample were dropped onto the surface of the hot carbon bed atomizer. The filter paper was completely burned and the sample decomposed and atomized. Standards were analyzed in an identical manner.
c. Blank and Background Measurements

Several blank filter paper disks were analyzed routinely so that the absorbance due to the blank could be subtracted from the sample signal. A blank analysis also was run on the sample collection vials to establish that no contamination was introduced into the blood sample from the Vacutainer vial or the EDTA anti-coagulant it contained. Vacutainer vials were filled with deionized water and this solution analyzed for cadmium by direct injection of a 5-µl aliquot. The absorption signals obtained were equal to those seen for 5 µl of deionized water alone, indicating that no cadmium was introduced from the glass container or the anticoagulant.

Molecular absorption was measured using the 2266Å non-resonance line of cadmium.

d. Calibration Procedures

Calibration was first attempted using aqueous standards. The standards were introduced into the atomizer in the same manner as were the blood samples; that is, 1 µl was placed on a filter paper disk and dropped onto the surface of the carbon bed. Although linear calibration curves were obtained in this manner, it was felt that use of standards with such a different matrix from that of the sample being analyzed would not yield reliable results. Consequently, calibration was carried out using the method of standard additions.
In the preparation of standard additions, a 0.5-ml aliquot of blood was spiked with 50 µl of an aqueous cadmium standard. Concentrations of standards used were 0.1, 0.2, 0.5, and 1.0 ppm Cd. One-µl aliquots of the spiked sample were taken for analysis on a filter paper disk. A typical calibration curve is shown in Figure 11. Later this procedure was altered slightly such that 1, 2, 3, and 5 µl aliquots of a 10 ppm Cd standard were added to 0.5-ml aliquots of blood. This practice further minimized the effect of dilution of the original sample with the added standard. Comparable calibration curves were obtained for the two methods.

The same blood sample was used in preparing all four standards. Aqueous standards used for the standard additions were made up weekly and stored in polyethylene vials. All glassware and storage containers were pre-equilibrated with cadmium solutions at these concentrations and were routinely used for preparation of the same standards. This practice minimized the loss of cadmium from the solutions to the walls of the containers.

A standard was repeated between each different blood sample to give a measure of the sensitivity of the carbon bed as a series of analyses proceeded. In this way, the calibration curve could be adjusted for a gradual change in the sensitivity of the carbon bed with time. A new calibration curve was prepared each day or each time the
FIGURE II

This figure shows an example calibration curve obtained for standard additions to whole blood. The samples were analyzed by the filter paper disk technique.
atomizer bed was cleaned and replaced with fresh carbon.

C. RESULTS

1. Absorption Traces Obtained in the Analysis of Whole Blood

A typical absorption trace from the determination of cadmium in whole blood is presented in Figure 12, together with the background absorption signals recorded for the same sample. It can be seen that absorption signals were sharp and triangular in shape, allowing the measurement of peak height as representative of the absorption of the sample.

2. Blank and Background Measurements

Blank filter paper disks were analyzed routinely after the cleaning procedure was completed and before their use for sample analysis. The cleaning method described was found to reduce the absorption signal from blank disks to approximately the level of the signal obtained from a 5-µl injection of deionized water, or about 0.03 absorbance units.

Molecular absorption was also routinely measured during each analysis series. Although a significant background was present, its level was constant and less than one-half of the resonance absorption signal. Background absorbance could therefore be subtracted from the resonance absorbance with little increase in the uncertainty of
FIGURE 12

Typical absorption signals obtained for the analysis of 1 µl of whole blood using the filter paper disk technique.

(a) Resonance absorption (2288 Å).  (b) Background (2266 Å).
the measurement. The average value of the background was 0.037 absorbance units. This value corresponds to a signal of approximately 8% absorption. The background absorption was found not to vary between blood samples.

3. Average Concentration of Cadmium in Whole Blood

In practice, at least 12 successive aliquots of a blood sample or of a spiked standard were injected into the atomizer using filter paper disks. This made possible the calculation of a mean and standard deviation for each sample. Blank and background absorbance measurements were subtracted from the resonance absorbance to obtain the true absorbance of the cadmium in the sample. The average total signal recorded for 1 µl of whole blood using the resonance line was approximately 18% absorption. The relative standard deviation of the total absorption signal was 15%, which corresponds to an average deviation of 2.7% in the total absorption measurement. If this deviation is applied only to the part of the absorbance signal due to cadmium, the relative standard deviation of the cadmium absorbance measurement is approximately 60%. This corresponds to an error of approximately ± 6 ppb Cd. This precision could be significantly improved with the use of automatic background correction.

Figure 13 illustrates the distribution of the calculated cadmium concentrations of 41 whole blood samples. It can be seen that there is a range of concentrations varying
FIGURE 13

This figure shows the distribution of the cadmium concentrations found in whole blood for both smokers and nonsmokers.
from 1 to 25 ppb Cd, with a mean concentration of
approximately 10 ppb. Most samples were found to contain
cadmium in a concentration between 5 and 20 ppb.

   Nonsmokers

   The data in Figure 13 has been separated according
to smoking habits in order to determine whether smoking
affected blood cadmium levels. It was found that, on the
average, the concentration of cadmium in the blood of
smokers (approximately 11.5 ppb) was higher than that of
nonsmokers (approximately 9 ppb). Each sampling group
exhibited its own range of concentrations of cadmium;
these ranges partially overlapped one another as can be
seen in Figure 13. The range of cadmium values for non-
smokers was found to be from 1 to 18 ppb; furthermore,
the values exhibited a fairly normal distribution within
this range. The standard deviation from the mean for
the group of nonsmokers was calculated to be 4.6 ppb
(mean [Cd] = 9.0 ppb).

   The range of values for cadmium in the blood of
smokers, on the other hand, was much wider, extending from
2 to 25 ppb Cd. The mean cadmium concentration for this
group was 11.5 ppb, and the standard deviation from the
mean was 6.8 ppb.

   The "standard deviation from the mean" has been cal-
culated here only as an indication of the amount of varia-
tion in blood cadmium levels between the individuals in each group. It must be emphasized that this value is not a measure of the precision of the method, nor is it a true measure of a deviation. Each sample analyzed in the collection of this data was unique; no "true value" for the concentration of cadmium in whole blood is known. The calculated value, therefore, may be considered only as an indication of the range, and the distribution within that range, for each group sampled.

The data in Figure 13 indicated that there was a difference in blood cadmium levels between smokers and nonsmokers. The general observation could be made that the average smoker showed a higher cadmium concentration in the blood than the average nonsmoker.

D. DISCUSSION

1. Concentrations of Cadmium Obtained for Whole Blood

The values obtained for the concentration of cadmium in whole blood were similar to those reported by other researchers. Table 4 lists the average concentration of cadmium in blood as determined by this method along with the concentrations reported using several other methods. It could be seen in general that those methods which employed dry-ashing steps, such as graphite furnace atomic absorption with a 3-step temperature program, tended to yield results lower than those obtained by the method described here. On the other hand, those methods
### Table 4

**COMPARISON OF LITERATURE VALUES FOR CADMIUM IN WHOLE BLOOD**

<table>
<thead>
<tr>
<th>Conc. Cd (ppb)</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>electrothermal AAS, quartz &quot;T&quot; atomizer</td>
<td>105</td>
</tr>
<tr>
<td>1.3</td>
<td>graphite furnace AAS</td>
<td>93</td>
</tr>
<tr>
<td>7.5-9.5</td>
<td>graphite furnace AAS</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>punched disk, graphite furnace AAS</td>
<td>71</td>
</tr>
<tr>
<td>10.6±4.9</td>
<td>wet-ash, graphite furnace AAS</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>1:10 dilution, graphite furnace AAS</td>
<td>87</td>
</tr>
<tr>
<td>14</td>
<td>1:50 dilution, graphite furnace AAS</td>
<td>86</td>
</tr>
<tr>
<td>6.6±2.5</td>
<td>flame AAS</td>
<td>106</td>
</tr>
<tr>
<td>5.3</td>
<td>APDC-MIBK extraction, flame AAS</td>
<td>80</td>
</tr>
<tr>
<td>3.5±0.9</td>
<td>Ta boat</td>
<td>81</td>
</tr>
<tr>
<td>5</td>
<td>Delves cup</td>
<td>82</td>
</tr>
<tr>
<td>0.6</td>
<td>Delves cup</td>
<td>83</td>
</tr>
<tr>
<td>3.1</td>
<td>1:5 dilution, atomic fluorescence</td>
<td>102</td>
</tr>
</tbody>
</table>
which employed wet digestions could be seen to have resulted in somewhat higher values for cadmium concentrations. This observation was not unexpected in view of the fact that dry ashing had been shown to result in significant losses of volatile cadmium, leading to low results. Wet ashing, however, often causes high results due to contamination of the sample from the reagents added.

The average concentration of cadmium in the blood of smokers (11.5 ppb) was found in this study to be higher than that of nonsmokers (9 ppb). The difference in average blood cadmium levels between these two groups was 27%. This difference was significantly less than that reported by Einbrodt, et al., who found that the average cadmium concentration in the blood of smokers was 50% greater than that of nonsmokers.61 It must be noted, however, that the cadmium levels reported by these workers (2-3 ppb) were approximately one-fourth of the levels found in this research. Furthermore, the method used to obtain the data was not described.

The overall precision of the analytical method for the determination of cadmium in whole blood was calculated to be approximately 60%. It must be noted that this precision could be greatly improved by the use of automatic background correction. Using the equipment available for this study (no automatic background correction), separate
 aliquots of blood had to be used to independently measure the molecular background and the resonance absorption. The deviation of the resonance signal, therefore, included the deviation of the blank signal and the background signal, as well as the deviation of the actual absorption due to cadmium. The error in the measurement of the absorption signal thus is compounded.

The use of an automatic background corrector would subtract the deviation of the background measurement from that of the resonance signal. The background absorption of each individual sample aliquot would be subtracted at the same time as the absorption of the resonance line was recorded. The deviation in the resonance absorption signal therefore would be reduced, and the overall precision of the measurement would be improved.

Automatic background correction is generally included on commercial instruments; unfortunately, it was not available for use in this study.

2. Strengths and Weaknesses of the Filter Paper Disk Technique

The filter paper disk technique was shown to be simple, reliable, and accurate for the direct determination of cadmium in whole blood. The technique possessed many of the characteristics which were previously stated to be desirable for a valuable method of analysis. No sample pretreatment was necessary, eliminating the danger
of contamination of the sample from added reagents or the loss of cadmium due to sample transfer or volatilization. The blank absorption was reduced to a minimum so that corrections could easily be made in the absorbance values of both standards and samples. The background absorption and reproducibility were also improved by this technique; however, these parameters were still found to present some problems in the routine application of the analysis method. It was also observed that the sensitivity of the analysis seemed to change with time. These problems are individually discussed in the following sections.

a. Background Absorption Due to the Matrix

Although absorption due to the organic matrix was dramatically reduced by the use of filter paper disks for sample introduction, a small background signal was still observed when analyzing whole blood by this method. A small signal would always be expected due to the absorption of CO and H₂ formed upon breakdown of the organic matrix. Part of the background signal also may have been due to incomplete degradation of the matrix.

Some of the background absorption could also have resulted from light scattering by carbon dust entering the light path. As a filter paper disk hit the surface of the carbon bed, the impact may cause some dislodge of carbon dust within the bed; the air flow through the cell could draw this dust into the light path of the atomizer.
It was in fact observed that after several hours of use a small pile of carbon dust built up in the crosspiece of the quartz "T" below the atomizer bed (see Figure 1). This evidence indicated that some small amount of carbon dust did enter the light path during analysis.

The absorption due to incomplete decomposition of the matrix could be further reduced by increasing the contact time of the sample with the carbon bed. This could be accomplished by decreasing the flow rate through the cell or increasing the size of the bed. Although these adjustments have the advantage of lowering the molecular absorption, there were also disadvantages in applying either change to the described system.

During studies with direct injection methods, decreasing the flow rate through the cell was observed to widen the absorption peaks and decrease the reproducibility of the signals. Broad, oddly-shaped signals were much more difficult to measure accurately. In addition to this, decreased flow increased the possibility of loss of some of the vaporous sample due to diffusion out of the top of the atomizer.

The second means of decreasing background absorption; that is, by increasing the size or height of the carbon bed, was difficult to accomplish without a major change in the design of the atomizer. The carbon bed was normally packed to a height corresponding to the edge of the second topmost
winding of the RF coil around the cell. This bed depth assured good coupling between the RF generator and the carbon, resulting in uniform heating of the atomizer bed. In order to change the depth of the bed, the height of the RF coils would have to be altered.

The analysis technique described exhibited the lowest background absorption of any of the tested procedures. The amount of molecular absorption was considered to be acceptable in view of the steps necessary to make further reduction of the background possible. The changes suggested above, of course, would not guarantee a decrease in background absorption if they were implemented.

b. Reproducibility of the Absorption Signal

Reproducibility remained the biggest problem in the analysis of blood by this method. However, this problem was not unique to the filter paper disk technique. Rather, lack of precision has been the major disadvantage in most of the reported methods for analysis of biological matter. The inconsistency of absorption signals that was observed in blood analysis using filter paper disks could be attributed to several factors.

i. Differences in blank absorption levels. Variations in the level of absorption due to blank filter paper disks resulted in corresponding variations in the absorption signals of blood samples.
ii. Differences in sample size. Any variation in the size of the sample aliquot measured or delivered for analysis would cause differences in the absorption signals recorded.

iii. Differences in the reaction of the sample with the bed. Much of the variation in absorption signals (both resonance and background) could be accounted for in the way the sample hit the surface of the carbon bed. The bed surface was not a homogeneous one; that is, some carbon pieces were hotter than others. Dust which accumulated around the edges of the bed and in the spaces between carbon pieces was a different temperature than the harder carbon. The place of contact of the sample with the bed thus directly affected the speed and efficiency of sample breakdown and atomization. A non-homogeneous temperature of the bed surface was most likely the greatest cause of variation in absorption signals.

The mode of contact of the filter paper disk; that is, whether the disk fell edgewise or flat onto the carbon surface, also changed the speed of sample combustion. In the same way, the position of the blood spot on the filter paper circle affected the shape and size of the signal. That part of the sample nearer the edge of the paper burned faster than that nearer the center if the disk made contact with the bed edge-on.
Another major influence on signal reproducibility was whether the sample and disk touched the side of the atomizer as they fell toward the carbon bed. It was observed that those samples which touched the sides of the quartz inner sleeve before contacting the bed produced a higher cadmium absorption signal. Apparently the disk released some cadmium from the surface of the quartz, or became contaminated from contact with the sides. This indicated that in some cases contamination was not released from surfaces, such as the quartz or carbon, unless dislodged by physical contact. This was significant because it implied that contact of any sample with the sides of the atomizer or the carbon itself may release some cadmium not originally contained in the sample. In this case, every sample may give some small signal, even if no cadmium is present. This absorbance by cadmium, of course, should be subtracted from the sample absorbance along with that of the blank.

c. Sensitivity Changes with Time

It was observed that the sensitivity of the analysis of whole blood for cadmium changed as the length of analysis time, and thus the age of the carbon bed, increased. This was indicated by a change in the absorbance of a standard analyzed between each blood sample. This variation, which was within a 6% absorption range, could be explained by changes in the efficiency of atomization.
caused by differences in the temperature or surface character of the bed. As the carbon bed became dusty and more porous, the coupling efficiency of the carbon to the RF generator decreased and the temperature dropped. This in turn changed the efficiency of atomization and thus the size of the absorption signal. As was previously mentioned, this change was accounted for by measurement of a standard sample between each blood sample. Any changes in sensitivity could subsequently be adjusted for in the calibration curve.

E. CONCLUSIONS AND SUMMARY

1. It was shown that the use of the carbon bed atomizer together with sample introduction on a filter paper disk was a feasible and successful technique for the direct determination of cadmium in whole blood. Carbon bed atomization allowed a more complete breakdown of the organic matrix, which successfully reduced molecular background. The technique also was sufficiently sensitive so that preconcentration steps were unnecessary. Sample pretreatments, and the errors associated with these procedures, were thus avoided. In addition, the technique was simple and analysis rapid.

2. This technique would be easily applicable to other biological materials.

3. The concentration of cadmium in whole blood for a population not occupationally exposed to the metal was
found to be between 1 and 25 ppb, with a mean concentration of 10 ppb. The relative standard deviation of the measurement of the cadmium concentration of the sample was approximately 60%.

4. The average cadmium concentration in the blood of smokers was found to be 11.5 ppb. This was higher than the average concentration found for nonsmokers (9 ppb Cd).
CHAPTER 2
THE DIRECT DETERMINATION OF CADMIUM IN URINE

A. INTRODUCTION

Much of the concern over the toxicity of cadmium has stemmed from the fact that it is a cumulative toxin. Cadmium is known to deposit in the body, particularly in the liver and kidneys, and is excreted only very slowly. Since urine was known to be excreted through the kidneys, interest had been aroused in the possible importance of urinary cadmium levels. Any study of cadmium in urine, however, required a method of analysis that was accurate and reliable. The filter paper disk technique, developed for the direct analysis of whole blood, was found to be easily applicable to a study of cadmium concentrations in urine.

Urine is an easily obtainable sample and would be convenient for routine analysis in industry. There has been a continuous demand for analytical methods suitable for routine monitoring of workers exposed to chemical toxins. A simple and reliable method for metals analysis in urine could expand the use of this particular sample in industrial toxicology.

1. Relationship Between Urine Cadmium Levels and Body Burden
   a. Animal Studies

   Much debate has centered on the relationship
between the cadmium concentration in the urine and the individual body burden or exposure level. Animal studies have shown that animals receiving a single oral dose of cadmium excreted 0.6-1.2% of the cadmium during the first few days. Chronic subcutaneous exposure in animals caused an increase in urinary cadmium after several months, coinciding with renal dysfunction and proteinuria. These studies, however, did not prove that urinary cadmium levels could be directly related to an individual's exposure to the metal or to the body burden. Other studies, in fact, have indicated that actual cadmium accumulation in the kidneys varies with age, with younger animals showing lower accumulation.

b. Human Studies

Researchers have reported that the average concentration of cadmium in the urine of human subjects appears to be higher in an "exposed" group than in a group considered to be "nonexposed." These studies indicated that urinary cadmium concentrations could be used as an indicator of exposure, on a group basis, under conditions of long-term, low-level exposure. At the same time, it was found to be difficult to relate urinary cadmium to exposure on an individual basis. Studies showed a wide scatter among individual concentrations of cadmium in the urine. The average concentration increased with age and was higher among smokers than among nonsmokers.
The daily total cadmium excretion also increased with age and among smokers.\textsuperscript{109}

An interesting study of urine and blood cadmium concentrations under conditions of industrial exposure has been reported by Lauwerys, et al.\textsuperscript{110,111} These researchers studied the cadmium concentrations in the urine and blood of workers in a factory producing cadmium salts. The authors reported scatter among individual urine cadmium levels, although the average urinary concentration among subjects exposed for greater than 250 days was within the same range. A monitoring of newly exposed individuals with time showed three phases of urinary cadmium levels. At the onset of exposure, cadmium in the urine increased rapidly to a level of approximately 15 $\mu$g Cd/g creatinine. (Creatinine was used as a type of internal standard to correct for variation in the fluid content of the samples.)\textsuperscript{109} From 15 to 120 days of exposure, the cadmium level in urine increased slowly; past this period of time there was a more rapid increase in cadmium concentration which seemed to be influenced by recent exposure.\textsuperscript{110} It was proposed that the first two phases represented an increase in cadmium body burden and induced detoxification mechanisms, such as production of metallothionein, a cadmium-binding protein. The third stage was explained as the phase where the body's binding sites were saturated and the cadmium level in the urine was more representative of
recent exposure. Thus, these researchers proposed that urinary cadmium may indicate an equilibrium with the body burden when exposure is low and current exposure when exposure is high.\textsuperscript{110,111}

2. Analytical Problems in the Determination of Cadmium in Urine

Urine was a very complex sample. It was known to consist of a wide variety of inorganic salts in varying concentrations. Some of the ions present include sodium, potassium, magnesium, calcium, ammonium, chloride bicarbonate, phosphate, and sulfate.\textsuperscript{63} Again, sodium and potassium are the predominant cations and chloride is the most abundant anion. Urine also contains significant amounts of organic compounds such as urea, uric acid, and creatinine. The concentrations of each of these components may change depending on the individual and on fluid intake. A list of the approximate amounts of the major components of a normal 24-hour urine sample can be found in Table 5.

Urine analysis presented many of the same problems as were encountered with whole blood. Although urine contained less organic material than blood, the high salt content caused serious background effects if the matrix was not completely decomposed. Sodium chloride in particular had been shown to interfere with cadmium analysis by flame atomic absorption spectroscopy when present in concentra-
Table 5

APPROXIMATE COMPOSITION OF 24-HOUR URINE
IN A NORMAL ADULT

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>2-4 g</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.5-2.0 g</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.1-0.3 g</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.1-0.2 g</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.4-1.0 g N</td>
</tr>
<tr>
<td>Chloride</td>
<td>100-250 meq</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>0-50 meq</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.7-1.6 g P</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0.6-1.8 g S</td>
</tr>
<tr>
<td>Urea</td>
<td>6-18 g N</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.3-0.8 g N</td>
</tr>
</tbody>
</table>

Normal pH = 5.5-6.5

Normal volume of 24-hour urine = 600-2500 ml/day
tions greater than 0.01 M. The high concentration of NaCl as well as other inorganic salts in urine samples caused nonspecific absorption for which correction was required. 


The most common method used for the determination of cadmium in urine samples is atomic absorption spectroscopy. Some researchers have used flame atomic absorption, either after dilution or after ashing and extraction procedures. Graphite furnace atomic absorption is the more widely used technique, however. Several of the reported methods involve previous wet-ashing steps using concentrated HNO₃. The dry residue is then dissolved in HNO₃ and either analyzed directly or following extraction with ammonium-l-pyrrolidine dithiocarbamate (APDC) into methylisobutyl ketone (MIBK). Other methods involve only chelation (typically using APDC or sodium diethyldithiocarbamate) and extraction prior to analysis using the graphite furnace. Some researchers simply diluted the sample before analysis, using deionized water or HNO₃ in order to reduce the background absorption. All of these procedures carried with them the risk of contamination of the sample from added reagents.

Ross and Gonzalez, and Carmack and Evenson reported a direct determination of cadmium in urine using
a graphite furnace with a three-step atomization program. The methods described relied on automatic background correction using a deuterium lamp to subtract molecular absorption. The molecular absorption, however, was often a major portion of the total signal. A small error in the measurement of background absorption thus would cause a significant error in the measurement of absorption due to cadmium. For example, if a 90% total absorption signal was recorded, 80% of which was background, a 2% error in the background measurement would result in a 20% error in the percent absorption attributed to cadmium.

The temperatures reported for the three stages of programmed atomization using a graphite furnace differed considerably between published procedures. The drying stage was typically carried out at 125°-150°C for 30 to 60 seconds. Ashing temperatures, however, varied between 300°C and 450°C. As has been previously stated, significant losses of cadmium can occur at these temperatures. Vesterberg and Wrangskogh reported irregular loss of cadmium at temperatures over 400°C; they suggested an ashing temperature of 350°C for the best sensitivity and least background.

Atomization temperatures, also, varied considerably between reported methods, with some programs calling for atomization at 900°C or 1300°C and others suggesting 1950°C or even 2150°C. It has been stated that the
most efficient atomization temperature depends on the individual sample matrix, with differing salt contents contributing to the matrix effect. It should be stated that background correction is essential when analyzing urine by these methods and that calibration must be carried out using standard additions.

Some novel methods of preconcentration of the cadmium in urine samples prior to analysis using atomic absorption have been reported in the literature. Dipivaloylmethane has been used as a chelating agent. Lund and Larsen used electrodeposition onto a platinum wire to concentrate cadmium from urine. The wire containing deposited cadmium was then placed within the light path of the atomic absorption spectrophotometer and the cadmium signal was recorded while voltage was applied through the filament. This method inherently measures only unbound cadmium. The results reported by these workers were questionable due to the fact that they added EDTA to each sample upon collection to prevent plating out of the metal onto the walls of the container. Even though the samples were acidified to pH 2 before electrodeposition was carried out, it seemed likely that some chelated cadmium would not be detected.

Preconcentration of heavy metals from urine using a poly(dithiocarbamate) resin has been described by Barnes and Genna. A 250-ml volume of urine was passed
through the resin; recovery from the resin was accomplished by total digestion with nitric and sulfuric acids. Finally, analysis was carried out using inductively-coupled plasma emission. There were obviously many sources of error in this procedure. Contamination could occur both from the resin and from the acids added for digestion. Extensive sample treatment prior to analysis made this technique inconvenient and of questionable accuracy.

Urine also has been analyzed using atomic fluorescence spectrometry by Michel, et al.\textsuperscript{102} The primary difficulty in this technique was a high level of scatter.

Finally, an indirect method of identifying cadmium poisoning made use of polyacrylamide gel electrophoresis. In this technique, separation of the proteins excreted in the urine by isoelectric focusing showed distinctive patterns; elevated levels of a specific protein called $\beta$-2-microglobulin indicated cadmium poisoning.\textsuperscript{120} Although the technique was very selective, it provided only an indication of high cadmium exposure and did not measure the concentration of the metal itself. It was possible that chemicals other than cadmium could cause increased levels of $\beta$-2-microglobulin in the urine.

4. Need For An Improved Analytical Procedure

It can be seen that all the reported methods for the determination of cadmium in urine have significant disadvantages. The method developed for the direct
analysis of whole blood using the quartz "T" atomizer could be applied easily and effectively to the direct analysis of urine samples. This technique eliminated both positive errors due to reagents and other contamination during sample handling, and negative errors inherent in dry-ashing and extraction procedures. By using the filter paper disk technique, urine samples could be analyzed directly and accurately.

This chapter describes the application of the filter paper disk technique to urine samples. Included are data obtained for the concentration of cadmium in the urine of a group of individuals who were not occupationally exposed to the metal.

B. EXPERIMENTAL

1. Equipment

The equipment used in the analysis of urine samples was identical to that previously described for the determination of cadmium in whole blood. The components of the atomic absorption spectrophotometer were unchanged; syringes and other apparatus were the same as those previously employed.

2. Procedure

a. Sampling Techniques

Spot urine samples were collected at random times from individuals who were not occupationally exposed
to cadmium. All subjects sampled were members of the university population in Baton Rouge. Specimens were collected in polyethylene vials which were previously cleaned in HNO$_3$ and rinsed with multiple portions of distilled deionized water. All samples were analyzed within four hours of collection. The average volume of urine collected was 5 ml.

Urine samples were introduced into the atomizer in the same manner as was described for whole blood samples. One microliter of urine was placed on a filter paper disk using a Hamilton microliter syringe. The disk containing the sample was then dropped directly onto the surface of the carbon bed, which was heated to approximately 1500°C. At this time, decomposition and atomization occurred.

Absorption measurements were made at the cadmium resonance line of 2288Å, while background absorbance was monitored using a non-absorbing cadmium line occurring at 2266Å. The cadmium absorbance of blank filter paper disks was also recorded so that the absorbance due to both the blank and to molecular background could be subtracted from the total sample absorbance at the resonance line. The remaining absorbance value was directly proportional to the concentration of cadmium in the sample.
Filter paper disks were cut from Whatman #41 filter paper and cleaned in 10% HNO₃ as has been previously described.

b. Calibration Procedures

Calibration was first attempted using aqueous cadmium standards. These standards were introduced into the atomizer in the same manner as were the urine samples; that is, by placing 1 µl onto a filter paper disk. However, studies soon showed that the calibration curves obtained using aqueous standards and those obtained using the standard addition method of calibration differed in slope, indicating a matrix effect. This effect is illustrated in Figure 14. Due to the matrix effect, calibration was carried out thereafter using the method of standard additions exclusively.

In preparing standard addition standards, microliter amounts of a 10 ppm or 20 ppm aqueous cadmium standard were added to a 0.5 ml aliquot of urine. It was found that the addition of 1, 3, and 5 µl aliquots of a 10 ppm cadmium standard provided the best calibration curve for most samples. Those samples which contained a higher (greater than 20 ppb) cadmium concentration were more accurately analyzed by additions of a 20 ppm cadmium standard. Attempt was made to carry out the calibration such that additions of cadmium were approximately equal to the amount already present in the sample; it was known
FIGURE 14

This figure shows the different slopes obtained for calibration curves constructed using aqueous standards (●), and standard additions to a urine sample (▲).
that this technique provided the most reliable calibration data.\textsuperscript{121}

Approximately one out of four urine samples was analyzed by constructing a complete standard addition calibration curve. The cadmium concentration of the remaining samples was determined by analysis of the original sample and an aliquot containing a single standard addition. The full calibration curves constructed with at least one sample per day were consistently linear. Therefore, it was felt that those curves constructed using only the original sample and one spiked aliquot were also satisfactory.

C. RESULTS

1. Concentration Range for Cadmium in the Urine of Unexposed Individuals

Spot urine samples were analyzed from a number of individuals who were not occupationally exposed to cadmium using the filter paper disk technique. Most of these individuals were male LSU faculty members. The results of the analyses are compiled in Table 6. For 32 subjects, the mean value for the concentration of cadmium in the urine was 20 ppb; the values ranged from 0 (non-detectable) to 67 ppb cadmium. Figure 15 illustrates the distribution of the cadmium concentrations found in urine. For the purposes of constructing this
Table 6
VALUES FOR CONCENTRATION OF CADMIUM FOUND IN THE URINE
OF UNEXPOSED INDIVIDUALS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Conc. Cd (ppb)</th>
<th>Subject</th>
<th>Conc. Cd (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23</td>
<td>12</td>
<td>insufficient sample</td>
</tr>
<tr>
<td>B</td>
<td>24</td>
<td>13</td>
<td>67*</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>14</td>
<td>N.D.</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>F</td>
<td>27</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>N.D.</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>66*</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td>insufficient sample</td>
<td>28</td>
<td>18</td>
</tr>
</tbody>
</table>

n = 32
mean = 20 ppb
range = 0-67 ppb

* Discarding two high values, mean = 17 ppb.
FIGURE 15

This figure shows the distribution of the cadmium concentrations found in the urine of individuals not occupationally exposed to cadmium.
figure, the values listed in Table 6 were rounded to the nearest 5 ppb. It can be seen from Figure 15 that the great majority of the urine samples analyzed contained between 0 and 30 ppb cadmium. If the two abnormally high values were discarded (Subject 6 and Subject 13, whose urine contained 66 and 67 ppb Cd, respectively), the mean urinary cadmium concentration for the population sampled became 17 ppb.

2. Variations in the Cadmium Concentration in the Urine of An Individual

It was found that the concentration of cadmium in the urine of an individual varied on a day-to-day basis. The urine of two subjects was analyzed regularly for a period of approximately two months in order to study this individual variability. The data accumulated are presented in Table 7. The distribution of these values for the concentration of cadmium in urine is illustrated in Figure 16. It can be seen that, aside from two abnormally high values, the urinary cadmium concentrations for these individuals were randomly distributed about a mean value of approximately 20 ppb cadmium. Discarding the two high values, the average urinary concentration (mean ± σ) was found to be 20 ± 7 ppb Cd for Subject A and 22 ± 9 ppb for Subject B.

The "standard deviations" calculated above are not an indication of precision. It must be emphasized that
Table 7
DAILY VARIATIONS IN THE CONCENTRATION OF CADMIUM IN THE URINE OF TWO INDIVIDUALS

<table>
<thead>
<tr>
<th>Date</th>
<th>Subject A</th>
<th>Subject B</th>
</tr>
</thead>
<tbody>
<tr>
<td>03-10-80</td>
<td>30</td>
<td>no sample collected</td>
</tr>
<tr>
<td>03-14-80</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>03-17-80</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>03-19-80</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>03-24-80</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>03-26-80</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>03-28-80</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>03-31-80</td>
<td>13</td>
<td>no sample collected</td>
</tr>
<tr>
<td>04-16-80</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>04-18-80</td>
<td>62*</td>
<td>31</td>
</tr>
<tr>
<td>04-21-80</td>
<td>27</td>
<td>53*</td>
</tr>
<tr>
<td>04-23-80</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>04-28-80</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>04-30-80</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
</table>

\[ \begin{align*}
  n &= 14 & n &= 12 \\
  \text{mean} &= 23 \text{ ppb} & \text{mean} &= 24 \text{ ppb} \\
  \sigma &= 13 & \sigma &= 13 \\
  \text{range} &= 5-62 \text{ ppb} & \text{range} &= 14-53 \text{ ppb}
\end{align*} \]

* Discarding one high value in each group:

\[ \begin{align*}
  \text{Subject A} & \quad \text{Subject B} \\
  \text{mean} &= 20 \text{ ppb} & \text{mean} &= 22 \text{ ppb} \\
  \sigma &= 7 & \sigma &= 9
\end{align*} \]
FIGURE 16

This figure shows the distribution of the cadmium concentrations found in the urine of two individuals. Samples were collected regularly over a 2-month period.
each sample analyzed and reported in Table 7 was unique. The variations in cadmium concentration between these samples were not due to random error but rather to real differences between specimens. The "standard deviation" has been calculated above only as an indication of the amount of variation in urinary cadmium concentrations for each individual on a day-to-day basis. This value simply serves as a numerical indicator of the distribution of the values for cadmium concentration within the observed range.

3. Precision of the Analytical Method

A measure of the precision of the filter paper disk technique in the analysis of urine was obtained by repetitive analysis of a single urine sample. The data collected are presented in Table 8. At a level of 15 ppb Cd in the sample, the relative standard deviation of the total absorbance signal was 9.5% (n = 22). The average total signal recorded for this sample was approximately 21% absorption. The relative standard deviation of the absorbance due only to cadmium was approximately 28%, which corresponds to an error of approximately ± 4 ppb Cd.
### Table 8
VALUES OBTAINED FOR REPEATED ANALYSIS OF A SINGLE URINE SAMPLE

<table>
<thead>
<tr>
<th>Aliquot</th>
<th>Total % Absorption</th>
<th>Aliquot</th>
<th>Total % Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>14</td>
<td>15.5</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>15</td>
<td>21.5</td>
</tr>
<tr>
<td>5</td>
<td>23.5</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>20.5</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>20.5</td>
<td>18</td>
<td>21.5</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>19.5</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>21</td>
<td>22.5</td>
</tr>
<tr>
<td>11</td>
<td>20.5</td>
<td>22</td>
<td>25</td>
</tr>
</tbody>
</table>

Average Total % Absorption = 20.9; $\sigma = 2.2$

Average Total Absorbance = 0.102; $\sigma = 0.0097$

Relative standard deviation of total absorbance = 9.5%

Absorbance due to Cd alone $\simeq 0.034$

Relative standard deviation of Cd absorbance $\simeq 28\%$
D. DISCUSSION

1. General Considerations Concerning the Determination of Cadmium in Urine

a. Advantages of the Method

The use of the described technique for the direct analysis of urine eliminated many of the errors inherent in conventional methods. Both positive errors due to contamination and negative errors due to ashing procedures were avoided because no sample pretreatment or preconcentration was necessary.

A major difficulty encountered in the analysis of urine samples was that of background or molecular absorption. Several researchers have reported that inorganic salts such as NaCl and KCl severely depressed cadmium absorbance.\textsuperscript{113,114,117} Carmack and Evenson\textsuperscript{117} reported that 70-90\% of the background absorption from urine was due to NaCl and KCl. The background absorption often exceeded that which could be accommodated by automatic background correctors.\textsuperscript{114}

The filter paper disk technique for sample introduction and the use of the quartz "T" atomizer made possible the direct determination of cadmium in urine by significantly reducing background absorption. For the urine samples analyzed in this research, the average background absorbance was approximately 0.04 absorbance units, or about 8\% absorption. Urine analysis was
therefore possible without prior dilution or ashing steps.

As was described in the RESULTS section, the relative precision of the analytical technique was approximately 28% at the 15 ppb level. This precision was considered to be acceptable considering the low levels of cadmium being determined and the difficult matrix involved. The precision of the method could be improved by the use of an automatic background corrector, as has been discussed in Chapter 1.

b. Matrix Effects

It was found that the urine matrix affected not only the molecular background but also the efficiency of atomization of the cadmium in the sample. This effect was demonstrated by the fact that standard addition calibration curves exhibited a different slope from that of aqueous cadmium standards. A comparison of the two types of calibration curves is illustrated in Figure 14. It was also found that calibration curves generated by standard additions to different urine samples differed in slope. This effect is illustrated in Figure 17. The difference in slope for different urine samples indicated that the matrix not only influenced the efficiency of atomization, but that this effect varied between urine samples. It was evident that some constituent of the urine caused a chemical interference. This interference presumably would bind to the cadmium present in or
This figure shows the different slopes obtained for calibration curves generated by standard additions to two different urine samples.
added to the sample, changing its rate of atomization. The fact that some samples exhibited widely different slopes for their standard addition curves indicated the presence of different chemical forms of cadmium.

It would be of great interest to determine if the cadmium in the urine was inorganic or organic in form. The standard additions made to urine samples in this work were of aqueous solutions of CdSO₄; it was doubtful that this was the form originally present in the urine. It might be expected, therefore, that the original cadmium and the added cadmium would be atomized to different extents upon analysis. This was a possible contributing factor to the observed matrix effect.

It was not possible to determine the chemical form of cadmium using the technique described here. Some method of separation of different cadmium compounds prior to atomization would be necessary to establish if the form of the metal was indeed different in various urine samples. Preliminary work in the area of metal speciation was pursued by the author and will be described in Part II of this dissertation. It was obvious that further characterization of the chemical form of metals in biological samples was essential for an understanding of their absorption, excretion, and effect in the body.
2. Concentration Range for Cadmium in the Urine of Unexposed Individuals

It was found that the average value for the concentration of cadmium in the urine of the subjects sampled was 20 ppb. It can be seen (Figure 15) that the sample distribution was skewed toward lower cadmium concentrations and included two abnormally high values. A comparison between the mean value obtained here and those reported in the literature is presented in Table 9. Many of the literature values for urinary cadmium were considerably lower than those obtained in this research. However, it must be considered that most of these values were obtained by conventional graphite furnace atomic absorption preceded by wet-ashing, dilution, or extraction. These procedures characteristically lead to erroneous results due to loss of some of the cadmium in the sample. It was believed that the data obtained using the quartz "T" atomizer and the filter paper disk technique more accurately reflected the normal concentration of cadmium in the urine.

No samples were analyzed from individuals who were known to be occupationally exposed to cadmium or its compounds. Studies including such population groups would be necessary to establish whether urine analysis would be an effective screening method for high cadmium exposure or body burden. It appeared from these studies
Table 9

COMPARISON OF LITERATURE VALUES FOR CADMIUM IN URINE

<table>
<thead>
<tr>
<th>Conc. Cd (ppb)</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>electrothermal AAS</td>
<td>in press</td>
</tr>
<tr>
<td></td>
<td>quartz &quot;T&quot; atomizer</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>graphite furnace AAS</td>
<td>117</td>
</tr>
<tr>
<td>0.4-3.7</td>
<td>graphite furnace AAS</td>
<td>90</td>
</tr>
<tr>
<td>0.7</td>
<td>1:1 dilution, graphite furnace AAS</td>
<td>122</td>
</tr>
<tr>
<td>10</td>
<td>1:10 dilution, quartz &quot;T&quot; AAS</td>
<td>73</td>
</tr>
<tr>
<td>&lt;3</td>
<td>1:50 dilution, graphite furnace AAS</td>
<td>86</td>
</tr>
<tr>
<td>1.7</td>
<td>extraction, graphite furnace AAS</td>
<td>115</td>
</tr>
<tr>
<td>1.25±0.84</td>
<td>wet-ash, graphite furnace AAS</td>
<td>89</td>
</tr>
<tr>
<td>44</td>
<td>flame AAS</td>
<td>123</td>
</tr>
<tr>
<td>3-12</td>
<td>dilution, flame AAS</td>
<td>112</td>
</tr>
<tr>
<td>2.04</td>
<td>dry-ash, extraction, flame AAS</td>
<td>80</td>
</tr>
<tr>
<td>0.5</td>
<td>atomic fluorescence</td>
<td>102</td>
</tr>
<tr>
<td>23.6±3.5</td>
<td>ion-exchange preconcentration, inductively-coupled plasma emission</td>
<td>119</td>
</tr>
</tbody>
</table>
that the range of cadmium concentrations for non-
on-occupationally exposed individuals was sufficiently
narrow such that differences for an exposed population
may be readily discernible.

3. Variations in the Cadmium Concentration in the Urine
of an Individual

The concentration of cadmium in the urine of an individual was found to vary on a day-to-day basis. It can be seen from Figure 16 that the amount of this variation was different for the two subjects studied. Aside from one abnormally high value in each case, the average urinary concentration for Subject A was found to be 20 ± 7 ppb and that of Subject B to be 22 ± 9 ppb. This indicated an average daily variation of 35-40%.

It seemed plausible that daily excretion of cadmium in the urine would vary, with many factors influencing this variation. Diet, fluid intake, exercise, and the levels of many other compounds in the body were thought to affect the daily balance of cadmium. Of course, fluid intake would directly affect the concentration of cadmium found in the urine by altering the amount of fluid passed through the kidneys. One possible means of accounting for variable fluid content would be to measure the specific gravity of each urine sample. A possible internal correction would be to determine the cadmium concentration in relation to some other
component in the urine, such as creatinine.\textsuperscript{110}

In view of the fact that spot urine samples were analyzed in this study, it was not surprising that daily variations were noted for the cadmium concentration. Samples were not always collected at the same time of the day, although most often this was done in the afternoon. It was possible that changes in factors such as food or fluid ingested, metabolic activity, or exercise could cause differences in cadmium excretion at different times of the day.

Some of the variation that was observed between separate individuals for urinary cadmium undoubtedly also was due to differences in fluid intake and excretion.

4. Estimated Daily Excretion of Cadmium Through the Urine

The average daily excretion of cadmium through the urine could be estimated based on the data obtained in these studies. The average concentration of cadmium in the urine of the individuals sampled was 20 ppb. If the normal volume of urine excreted in 24 hours is taken to be 1500 ml,\textsuperscript{63} then the average amount of cadmium excreted per day would be approximately 30 µg.

5. Correlations Between the Cadmium Concentration in the Urine and That in Perspiration

It was of interest to discover whether a correlation existed between the concentration of cadmium in
the urine and that in other excretory fluids. Therefore, studies were undertaken in which sweat and urine specimens were taken at the same time from a number of subjects. These samples were subsequently analyzed and compared as to both cadmium concentration and matrix effects.

The results of these studies will be described in detail in the following chapter.

E. CONCLUSIONS AND SUMMARY

1. The use of the carbon bed atomizer and the filter paper disk method of sample introduction was an effective technique for the direct analysis of urine samples. Efficient reduction of the background absorption eliminated the need for previous ashing steps. In addition, the technique was sufficiently sensitive so that 1 μl of urine could be analyzed without preconcentration.

2. The average urinary cadmium concentration for individuals who were not occupationally exposed to the metal was found to be approximately 20 ppb. The relative precision of the measurement of the cadmium concentration in urine by this method was approximately 28%.

3. The urinary cadmium concentration for a given individual varied on a day-to-day basis. This variation was approximately 35-40%.
4. The average daily excretion of cadmium through the urine was estimated to be 30 µg.
CHAPTER 3
THE DIRECT DETERMINATION OF CADMIUM IN PERSPIRATION

A. INTRODUCTION

Excretion of trace metals in sweat is of potential importance in the balance of certain elements in the body. The loss of essential trace elements can be significant in nutrition, while excretion of toxic metals is a factor in toxicity studies. It has been suggested that the excretion of organics in the sweat is a means of ridding the body of non-essential components.\textsuperscript{124} Since a proportion of normal human sweating is insensible,\textsuperscript{125} this mode of loss is possibly more significant than generally believed for many metals. Little research has been published on the concentration levels of metals, particularly cadmium, in human perspiration.

1. Characteristics of Human Perspiration

a. Sensible and Insensible Sweating

Normal human perspiration consists both of sensible and insensible sweating.\textsuperscript{125} Insensible sweating is that elimination of moisture which occurs without the knowledge or awareness of the subject. Sensible perspiration, on the other hand, is sweating which can be felt by the individual.

Insensible perspiration includes elimination both through respiration and through the skin. The mechanisms
for these two pathways differ, but both are known to be influenced by factors such as metabolic activity, atmospheric conditions, and body size.\textsuperscript{125} Total insensible perspiration has been estimated to be about 23 g/hr per square meter of body surface area. The cutaneous portion of this total was considered to be approximately 16 g/hr per m\textsuperscript{2}. Respiratory excretion was therefore estimated to be between 6 and 19 g/hr, depending on the volume of respiration and the humidity.\textsuperscript{125}

Cutaneous insensible perspiration varies with the region of the body.\textsuperscript{125} The palm and sole of the foot have been found to be 5-20 times more active in insensible excretion than the general body surface. It also is known that there are two types of insensible sweating, which differ in the region of the body which exhibit them.\textsuperscript{125} Thermal sweating occurs over the general body surface, with the exception of the palms and soles. Mental or emotional sweating, however, is demonstrated primarily at the palms and soles, and to a lesser extent at the axilla, forehead, and some other regions. The composition of sweat is believed to vary depending on the region of the body.\textsuperscript{124,125}

b. Mechanisms of Sweat Excretion

Sensible and insensible perspiration are known to differ in their mechanism of excretion.\textsuperscript{125} Insensible perspiration occurs for the most part without the involve-
ment of the sweat glands. In the skin, the epidermis is supplied with moisture from the blood vessels. Insensible sweating involves primarily the evaporation of this moisture through the skin. Sweat glands are believed to be involved only to a limited extent in insensible perspiration, primarily on the palms and soles, and to a small extent on the rest of the body surface.

In contrast, sensible perspiration is always a result of the secretion of the sweat glands. It is known that sensible perspiration is excreted from two types of sweat glands. The more numerous type, the eccrine glands, are distributed over the whole body and secrete a dilute fluid with a low concentration of many substances. The apocrine glands, on the other hand, are found in association with hair follicles in a few restricted areas of the body, such as the axilla. The secretion of the apocrine sweat glands contains many organic substances which vary in different glands and with different individuals. Apocrine glands are activated by intense pain or fear.

c. Composition of Sweat

It might be expected that sensible and insensible sweat would differ significantly in composition. Sensible sweat has been said to contain all the minerals found in the blood. It was not known whether insensible sweat also contained these same elements.
It has been stated that sweat excreted during the initial stages of perspiration contains both secretions from the sweat glands and contaminants from the skin tissue. Sweat obtained during more profuse perspiration, however, was thought to contain only the excretion from the sweat glands.\textsuperscript{125}

d. Average Amount of Perspiration

The average amount of perspiration for humans has been estimated by Kuno\textsuperscript{125} to be between 3.0 and 3.3 kg/day for a 65-kg (165-lb) man under conditions of approximately 29°C. Sensible perspiration was believed to account for approximately 2.3 kg of this total. Of course, many factors influence the amount of perspiration expected for an individual including atmospheric conditions, metabolic rate, and body size. The maximum amount of perspiration was reported to be 1.5 to 2.0 kg/hr.\textsuperscript{125}

Mitchell and Hamilton reported an experimental insensible perspiration loss of 117 g/hr under "comfortable" conditions and 720 g/hr under "hot, humid" conditions.\textsuperscript{126} These estimates are significantly higher than those of Kuno mentioned above. It is possible that some of the sweating regarded as insensible by Mitchell and Hamilton was in fact sensible perspiration.
2. Analytical Problems in the Analysis of Sweat

The analysis of perspiration was made difficult by several factors. The first problem was in the collection of a representative sample. Care must be taken to prevent contamination not only from the collection vessel but also from the skin. The danger of loss through evaporation also was present. The possibility that the composition of sweat varied depending on the region of the body also complicated sample collection. Several authors have recommended a total-body washdown procedure for collection of sweat. In this technique, subjects were exposed to a controlled-temperature chamber for a measured amount of time, after which the whole body was washed to collect sweat solutes. The amount of sweat was calculated by measuring the net loss in body weight during the collection period. Other investigators collected perspiration in a polyethylene bag attached to the subject's forearm. Cohn and Emmett reported that specimens collected by the arm-bag method gave higher and more variable results than those collected from the whole body. It seemed that both procedures left much room for error.

Another difficulty in the analysis of sweat samples was similar to one encountered in urine analysis. The presence of high concentrations of inorganic salts, particularly NaCl and KCl, could interfere with the
absorbance of certain metals if absorption techniques were used for analysis. This interference was known to be a problem for cadmium in particular. The approximate concentrations of some of the electrolytes found in human sweat are listed in Table 10. Salts such as NaCl have been shown to seriously interfere with cadmium absorption at concentrations greater than 1 mg/ml; it can be readily seen from Table 10 that the concentrations of several salts in perspiration were well within the range to exhibit interference.

3. Common Methods for the Analysis of Sweat

Few examples of sweat analysis were to be found in the literature. Several older papers reported the analysis of perspiration for elements such as iron, zinc, nitrogen, and calcium by various wet methods and colorimetric procedures. Consolazio, et al., estimated the losses of several trace metals in sweat, measured by various procedures. Cadmium was not included among the metals determined. A list of some of the metals included in the study and their reported rate of loss through perspiration is presented in Table 11.

Several elements have been determined in sweat by X-ray methods. Barbier, et al., determined bromine in sweat by X-ray fluorescence, while Quinton used an energy-dispersive X-ray detector on a scanning electron microscope to determine several of the major elements.
Table 10

APPROXIMATE CONCENTRATION OF THE ELECTROLYTES IN SWEAT

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal Conc. (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>200</td>
</tr>
<tr>
<td>Potassium</td>
<td>20</td>
</tr>
<tr>
<td>Calcium</td>
<td>2</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1</td>
</tr>
<tr>
<td>Chloride</td>
<td>320</td>
</tr>
<tr>
<td>Urea-N</td>
<td>15</td>
</tr>
<tr>
<td>Amino acid-N</td>
<td>1</td>
</tr>
<tr>
<td>Ammonia</td>
<td>5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>2</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>25</td>
</tr>
</tbody>
</table>

Normal pH = 4.5-7.5

\(^{125}\)
Table 11

ESTIMATED LOSSES OF TRACE METALS IN SWEAT

<table>
<thead>
<tr>
<th>Element</th>
<th>Loss (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>0.059</td>
</tr>
<tr>
<td>Mn</td>
<td>0.097</td>
</tr>
<tr>
<td>Fe</td>
<td>0.5</td>
</tr>
<tr>
<td>Co</td>
<td>0.017</td>
</tr>
<tr>
<td>Cu</td>
<td>1.59</td>
</tr>
<tr>
<td>Zn</td>
<td>5.08</td>
</tr>
<tr>
<td>Se</td>
<td>0.34</td>
</tr>
<tr>
<td>Sr</td>
<td>0.96</td>
</tr>
<tr>
<td>Mo</td>
<td>0.061</td>
</tr>
<tr>
<td>I</td>
<td>0.006</td>
</tr>
<tr>
<td>F</td>
<td>0.65</td>
</tr>
<tr>
<td>Ni</td>
<td>0.083</td>
</tr>
<tr>
<td>Pb</td>
<td>0.256</td>
</tr>
<tr>
<td>Hg</td>
<td>0.0009</td>
</tr>
<tr>
<td>Sb</td>
<td>0.011</td>
</tr>
<tr>
<td>Sn</td>
<td>2.23</td>
</tr>
<tr>
<td>Ba</td>
<td>0.085</td>
</tr>
<tr>
<td>Br</td>
<td>0.2</td>
</tr>
<tr>
<td>Al</td>
<td>6.13</td>
</tr>
<tr>
<td>Ti</td>
<td>0.001</td>
</tr>
<tr>
<td>Nb</td>
<td>0.003</td>
</tr>
</tbody>
</table>
in sweat.\textsuperscript{132}

Atomic absorption spectroscopy has also been used to determine metals in sweat.\textsuperscript{127,128} Cohn and Emmett\textsuperscript{127} reported a mean concentration of 23 µg/l (23 ppb) for cadmium in the sweat of 6 males and 2 females. Asayama, \textit{et al.}, reported a high correlation among the concentrations of nine metals in perspiration. The authors proposed that the metals were excreted by similar mechanisms.\textsuperscript{128} Both of these papers reported that the concentration of most metals (including cadmium) in perspiration was higher than the concentration found in urine.\textsuperscript{127,128}

4. Reasons for This Study

In view of the limited data available on the concentration of metals, particularly cadmium, in human perspiration, it was thought to be of interest to investigate this excretory mode more closely. It has been stated that the levels of cadmium in sweat are higher than those in urine. This suggested that excretion of cadmium in the sweat could be a significant factor in the balance between uptake and secretion of the metal; the amount of cadmium excreted through perspiration would directly affect the body burden of this metal in man.

Graphite furnace atomic absorption spectroscopy using the quartz "T" atomizer could be easily applied
to the analysis of sweat samples. In conjunction with sample introduction on filter paper disks, the described apparatus provided a rapid and accurate means of analysis for a potentially difficult biological matrix.

B. EXPERIMENTAL

1. Equipment

The equipment used in the analysis of sweat samples was identical to that previously described for the analysis of urine and blood. The atomic absorption spectrophotometer was unchanged; all components and auxiliary equipment were the same as those previously mentioned.

2. Procedure

a. Sampling Techniques

Sweat samples were collected under supervision in a sauna at the LSU Field House, from volunteers who were members of the university population. Individuals sampled had undergone an exercise program and then showered before entering the sauna. Sweat samples were collected only after profuse sweating had begun in order to avoid collection of skin contaminants. Collection was made by catching drops of perspiration from the nose or forehead in a previously cleaned polyethylene vial. No contact was made between the vial and the skin itself. The average volume of sweat collected was
approximately 2 ml over a 10-minute period.

Collection of most samples was made in early afternoon; some samples were obtained in late afternoon. In most cases, urine samples were collected from each subject within minutes of the collection of sweat specimens. It thus was possible to compare the cadmium content of these two excretory fluids collected at essentially the same time under the same conditions for each individual.

All samples were analyzed within four hours of collection using the technique previously described for blood and urine specimens. One microliter of sweat was placed on a filter paper disk using a Hamilton syringe. Disk and sample were subsequently dropped onto the surface of the hot carbon bed where atomization occurred. The concentration of cadmium was determined by monitoring the percent absorption of the cadmium resonance line at 2288Å. This signal represented both absorption due to cadmium and non-specific absorption due to molecular background. Background correction was accomplished by measuring the absorption by the sample of a non-resonance line of the cadmium hollow cathode found at 2266Å. Absorbance at this wavelength was due only to molecular species. The difference between the two signals was a measure of absorbance due to cadmium and could be related to the concentration of the metal.
Filter paper disks were cut from Whatman #41 filter paper and cleaned of cadmium contamination by leaching with 10% HNO₃ as previously described. The absorbance of blank filter paper disks was routinely recorded and subtracted from the sample absorbance.

b. Calibration Procedures

Calibration was carried out using the method of standard additions. It was observed early in this study that the calibration curves constructed by standard additions to different sweat samples differed in slope. This phenomenon had also been observed when analyzing urine specimens. It was apparent that the matrix effects for different sweat samples varied; this fact made it necessary to generate separate calibration curves for each sample.

Standard addition calibration was accomplished by spiking 0.5 ml aliquots of a single sweat sample with microliter amounts of a 20 ppm aqueous cadmium standard. A 20 ppm standard was selected because additions of this solution would approximately double the concentration of cadmium originally present in most sweat samples. Generally, 1, 3, and 5 µl aliquots of the aqueous standard were added. It was observed as data collection progressed that often the standard prepared by the addition of 5 µl of the 20 ppm cadmium solution was beyond the linear range of the analytical method. For
this reason, subsequent calibration curves were constructed using only the original sample plus the two standards prepared by adding spikes of 1 and 3 μl of the aqueous cadmium.

A complete standard addition calibration curve was constructed for approximately one out of four perspiration samples. The remaining samples were analyzed using the original sample and one aliquot containing a standard addition. The volume of many samples was insufficient to provide more than one 0.5 ml aliquot for the purpose of standard additions. However, the complete calibration curves constructed with at least one sample per day were consistently linear. Therefore, it was felt that those curves constructed using the original sample and one spiked aliquot were also satisfactory.

C. RESULTS

1. Concentration Range for Cadmium in the Perspiration of Unexposed Individuals

Sweat samples were collected from a number of individuals who were not occupationally exposed to cadmium. All subjects sampled in this study were male and most were between 40 and 60 years of age. Perspiration samples were analyzed for cadmium using the technique previously described. The results of these analyses are presented in Table 12. The mean concentration of
Table 12

VALUES FOR CONCENTRATION OF CADMIUM FOUND IN THE SWEAT OF UNEXPOSED INDIVIDUALS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Conc. Cd (ppb)</th>
<th>Subject</th>
<th>Conc. Cd (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>41</td>
<td>12</td>
<td>70</td>
</tr>
<tr>
<td>B</td>
<td>90</td>
<td>13</td>
<td>68</td>
</tr>
<tr>
<td>C'</td>
<td>28</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>D'</td>
<td>161</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>E'</td>
<td>12</td>
<td>16</td>
<td>85</td>
</tr>
<tr>
<td>F'</td>
<td>50</td>
<td>17</td>
<td>205</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>18</td>
<td>175</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>19</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>21</td>
<td>110</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>22</td>
<td>insufficient sample</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>23</td>
<td>194</td>
</tr>
<tr>
<td>7</td>
<td>98</td>
<td>24</td>
<td>54</td>
</tr>
<tr>
<td>8</td>
<td>83</td>
<td>25</td>
<td>126</td>
</tr>
<tr>
<td>9</td>
<td>59</td>
<td>26</td>
<td>195</td>
</tr>
<tr>
<td>10</td>
<td>58</td>
<td>27</td>
<td>68</td>
</tr>
<tr>
<td>11</td>
<td>180</td>
<td>28</td>
<td>22</td>
</tr>
</tbody>
</table>

n = 33

mean = 84 ppb

range = 11-205 ppb
cadmium for all 33 individuals was 84 ppb; the range of values extended from 11 ppb to 205 ppb Cd.

The distribution of these values for the cadmium concentration in sweat is illustrated in Figure 18. To obtain this figure, the values listed in Table 12 were rounded to the nearest 10 ppb and presented as a function of the frequency of observation. It can be observed that the majority of sweat samples contained a cadmium concentration of between 10 and 130 ppb; these samples exhibited a fairly normal distribution pattern about a mean value of 60 ppb Cd. Six values appeared to be abnormally high, falling outside this distribution.

2. Variations in the Cadmium Concentration in the Perspiration of an Individual

The perspiration of two individuals was analyzed repeatedly over a period of approximately two months in order to follow day-to-day changes in the concentration of cadmium. It was observed that the cadmium concentration did indeed change with time. The results of this study are presented in Table 13. Figure 19 illustrates the distribution of the values for the cadmium concentrations for these two subjects. It can be seen that the distribution range differed between the two subjects, with Subject B having a higher average concentration of cadmium in the sweat than Subject A. There appeared to be one abnormally high value for each
FIGURE 18

This figure shows the distribution of the cadmium concentrations found in the sweat of individuals not occupationally exposed to cadmium.
Table 13
DAILY VARIATIONS IN THE CONCENTRATION OF CADMIUM
IN THE SWEAT OF TWO INDIVIDUALS

<table>
<thead>
<tr>
<th>Date</th>
<th>Subject A</th>
<th>Subject B</th>
</tr>
</thead>
<tbody>
<tr>
<td>02-08-80</td>
<td>16</td>
<td>51</td>
</tr>
<tr>
<td>03-10-80</td>
<td>18</td>
<td>140</td>
</tr>
<tr>
<td>03-14-80</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>03-17-80</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>03-19-80</td>
<td>32</td>
<td>43</td>
</tr>
<tr>
<td>03-24-80</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>03-26-80</td>
<td>52</td>
<td>86</td>
</tr>
<tr>
<td>03-28-80</td>
<td>23</td>
<td>62</td>
</tr>
<tr>
<td>03-31-80</td>
<td>68</td>
<td>no sample collected</td>
</tr>
<tr>
<td>04-16-80</td>
<td>105</td>
<td>48</td>
</tr>
<tr>
<td>04-18-80</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>04-21-80</td>
<td>38</td>
<td>205</td>
</tr>
<tr>
<td>04-23-80</td>
<td>46</td>
<td>128</td>
</tr>
<tr>
<td>04-28-80</td>
<td>22</td>
<td>70</td>
</tr>
<tr>
<td>04-30-80</td>
<td>26</td>
<td>77</td>
</tr>
</tbody>
</table>

n = 15  
mean = 41 ppb  
σ = 24  
range = 16-105 ppb

n = 14  
mean = 90 ppb  
σ = 45  
range = 43-205 ppb
FIGURE 19

This figure shows the distribution of the cadmium concentrations found in the sweat of two individuals. Samples were collected regularly over a 2-month period.
individual. The mean cadmium concentration in the sweat of these two individuals was found to be 41 ppb for Subject A and 90 ppb for Subject B. If the high value for each subject was excluded, the average cadmium concentration (mean ± σ) was found to be 36 ± 17 ppb for Subject A and 82 ± 32 ppb for Subject B. This indicated an average daily variation of approximately 40-45%.

The "standard deviation" has been calculated here as a measure of the amount of variation in the concentration of cadmium found in the sweat on a day-to-day basis. It must be emphasized, however, that a different sample was analyzed to obtain each of the reported values; therefore, the values calculated above are not a measure of precision nor of true deviation. These values only serve as an indication of the range of cadmium levels found in sweat and the distribution within that range.

The data indicated that on one day (4-16-80), the perspiration samples collected from the two subjects may have been reversed. Unfortunately, it was impossible to verify this possibility.

3. Precision of the Analytical Method

A single perspiration specimen was analyzed repeatedly in order to determine the precision of the filter paper disk technique in the analysis of this type
of sample. The data obtained are presented in Table 14.

At a level of 22 ppb Cd in the sample, the relative standard deviation of the total absorbance measurement was 12%. The average total signal recorded for the sample was 14% absorption. The relative standard deviation of the absorbance signal due only to cadmium was approximately 22%, which corresponds to an error of approximately ± 5 ppb Cd.

4. Comparison of the Cadmium Concentrations Found in Sweat and Urine

In most cases, urine samples were collected in conjunction with sweat specimens for each individual tested. Both sweat and urine samples were analyzed within four hours of collection. The concentration of cadmium in these two excretory fluids could thus be compared for each subject; these data are presented in Table 15. It can be seen that in all cases the urinary cadmium concentration was less than that in the sweat. A comparison of the two values by means of a ratio ([Cd] in sweat/[Cd] in urine) showed no distinct correlation between the cadmium concentration of the two samples. A graphical presentation of the correlation data can be found in Figure 20.

Sweat and urine specimens were also collected together for the two subjects that were sampled repeatedly for variability studies. The analyses of these samples made
Table 14
VALUES OBTAINED FOR REPEATED ANALYSIS OF A
SINGLE SWEAT SAMPLE

<table>
<thead>
<tr>
<th>Aliquot</th>
<th>% Absorption</th>
<th>Aliquot</th>
<th>% Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>12</td>
<td>13.5</td>
</tr>
<tr>
<td>3</td>
<td>15.5</td>
<td>13</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>13.5</td>
<td>14</td>
<td>13.5</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>19</td>
<td>14.5</td>
</tr>
<tr>
<td>10</td>
<td>14.5</td>
<td>20</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Average Total % Absorption = 14.1; $\sigma = 1.8$

Average Total Absorbance = 0.066; $\sigma = 0.0079$

Relative standard deviation of total absorbance = 12%

Absorbance due to Cd alone = 0.035

Relative standard deviation of Cd absorbance = 22%
Table 15
COMPARISON OF THE CONCENTRATION OF CADMIUM IN THE URINE AND IN THE SWEAT OF UNEXPOSED INDIVIDUALS

<table>
<thead>
<tr>
<th>Subject</th>
<th>([\text{Cd}] \text{ in Sweat (ppb)})</th>
<th>([\text{Cd}] \text{ in Urine (ppb)})</th>
<th>Sweat (<a href="%5Ctext%7Bppb%7D">\text{Cd}</a>)</th>
<th>Urine (<a href="%5Ctext%7Bppb%7D">\text{Cd}</a>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>41</td>
<td>23</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>90</td>
<td>24</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>28</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>N.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>4</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>24</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>7</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>66</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>98</td>
<td>20</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>83</td>
<td>19</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>59</td>
<td>12</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>58</td>
<td>18</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>68</td>
<td>67</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>N.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>42</td>
<td>18</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>85</td>
<td>10</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>205</td>
<td>28</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>175</td>
<td>11</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>45</td>
<td>12</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>45</td>
<td>19</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>110</td>
<td>25</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>194</td>
<td>32</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>54</td>
<td>9</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>126</td>
<td>27</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>195</td>
<td>17</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>68</td>
<td>22</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>22</td>
<td>18</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 20

This figure shows the poor correlation observed between the cadmium concentration in the urine and that in the sweat of individuals not occupationally exposed to cadmium.
possible a comparison of the day-to-day changes in the cadmium concentration of these two fluids. The data obtained, which represents a composite of Tables 7 and 13, are presented in Table 16. Again, urinary cadmium concentrations were less than those in sweat and the ratio between the cadmium concentrations in the two samples ([Cd] in sweat/[Cd] in urine) was highly variable.

The cadmium concentration for the sweat and urine of Subject A as a function of the date is presented graphically in Figure 21. A similar illustration for Subject B can be found in Figure 22.

D. DISCUSSION

1. General Considerations Concerning the Determination of Cadmium in Perspiration

It has been previously mentioned that the determination of cadmium in perspiration was made difficult by the presence of a high concentration of inorganic salts, such as NaCl and KCl, as well as by other constituents of the matrix. The filter paper disk technique utilized in this research allowed the direct determination of cadmium in perspiration by providing a more complete breakdown of the sample matrix, thereby minimizing background absorbance. For all sweat samples analyzed, the average background absorbance was approximately 0.03 absorbance units, which cor-
Table 16

COMPARISON OF THE CONCENTRATION OF CADMIUM IN THE URINE AND IN THE SWEAT OF TWO INDIVIDUALS (REPEATED SAMPLING)

<table>
<thead>
<tr>
<th>Date</th>
<th>Subject A</th>
<th>Subject B</th>
</tr>
</thead>
<tbody>
<tr>
<td>03-10-80</td>
<td>18/30 = 0.6</td>
<td>no samples collected</td>
</tr>
<tr>
<td>03-14-80</td>
<td>18/18 = 1.0</td>
<td>60/16 = 3.7</td>
</tr>
<tr>
<td>03-17-80</td>
<td>50/22 = 2.3</td>
<td>75/20 = 3.7</td>
</tr>
<tr>
<td>03-19-80</td>
<td>32/24 = 1.3</td>
<td>43/22 = 2.0</td>
</tr>
<tr>
<td>03-24-80</td>
<td>42/28 = 1.5</td>
<td>100/40 = 2.5</td>
</tr>
<tr>
<td>03-26-80</td>
<td>52/25 = 2.1</td>
<td>86/16 = 5.4</td>
</tr>
<tr>
<td>03-28-80</td>
<td>23/5 = 4.6</td>
<td>62/17 = 3.6</td>
</tr>
<tr>
<td>03-31-80</td>
<td>68/13 = 5.2</td>
<td>no samples collected</td>
</tr>
<tr>
<td>04-16-80</td>
<td>105/18 = 5.8</td>
<td>48/35 = 1.4</td>
</tr>
<tr>
<td>04-18-80</td>
<td>60/62 = 1.0</td>
<td>120/31 = 3.9</td>
</tr>
<tr>
<td>04-21-80</td>
<td>38/27 = 1.4</td>
<td>205/53 = 3.9</td>
</tr>
<tr>
<td>04-23-80</td>
<td>46/20 = 2.3</td>
<td>128/14 = 9.1</td>
</tr>
<tr>
<td>04-28-80</td>
<td>22/15 = 1.5</td>
<td>70/15 = 4.7</td>
</tr>
<tr>
<td>04-30-80</td>
<td>26/15 = 1.7</td>
<td>77/14 = 5.5</td>
</tr>
</tbody>
</table>

n = 14  n = 12
mean = 2.3 mean = 4.1
FIGURE 21

This figure shows the concentration of cadmium found in the urine and in the sweat of Subject A as a function of the date.
FIGURE 22

This figure shows the concentration of cadmium found in the urine and in the sweat of Subject B as a function of the date.
responds to a signal of about 6% absorption. Preliminary ashing steps or dilution of the sample before analysis was unnecessary. The described technique therefore eliminated the errors associated with sample pretreatment, as well as greatly simplifying the analysis procedure.

The precision of the analytical method has been stated to be approximately 22% at the 22 ppb level. This precision was considered to be acceptable for the determination of cadmium at this low level in perspiration samples. As has been suggested in Chapter 1, the precision of the method could be improved by the use of an automatic background corrector.

As has been previously mentioned, it was observed that calibration curves obtained by standard additions to different sweat samples varied in slope. This observation had also been made when urine samples were analyzed and indicated a chemical interference by some constituent(s) of the sample matrix. Different chemical forms of a metal would be expected to differ in their efficiency of atomization under the same conditions; therefore, the change in slope for the standard addition calibration curves for different perspiration samples indicated a different chemical form of cadmium in these samples.
2. Concentration Range for Cadmium in the Perspiration of Unexposed Individuals

The distribution of values for the concentration of cadmium in the perspiration of the population sampled, shown in Figure 18, indicated two distribution ranges. The majority of samples analyzed contained between 11 and 126 ppb Cd and exhibited an essentially normal distribution within this range. An additional six samples, however, contained from 161 to 205 ppb Cd. It was possible that these individuals more efficiently excreted cadmium through the sweat glands.

Limited data were available in the literature regarding the level of cadmium in perspiration. Cohn and Emmett reported a mean value of $24 \pm 16 \mu g/l$ (24 ppb) Cd for 6 males. The method of analysis used by these researchers was flame atomic absorption spectroscopy. Other researchers who analyzed perspiration did not report the concentration of cadmium.

The values obtained for the concentration of cadmium in sweat by the described method were believed to be reasonable and accurate. Although Cohn and Emmett reported a significantly lower average value than that reported here, it must be considered that their method involved a much more complex sample collection procedure (total body washdown) and thereby considerable dilution of the sample. After collection and filtration, pre-
concentration of the sample was necessary in order to
determine cadmium in the ppb range. The method there-
fore was subject to many sources of error. It was
believed that direct collection of the sample, such as
was practiced in this study, and direct analysis by the
filter paper disk technique resulted in a more accurate
measure of the cadmium content of perspiration.

3. Estimated Daily Excretion of Cadmium Through Perspiration

The sweat samples collected for this study were
considered to represent sensible sweat. In fact, under
the conditions in which the samples were collected (in
a sauna), it might be considered that maximum sweating
took place. It has been estimated that maximum perspira-
tion takes place at a rate of approximately 1.5 kg/hr.\textsuperscript{125}
Under these conditions, an individual will excrete
approximately 90 \textmu g of cadmium per hour (using an average
value of 60 ppb for the cadmium concentration of sweat).
If this rate of sweating continued indefinitely, the
maximum amount of cadmium lost through perspiration
would be approximately 2 mg/day.

The level of cadmium in insensible sweat may be
different from those reported here due to the fact that
sensible and insensible sweating were believed to take
place by different mechanisms.\textsuperscript{125} However, if it is
assumed that the concentration of cadmium in insensible
sweat is the same as that measured here for sensible
sweat, an estimation of the total amount of cadmium excretion under more normal conditions can be made.

If the average amount of insensible perspiration is 1 kg/day, then approximately 60 μg of cadmium would be excreted daily by this means. The total cadmium excretion per 24-hour period would depend on the additional amount of sensible sweat excreted. This, in turn, would depend on individual characteristics and environmental conditions. As a rough estimation, it may be assumed that man excretes approximately 3 kg/day of both sensible and insensible sweat. Therefore, the amount of cadmium which may be excreted through the sweat can be estimated to be about 120 μg/day.

It seemed, based on the studies reported here, that excretion of cadmium through perspiration was a significant mode of loss of the metal.

4. Variations in the Cadmium Concentration of Sweat for an Individual

The cadmium concentration of perspiration was found to vary for an individual sampled on different days. As Figure 19 illustrates, the amount of variation observed was different for the two subjects studied. The concentration of cadmium found in the perspiration of Subject A was within a range of from 16 to 105 ppb Cd, while that of Subject B spanned a wider range of between 43 and 205 ppb Cd.
It was not surprising that the concentration of cadmium in sweat was found to vary on a day-to-day basis. Many factors could influence this variation, including individual characteristics such as diet, exercise, fluid intake, and the efficiency of the sweat glands. Diet could affect elimination of cadmium through perspiration by changing the intake of cadmium, as well as that of other metals or compounds which might be in balance with it in the body. Fluid intake also would directly influence the cadmium concentration in sweat by altering the fluid available for secretion and, depending on the individual, the amount of perspiration secreted. Of course, it has been well known that atmospheric conditions will affect the total amount of perspiration. Although this effect was minimized in this study by the collection of samples always under the same conditions, atmospheric variations would influence total perspiration, and therefore total excretion of cadmium, under normal daily circumstances. A difference in the amount of daily variation between individuals indicated a difference in the response of the individual's sweat glands to factors such as those mentioned above.

5. Comparison of the Cadmium Concentrations Found in Sweat and Urine

It was of interest to discover whether a
correlation existed between the concentration of cadmium in the sweat and in the urine, as representatives of two different excretory modes in man. As is illustrated in Figure 20, no direct correlation was evident between the absolute concentration of cadmium in these two excretory fluids for the total population studied. However, examination of Figures 21 and 22 suggested a rough correlation between the daily variations in the cadmium concentrations in these two fluids. It can be observed that, in general, elevations in urinary cadmium occurred in conjunction with elevations in sweat cadmium for both individuals studied. The same appeared to be true in regard to depressed cadmium values. This correlation, of course, was only a general tendency, and on certain days was not in evidence. The overall trend, however, indicated that an increase in cadmium excretion was reflected in an increase in both modes of elimination from the body. Increases or decreases in the cadmium level in these two fluids may reflect a corresponding increase or decrease in cadmium intake or exposure. It was also possible that a change in the level of some other component(s) in the body may cause a change in the rate of excretion of cadmium.

In the course of sweat and urine analyses, a general pattern was observed between the slopes of the standard addition calibration curves for the two types
of samples. It was noticed that repeated analysis of samples from the same individual (observed for Subjects A and B) always seemed to yield calibration curves of the same general slope. In those cases where the slope of the calibration curve changed from the normal, the concentration of cadmium found in that particular sample also deviated substantially from the mean. These effects can be observed from the data presented in Table 17.

It is also evident from the data in Table 17 that the slopes of the calibration curves for the two types of samples (urine and sweat) were similar in almost all cases. Again, it was noticed that deviations from the "normal" slope were reflected in substantial deviations from the average cadmium concentration for that particular type of sample.

A similar parallelism between the slopes of the standard addition curves for urine and sweat specimens was observed for other individuals sampled. The slopes of the calibration curves generally were near 0.0015 (A/ppb). Those samples with shifted slopes were found to contain unusually high or low concentrations of cadmium.

The similarity in slopes for the calibration curves for sweat and urine samples indicated that the matrix effects were similar for the two sample types. This suggested either that the cadmium secreted via these
Table 17

COMPARISON OF THE CADMIUM CONCENTRATION AND SLOPES OF THE CALIBRATION CURVES FOR URINE AND SWEAT ANALYSIS

<table>
<thead>
<tr>
<th>Date</th>
<th>Subject A Urine</th>
<th>Subject A Sweat</th>
<th>Subject B Urine</th>
<th>Subject B Sweat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><a href="ppb">Cd</a> Slope(A/ppb)</td>
<td><a href="ppb">Cd</a> Slope(A/ppb)</td>
<td><a href="ppb">Cd</a> Slope(A/ppb)</td>
<td><a href="ppb">Cd</a> Slope(A/ppb)</td>
</tr>
<tr>
<td>03-26-80</td>
<td>25 0.0015</td>
<td>52 0.0015</td>
<td>16 0.0034</td>
<td>86 0.0039</td>
</tr>
<tr>
<td>03-28-80</td>
<td>5 0.0020</td>
<td>23 0.0015</td>
<td>17 0.0020</td>
<td>62 0.0022</td>
</tr>
<tr>
<td>04-16-80</td>
<td>18 0.0019</td>
<td>105 0.0010</td>
<td>35 0.0015</td>
<td>48 0.0038</td>
</tr>
<tr>
<td>04-18-80</td>
<td>62 0.0007</td>
<td>60 0.0007</td>
<td>31 0.0014</td>
<td>120 0.0013</td>
</tr>
<tr>
<td>04-21-80</td>
<td>27 0.0017</td>
<td>38 0.0015</td>
<td>53 0.0006</td>
<td>205 0.0008</td>
</tr>
<tr>
<td>04-23-80</td>
<td>20 0.0020</td>
<td>46 0.0017</td>
<td>14 0.0016</td>
<td>128 0.0022</td>
</tr>
<tr>
<td>04-28-80</td>
<td>15 0.0020</td>
<td>22 0.0016</td>
<td>15 0.0020</td>
<td>70 0.0017</td>
</tr>
<tr>
<td>04-30-80</td>
<td>15 0.0015</td>
<td>26 0.0016</td>
<td>14 0.0019</td>
<td>77 0.0015</td>
</tr>
</tbody>
</table>
two excretory fluids occurred in the same chemical form, or that the matrices were indeed the same. This possibility was not considered unreasonable when the normal concentrations of the major components of sweat and urine were compared (Tables 5 and 10). The compositions of these two fluids were very similar; therefore, it would be expected that they would decompose with comparable efficiency when introduced into the atomizer. Differences in slope between subjects may reflect individual variability in the proportion of the various constituents of the matrix.

It was not possible to determine in what specific chemical form cadmium was excreted in the sweat and urine. However, it was suggested that, in those cases where the slope of the standard addition curve differed from the majority of samples, a different combined form of cadmium was being excreted. It was possible that, in these cases, some special mechanism of excretion was being observed. The toxicological significance of this observation was unknown; determination of the exact chemical form of the cadmium being excreted would be desirable as the first step in postulating a possible excretory pathway.

E. CONCLUSIONS AND SUMMARY

1. The use of the carbon bed atomizer together with sample introduction on a filter paper disk was a simple
and reliable technique for the direct analysis of sweat samples. Efficient breakdown of the sample reduced background absorption to an acceptable level. Pretreatment of the sample by ashing or dilution thus was not required. In addition, the technique was sufficiently sensitive so that no preconcentration of the sample was necessary.

2. The average value for the concentration of cadmium found in the sweat of all the individuals sampled was 84 ppb. The subjects sampled were members of the university community and were not considered to be occupationally exposed to cadmium. The relative precision of the measurement of cadmium in the sweat was approximately 22%.

3. The concentration of cadmium in the sweat of an individual varied on a day-to-day basis. This variation was approximately 40-45%.

4. The approximate rate of excretion of cadmium through perspiration under conditions of profuse sweating was calculated to be 90 µg/hr. Under maximum sweating conditions, therefore, the greatest amount of cadmium that would be excreted by this means would be about 2 mg/day.

5. If it was assumed that the composition of insensible sweat was the same as sensible sweat, the excretion of cadmium through insensible perspiration was estimated to be approximately 60 µg/day. The average total cadmium
excretion through the sweat was estimated to be 120 µg/day.

6. The concentration of cadmium in the sweat was greater than that in the urine for all individuals sampled.

7. A general correlation was observed between the daily fluctuations in urine and sweat cadmium levels. This indicated that an increase or decrease in cadmium excretion was reflected in a corresponding change in both modes of elimination from the body.

8. The slopes of the standard addition calibration curves for urine and sweat analyses were very similar for samples collected together from the same individual. This indicated that the matrix effects were similar for the two types of samples and that the cadmium excreted through these fluids may be in the same chemical form.
CHAPTER 4
THE DIRECT DETERMINATION OF CADMIUM IN HAIR

A. INTRODUCTION

Hair has long been considered a minor excretory tissue. Hair is recognized to be a metabolic end product which incorporates many elements into its structure as growth proceeds.\textsuperscript{134} The analysis of trace metals in hair has received increasing attention in individualization studies (the identification of hair as being from a particular individual),\textsuperscript{135-138} forensic science,\textsuperscript{136-138} nutritional studies,\textsuperscript{139,140} and clinical diagnosis.\textsuperscript{141} Great interest also has been aroused in the use of hair as a monitor of environmental exposure or body burden of certain elements.\textsuperscript{134,142-145}

There are many advantages to the use of hair as a sample for metal analysis. It is a stable sample which can be easily collected and stored. In addition, hair is composed of a nearly homogeneous matrix which is essentially constant between individuals. This characteristic makes possible the application of one analysis procedure to any hair sample.

Hair is known to be composed primarily of fibrous proteins. It is notable that approximately 14\% of the hair matrix consists of the amino acid cysteine.\textsuperscript{63} Since it has been proposed that cadmium interacts with
body proteins through binding to the cysteiny1 residues of the protein molecule,\textsuperscript{33-36} it was thought that hair would be a logical tissue to analyze as a possible measure of the body burden of this metal. However, little data have been published defining the normal level of cadmium in hair.

1. Common Methods for the Determination of Cadmium in Hair

Several analytical techniques have been used to determine cadmium in hair. One of the more common methods is neutron activation analysis (NAA).\textsuperscript{134-136} NAA is a multielement technique, and it has the additional advantage of a very low sensitivity for many elements. Radiochemical separation; that is, separation of the element of interest from other activated (radioactive) components of the sample, is not always necessary for a matrix such as hair. This is due to the absence of many of the easily activated inorganic salts encountered in other biological materials. The main disadvantages of NAA are the requirement for a neutron activation source, typically a nuclear reactor, and the length of time often required for activation and counting. For example, Jervis \textit{et al.} reported an irradiation time of 20-60 hours\textsuperscript{134} for the determination of heavy metals in hair, while Obrusnik \textit{et al.} described irradiation for 3 days or 24 days, depending on the sample and the element of interest.\textsuperscript{137} The determination of some less con-
centrated elements still required radiochemical separa-
tions.\textsuperscript{134}

Hair samples have also been analyzed by methods such
as anodic stripping\textsuperscript{147} and emission spectroscopy.\textsuperscript{148}
Each of these methods, however, is greatly influenced by
the matrix and requires ashing or oxidation of the
sample prior to analysis.

The most common method reported in the literature
for the determination of cadmium in hair is atomic
absorption spectroscopy.\textsuperscript{142-144,149} Flame atomic absorption is
the usual technique, always preceded by a wet-ashing
or dry-ashing procedure to break down the organic
matrix. Wet ashing procedures typically involve acid
digestion and evaporation,\textsuperscript{144,149} leading to the
possibilities both of contamination and loss. Often
dry-ashing procedures call for heating in a muffle
furnace for several hours at temperatures of 450°-
500°C.\textsuperscript{142,143} Significant losses of cadmium can occur
at these temperatures.

2. Analytical Considerations in the Determination of
Cadmium in Hair

a. Adsorption of Cadmium on the Hair Shaft

In the analysis of hair for cadmium, several
possible influences on the concentration of the metal
must be considered. First, there is the possibility of
external contamination of the sample from the environ-
ment; for example, adsorption of cadmium from shampoos and other hair treatments, dust, and other particulates in the air. Many researchers have recommended washing techniques designed to remove such surface contamination without leaching metals from within the hair matrix. These washing procedures are generally of two types. Some authors have recommended washing in an organic solvent, such as diethyl ether,\textsuperscript{138} a mixture of ether and ethanol,\textsuperscript{134,147} chlorothene,\textsuperscript{149} or carbon tetrachloride.\textsuperscript{143} It has been stated that this treatment removed surface contamination without removal of minerals from the hair shaft.\textsuperscript{143,149} Washing with such organic solvents appeared to cause only negligible changes in the concentration of cadmium when compared to unwashed hair, but these procedures were stated to be effective in removing oils, lacquers, and other surface contaminants.\textsuperscript{134}

Other researchers have suggested that washing of hair samples in a detergent was a more suitable procedure.\textsuperscript{142,146,150} This treatment had the advantage of being more similar to a normal washing procedure which might be practiced by persons donating hair samples; differences between previous sample treatments were thus minimized by subjecting all hair samples to a similar wash. Detergent washing was observed to decrease the concentrations of iron and magnesium in the hair.\textsuperscript{150}
There were some workers who employed combinations of the organic solvent and detergent treatments prior to analysis.\textsuperscript{144,151}

Nishiyama and Nordberg conducted experiments which indicated the extent of possible adsorption of cadmium by the hair shaft.\textsuperscript{152} Using radioactive $^{109}$Cd, it was shown that adsorption of the metal from an aqueous solution varied greatly between individuals and was pH-dependent. After adsorption, it was difficult to remove the cadmium from the hair; $1.0\text{N HNO}_3$ was found to be the best agent for removal. These researchers also studied the uptake of injected $^{109}$Cd into the hair matrix; the cadmium detected in the hair was termed endogenous cadmium. It was found that $1.0\text{N HNO}_3$ also removed endogenous cadmium from the hair.\textsuperscript{152}

b. Variations in the Concentration of Cadmium in Hair

The second possible influence on the concentration of cadmium in the hair is a reported variation between individuals under the same exposure conditions. Such individual differences in the adsorption and excretion of cadmium through the hair could limit the usefulness of hair analysis as a monitor of environmental exposure.\textsuperscript{143,145,153} Studies have been published which illustrate an increase in Cd concentration in the hair with age, the exact profile of
which varied with sex.\textsuperscript{144} Other authors have reported a decrease in cadmium concentration with age in females and a large variation according to color of hair.\textsuperscript{143} Kello and Kostial reported an increased concentration of Cd in the hair of young mice as opposed to older mice.\textsuperscript{145} Such studies indicated that the use of hair as a monitor of individual body burden or exposure to Cd must be made with caution.

On the other hand, it appeared that hair analysis might be used as a general indicator of exposed and nonexposed populations.\textsuperscript{134,142,153} Nordberg and Nishiyama reported experiments with mice in which the uptake of \textsuperscript{109}Cd in hair had a high correlation with the whole body concentration of the metal.\textsuperscript{153} Cadmium in the blood, however, decreased much more rapidly than the whole body concentration, indicating that blood was a poor indicator of body accumulation. The authors admitted that the application of these results to humans might be made difficult by differing affinities for cadmium between individuals based on age, sex, and environmental contamination.\textsuperscript{153} Subsequent work by Oleru\textsuperscript{142} and Jervis et al.\textsuperscript{134} showed significantly increased cadmium concentrations in hair samples from occupationally or environmentally exposed groups as opposed to nonexposed groups. Oleru also reported a positive correlation between the concentration of
cadmium in the hair and that in kidney and liver tissue in humans.\textsuperscript{142} These findings supported the use of cadmium levels in hair as an indicator of exposure and body accumulation of cadmium in man.

The interpretation of data concerning cadmium in the hair was further complicated by the fact that variations were found between single hairs taken from the same head and even among segments of the same hair strand.\textsuperscript{136,137} These variations differed between elements and among individuals for the same element. It was reported in general that the concentration of elements increased from the scalp toward the end of the hair strand.\textsuperscript{137}

These numerous variations were the source of much confusion over the significance of hair cadmium data. No study was found which established a general range of cadmium concentrations for an unexposed population. Also, no data were found comparing cadmium concentrations in hair to that in other excretory tissues such as urine and sweat. It was the purpose of this portion of this research to study the concentration of cadmium in the hair using the previously described quartz "$T$" atomizer for electrothermal atomic absorption spectroscopy. The individuals sampled were members of the university community and were not occupationally exposed to cadmium.
B. EXPERIMENTAL

1. Equipment

The equipment utilized in the analysis of hair samples was identical to that used for the previously discussed biological materials. All components of the atomic absorption spectrophotometer were unchanged. Due to the fact that hair was a solid material, filter paper disks were not needed for sample introduction. The only additional items used for hair analysis were a razor blade for cutting sections of a hair strand and a ruler for measuring the lengths of these sections.

2. Procedure

a. Sampling Techniques

Most samples were collected during normal grooming. Hair strands which were removed by brushing immediately after a shampoo were collected and transported to the laboratory in clean envelopes. Some samples were obtained by plucking a hair strand directly from the head. Only those hairs which had a visible and discernable root terminus were analyzed.

Hair samples were rinsed several times with deionized water to remove dust and surface contamination. Some samples were previously rinsed in acetone or in a detergent solution to allow comparison of these washing procedures. All equipment which would come in contact with the hair was cleaned with 10% HNO₃; handling of the
hair strands was done using cleaned tweezers.

The strands of hair were cut into 1-cm segments using a razor blade. These segments were then weighed to the nearest 0.01 mg using a Mettler H-10 analytical balance. A 1-cm segment of hair was taken for analysis because this amount of sample produced an absorption signal of moderate intensity. The average absorption signal recorded was approximately 30%, although hair segments from some subjects generated a signal of over 95% absorption.

Analysis was accomplished by simply dropping the piece of hair directly onto the surface of the carbon bed. No sample introduction vehicle was necessary; thus there was no sample blank. Resonance absorption was recorded using the cadmium resonance line at 2288 Å. Molecular background was monitored at 2266 Å.

b. Calibration Procedures

Calibration was carried out using aqueous cadmium standards. Standards over the range of 5 to 50 ppb were introduced into the atomizer by directly injecting a 5-μl aliquot using an Eppendorf pipette. Injection of these standards corresponded to an introduction of from $2.5 \times 10^{-5}$ to $2.5 \times 10^{-4}$ μg of cadmium. The absorbance of a 5-μl injection of distilled, deionized water was subtracted as a blank from the resonance absorbance of each standard solution.
Standards were made up fresh every two to three days from a CdSO₄ stock solution of 1000 ppm. The flasks and pipettes used for dilutions of the standards, as well as the containers in which they were stored, were pre-equilibrated with solutions of the concentrations they were to contain. Flasks and pipettes were used routinely for a single standard concentration.

C. RESULTS

Typical absorption traces for several 1-cm hair segments, illustrating both resonance absorption and molecular background, are presented in Figure 23. It can be seen that there was no significant absorption of the non-resonance line, indicating complete degradation of the sample matrix. Aqueous standards therefore could be used for calibration purposes. This was a distinct advantage of the analytical technique because of the difficulty found in other techniques in preparing standards that were representative of the hair matrix.¹⁵⁴

1. Variation in Cadmium Concentration Along the Hair Strand

Perhaps the most significant trend observed in this research was the fact that the concentration of cadmium in the hair varied along the length of the hair strand. Since only 1 cm of hair was taken for analysis, this variation was easily demonstrated.
FIGURE 23

Typical absorption signals obtained for 1-cm segments of human hair.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
Figure 24 illustrates the change in cadmium concentration along the length of one particular hair strand. It can be seen that the concentration of cadmium at the end of the hair was significantly higher than that at the root. The fact that this was a general trend is illustrated in Figure 25, which represents a composite of analyses of several hair strands from the same individual. Six consecutive 1-cm segments were cut for analysis, beginning at the root terminus of each hair strand. Correspondingly, 6 segments were analyzed from the end of each strand. The average strand length was 20 cm.

It was evident that the cadmium concentration found in hair was greatly dependent on the section of the hair strand taken for analysis. The concentration in the case illustrated in Figure 25, in fact, varied by a factor of ten between the 1-cm segment nearest the root ([Cd] ≈ 0.4 ppm) and the 1-cm segment nearest the end ([Cd] ≈ 4 ppm).

2. Variation in Cadmium Concentration Between Hair Strands from an Individual

In addition to the variation in cadmium concentration along a given hair strand, it was found that the concentration of the metal varied slightly for different hair strands from one individual. To demonstrate this, many hairs from the same individual were analyzed
FIGURE 24

This figure shows the change in cadmium concentration along the length of one particular hair strand.
FIGURE 25

This figure shows the change in cadmium concentration observed along the length of a hair strand. The data presented here represent a composite of analyses of seven hair strands from the same individual.
over the course of several weeks. The analysis of each hair included the determination of the cadmium concentration of the 1-cm segment nearest the root (designated 1-Root) and the next 1-cm segment near the root, one cm removed from the root terminus (designated 2-Root). Also analyzed were the two corresponding 1-cm segments nearest the end of each hair strand (1-End and 2-End). The data accumulated can be found in Table 18. It can be seen that although there was significant variation between hair strands, the average concentration of cadmium at the end of the hair (1.9 ppm) was greater than the average concentration at the root (0.4 ppm).

The calculated standard deviation from the mean has been presented as an indication of the variation in the concentration of cadmium determined for the individual segments within each group. It can be observed that, on the average, the relative standard deviation for a group of measurements was approximately 50%. It must be emphasized, however, that the standard deviation has been used here only as an indication of variation between samples, not as a measure of indeterminate error. Since, in fact, each sample segment analyzed was distinct and different, the variation between segments was not due solely to random error. Also, no true value for the concentration of cadmium in 1 cm of hair
Table 18
VALUES OBTAINED FOR ANALYSIS OF SEVERAL HAIR STRANDS
FROM ONE INDIVIDUAL

<table>
<thead>
<tr>
<th></th>
<th>Conc. Cd for 1-cm Segments (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-Root</td>
</tr>
<tr>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

n = 20 20 16 18
mean = 0.4 0.5 1.9 1.4
\( \sigma \) = 0.2 0.3 1.1 0.7
range = 0.1-0.6 0.1-1.1 0.6-3.9 0.5-3.3
was known. The variation seen, therefore, was not a measure of the precision of the method.

Unfortunately, no measurement of random error (short-term precision) was possible in the analysis of hair due to the fact that each hair segment could be analyzed only once. The precision in the measurement of the absorption signal of aqueous standards was measured and was found to be from 1-2%, depending on the concentration of the standard. This represented the best possible precision of the method. The precision in the analysis of hair samples, of course, was expected to be less than this value. The greatest error in the determination of cadmium in hair was introduced in the weighing of the 1-cm hair segment taken for analysis. The analytical balance used was capable of weight measurement to the nearest 0.01 mg. A 1-cm segment of hair generally weighed between 0.05 and 0.10 mg; the precision of the weighing step was therefore between 10-20%.

It should be noted that in the course of these analyses, a few values were obtained which were obviously spurious and so were disregarded in the calculation of the mean.

3. Range of Cadmium Concentration in the Hair of Individuals in a Non-Exposed Population

Hair from several individuals was analyzed in
order to determine the expected range of cadmium concentration in the hair for a non-exposed population. The sampling group included both males and females from the university population in Baton Rouge. Many different ages were represented within the approximate range of 18-60 years; however, most individuals sampled were within the 20-30 year age group.

Again, four 1-cm segments of each hair strand were analyzed for cadmium, the two 1-cm segments nearest the root and the two nearest the end of each strand. Segments 1-Root and 2-Root (see page 175) were then averaged to obtain a measure of the cadmium concentration at the root for each individual; likewise, segments 1-End and 2-End were averaged to obtain the concentration at the hair end. The resulting data can be found in Table 19.

Once again, it was observed that in nearly all cases the average concentration of cadmium at the end of the hair was higher than the average concentration at the root. In addition, a positive correlation seemed to emerge between the average concentration of cadmium at the end of the hair strand and the total length of the strand. This correlation is illustrated in Figure 26. It can be observed that the cadmium concentration at the end of the hair strand is generally related to the overall length.
Table 19

CONCENTRATION OF CADMIUM IN THE HAIR OF INDIVIDUALS IN A NON-EXPOSED POPULATION

<table>
<thead>
<tr>
<th>Sample</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length(cm)</td>
<td>Avg. [Cd] at End(ppo)</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>2.4</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>0.8</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>0.2</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>0.3</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>16</td>
<td>11</td>
<td>0.5</td>
</tr>
<tr>
<td>17</td>
<td>7</td>
<td>2.8</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Avg.:

Males: 9 | 0.6 | 0.37 ± 0.32
        | 0.4 |
Females: 25 | 2.2 | 0.3

Overall avg. [Cd] at Root = 0.34 ppm ± 0.3 ppm
FIGURE 26

This figure shows the correlation between the concentration of cadmium at the end of the hair strand and the total length of the strand.
Several studies had indicated that the accumulation of heavy metals in the hair varied with sex.\textsuperscript{143,144} The data in Table 19 have been separated into male and female groups in order to determine whether a difference in cadmium concentration could be observed. As indicated in Table 19, the overall average concentration of cadmium at the root for the two groups was nearly identical. The overall average concentration at the end of the hair strand, however, differed by approximately a factor of 3 between the group of males (average $[\text{Cd}] = 0.6 \text{ ppm, or approximately } 3 \times 10^{-5} \mu\text{g Cd/cm hair}$) and females (average $[\text{Cd}] = 2.2 \text{ ppm or about } 1 \times 10^{-4} \mu\text{g Cd/cm hair}$). The average increase in cadmium concentration per unit length (from the root to the end of the hair) was calculated in order to determine whether this unit increase was the same for males and females. The resulting data are presented in Table 20. It can be seen that the average increase in cadmium concentration over the hair strand was 0.04 ppm/cm for males and 0.07 ppm/cm for females. This corresponds to an average increase of approximately $2 \times 10^{-6} \mu\text{g Cd/cm of hair for males and about } 4 \times 10^{-6} \mu\text{g Cd/cm of hair for females.}$

The overall average concentration of cadmium at the root of the hair for the total population tested was calculated to be 0.3 ppm, or about $2 \times 10^{-5} \mu\text{g of}$
Table 20

CHANGE IN CADMIUM CONCENTRATION PER UNIT LENGTH OF HAIR

<table>
<thead>
<tr>
<th>Sample</th>
<th>Strand Length(cm)</th>
<th>Δ[Cd] Between Root &amp; End(ppm)</th>
<th>Δ<a href="ppm">Cd</a> cm</th>
<th>Sample</th>
<th>Strand Length(cm)</th>
<th>Δ[Cd] Between Root &amp; End(ppm)</th>
<th>Δ<a href="ppm">Cd</a> cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.4</td>
<td>0.04</td>
<td>21</td>
<td>20</td>
<td>0.9</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.2</td>
<td>0.02</td>
<td>22</td>
<td>25</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>0.1</td>
<td>0.01</td>
<td>23</td>
<td>30</td>
<td>5.6</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>1.7</td>
<td>0.1</td>
<td>24</td>
<td>14</td>
<td>0.1</td>
<td>0.007</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.0</td>
<td>0.0</td>
<td>25</td>
<td>18</td>
<td>0.7</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>0.3</td>
<td>0.02</td>
<td>26</td>
<td>17</td>
<td>0.3</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>-0.4</td>
<td>-0.1</td>
<td>27</td>
<td>36</td>
<td>3.7</td>
<td>0.1</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>-0.1</td>
<td>-0.01</td>
<td>28</td>
<td>55</td>
<td>2.6</td>
<td>0.05</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>0.2</td>
<td>0.02</td>
<td>29</td>
<td>50</td>
<td>9.1</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>0.6</td>
<td>0.07</td>
<td>30</td>
<td>28</td>
<td>1.7</td>
<td>0.06</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>0.1</td>
<td>0.01</td>
<td>31</td>
<td>50</td>
<td>2.5</td>
<td>0.05</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>0.1</td>
<td>0.01</td>
<td>32</td>
<td>26</td>
<td>0.7</td>
<td>0.03</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>0.1</td>
<td>0.01</td>
<td>33</td>
<td>25</td>
<td>0.7</td>
<td>0.03</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>0.1</td>
<td>0.01</td>
<td>34</td>
<td>9</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>0.0</td>
<td>0.0</td>
<td>35</td>
<td>16</td>
<td>1.1</td>
<td>0.07</td>
</tr>
<tr>
<td>16</td>
<td>11</td>
<td>0.2</td>
<td>0.02</td>
<td>36</td>
<td>14</td>
<td>1.0</td>
<td>0.07</td>
</tr>
<tr>
<td>17</td>
<td>7</td>
<td>1.5</td>
<td>0.2</td>
<td>37</td>
<td>17</td>
<td>0.5</td>
<td>0.03</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>0.5</td>
<td>0.06</td>
<td>38</td>
<td>15</td>
<td>1.0</td>
<td>0.07</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>-0.1</td>
<td>-0.01</td>
<td>39</td>
<td>23</td>
<td>2.3</td>
<td>0.1</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0.1</td>
<td>0.01</td>
<td>40</td>
<td>18</td>
<td>1.0</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Avgs: 9 0.4 0.04  Avgs: 25 1.9 0.07
cadmium per cm of hair. Figure 27 illustrates the distribution of cadmium concentration found at the root of the hair of males and females.

4. The Effect of Washing Procedures on the Concentration of Cadmium in Hair

As discussed in the introduction, it had been reported that various washing procedures for hair samples prior to analysis could affect the concentration of some metals both on the surface and within the hair shaft. A study was undertaken to determine if washing with an organic solvent (such as acetone) or with a detergent would affect the amount of cadmium found in the hair strand. In one experiment, several strands of hair were rinsed in acetone followed by distilled-deionized water while a parallel sample of hair from the same individual was rinsed in distilled-deionized water only. Both samples were analyzed consecutively. The results are presented in Table 21. No significant difference can be seen between the two sample groups. Although the concentration of cadmium found in the hair that had been rinsed with acetone was slightly higher than that for the hair rinsed only in water, these small differences were considered to be within expected experimental variation.

A similar experiment was performed whereby one sample of hair was washed with an anionic detergent,
FIGURE 27

This figure shows the distribution of the cadmium concentration found at the root of the hair for both males and females.

FREQUENCY OF OBSERVATION

AVG. CONC. OF Cd AT ROOT OF HAIR STRAND (ppm)

Males

Females
### Table 21

**EFFECT OF WASHING PROCEDURES ON THE CONCENTRATION OF CADMIUM IN HAIR**

**Acetone vs. Distilled-Deionized Water**

<table>
<thead>
<tr>
<th>Washing Procedure</th>
<th>Avg. [Cd] for 1-cm Segments (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-Root</td>
</tr>
<tr>
<td>Acetone, then purified H₂O</td>
<td>0.30</td>
</tr>
<tr>
<td>purified H₂O only</td>
<td>0.24</td>
</tr>
</tbody>
</table>

### Table 22

**EFFECT OF WASHING PROCEDURES ON THE CONCENTRATION OF CADMIUM IN HAIR**

**Detergent vs. Distilled-Deionized Water**

<table>
<thead>
<tr>
<th>Washing Procedure</th>
<th>Avg. [Cd] for 1-cm Segments (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-Root</td>
</tr>
<tr>
<td>Detergent, then purified H₂O</td>
<td>0.10</td>
</tr>
<tr>
<td>purified H₂O only</td>
<td>0.04</td>
</tr>
</tbody>
</table>
followed by rinsing in distilled-deionized water, while a parallel sample was rinsed only in distilled-deionized water. The results can be found in Table 22. It was observed that the concentration of cadmium in the hair washed with detergent was significantly higher than that of the hair rinsed only in distilled-deionized water, indicating contamination of the sample during the washing procedure.

5. The Concentration of Cadmium in Shampoo

The studies described above showed that contamination of hair samples was possible during treatment with detergents. It also had been considered that contact with shampoos and other hair treatments could account for a portion of the environmentally absorbed cadmium detected near the end of the hair strand. In view of these facts, it was desirable to determine the approximate concentration of cadmium in shampoo.

A shampoo solution was prepared using a weighed amount of a representative commercial shampoo (not a dandruff shampoo) in 10 ml of water. This solution was found to contain a cadmium concentration of 2.7 ppb. Back-calculation to the original amount of shampoo in the solution yielded a concentration of 0.17 µg of cadmium per gram of shampoo (0.17 ppm).
D. DISCUSSION

1. Advantages of the Use of the Quartz "T" Atomizer for the Determination of Cadmium in Hair

Use of the quartz "T" atomizer for electrothermal atomic absorption in the determination of cadmium in hair offered several advantages over other techniques of hair analysis. First, no sample pretreatment was necessary. The hair could be analyzed directly without previous ashing, solvation, or preconcentration. This eliminated errors due to contamination or loss during pretreatment stages. It also made the analysis more rapid and more convenient by decreasing the sample preparation that was necessary. Samples were simply rinsed and cut into 1-cm segments before being dropped directly onto the hot carbon bed.

The absence of significant background (molecular) absorption made possible the use of aqueous standard solutions for calibration purposes. Since the hair sample was completely broken down, as demonstrated by the lack of absorbance of a non-resonance line (2266Å), a direct comparison could be made between the absorbance of a hair sample and the absorbance of a known amount of injected cadmium. This calibration method was both simple and convenient, and it eliminated the severe problem of preparing standards with a matrix comparable to that of hair.
Another distinct advantage of the quartz "T" atomizer for analysis of hair was the fact that the sensitivity of the described technique was sufficient to allow the analysis of very small hair segments. This quality not only decreased the amount of sample needed for analysis, but also made possible the study of differences in the cadmium concentration along the length of an individual hair strand. These variations had been reported by other researchers and were confirmed by the studies described here.

2. Variation in Cadmium Concentration Along the Hair Strand

As was described in the RESULTS section, the concentration of cadmium in a hair strand was found to increase with increasing distance from the root. This fact had important implications.

Most common methods of cadmium determination in hair used bulk amounts of hair. The cadmium concentrations reported therefore were overall concentrations, averaging not only the concentration along a strand but also that for several strands of hair. If hair analysis is to be used as a means of monitoring human body burden and/or exposure to cadmium and other metals, it must be considered which section of the hair would reflect most accurately that cadmium actually excreted from the body.
The fact that cadmium concentration increased toward the end of the hair strand indicated that environmental exposure may contribute significantly to the cadmium present in the hair as it grows. This hypothesis was further supported by the general correlation between the average concentration of cadmium at the end of the hair strand and the total length of the strand (Figure 26). If it was assumed that the rate of hair growth was constant between individuals, one might expect a linear correlation between these two variables.

The data in Table 20 indicated that the increase in cadmium concentration per unit length from the root to the end of a hair strand differed considerably between individuals. The overall average for this unit increase was significantly lower for males (0.04 ppm/cm, or approximately $2 \times 10^{-6} \, \mu g \, Cd/cm$ hair) than for females (0.07 ppm/cm, or approximately $4 \times 10^{-6} \, \mu g \, Cd/cm$ hair). This suggested that there was a difference between the two groups in the amount of cadmium adsorbed by the hair from the environment. The differences could be due to several factors.

First of all, it was possible that there were individual variations in the ability of the hair matrix to absorb metals. Differences in matrix structure, protein content, and oil and moisture content were possible characteristics which could affect the
exposure of the hair to external cadmium through
shampoos and other hair treatments. Males and females
often have widely different hair care habits. Therefore, it was possible that the observed difference
in cadmium adsorption between the two groups could be
attributed to grooming practices and the resulting
exposure to external cadmium.

It was also possible that the ability of the hair
matrix to adsorb cadmium from the environment changed
as the hair grew longer. The portion of a hair strand
further from the scalp was older and thinner. Repeated
washing, brushing, and exposure to the environment
could change the resistance of the hair to external
contamination. It was possible that the hair shaft
could become more porous with age, thus increasing its
ability to retain metals from external sources. It was
observed (Table 20) that the increase in cadmium con-
centration per unit length of hair was higher for
females than for males. It was possible that this result
was partially due to the greater tendency of the longer
hair of females to adsorb environmental cadmium at
the ends of the strands. In this case, the increase in
cadmium per unit length of hair would not be linear
along the hair strand.

The amount of increase in cadmium concentration
with increased length of a hair strand could thus be
the result of many factors, including individual habits, hair structure, and environmental conditions.

The data indicated environmental contribution to the total cadmium present at the end of the hair. Analytical techniques which measured the cadmium concentration for the entire hair therefore would measure environmentally - contributed cadmium as well as that excreted from the body. Results reported when these methods were used would be difficult to interpret and even misleading. It was believed that the cadmium found in the section of the hair strand nearest the root would be most representative of the cadmium excreted from within the body. Therefore, if hair is to be used as a monitor of cadmium excretion, only the section of the strand nearest the root (perhaps the first 1-2 cm) should be taken for analysis.

3. Variation in Cadmium Concentration Between Hair Strands from An Individual

As indicated by the data in Table 18, variation was observed in the cadmium concentration of different strands of hair from the same individual. These variations can be attributed to several factors.

First of all, there is a variation in the length and in the growth rate of different hair strands from the same subject. The hairs taken for analysis were from different locations on the head; this also
contributed to differences in the length of individual hairs. Strands taken from different sections of the head also differed somewhat in thickness, or diameter, and texture. This fact indicated slight variations in the composition of the hair matrix depending on the location and, ultimately, on the cells responsible for the actual excretion of the proteins. All these factors would contribute to differences in the cadmium concentration determined for the particular hair strand.

Other factors which affected the variation in the measurement of the cadmium concentration were related to the method itself. These factors would contribute a random error to the variation already present between hair strands, and could increase the variation of the measurement both of excreted cadmium and of cadmium adsorbed from the environment.

First of all, differences in temperature and surface quality for different sections of the carbon bed contributed some variability to the signals obtained for consecutive analyses. These inhomogeneities in the carbon bed caused variations in the efficiency and speed of atomization of successive samples. Secondly, it was important that samples contacted the bed in the same manner each time a segment of hair was dropped into the atomizer. This factor, of course,
was impossible to control. Some samples, in fact, were dropped into the atomizer only to adhere to the inner wall of the quartz sleeve, due to the heat, and slowly burn and char away. The signal obtained when this occurred was merely a very broad deflection in the baseline.

The final limitation which contributed to the variation in the measurement of cadmium concentration was the fact that the 1-cm segments of hair that were analyzed were small and difficult to weigh. Weighing of the segments, in order to determine concentration, was performed on a Mettler H-10 analytical balance, capable of weight measurement to the nearest 0.01 mg. Due to the fact that 1-cm segments of hair were found to weigh between 0.05-0.10 mg, a 10-20% error could be introduced in the weighing step.

It was felt to be undesirable to increase the size of the segment taken for analysis for two reasons. First of all, this step would deemphasize one of the important advantages of the described analysis technique; that is, the ability to observe changes in cadmium concentration for different sections of the hair strand. Secondly, increasing the length of hair taken for analysis would increase the risk of introducing an amount of cadmium that would absorb nearly 100% of the incident light. A 1-cm segment of hair,
on the average, generated a signal of approximately 30% absorption. Some segments, however, produced signals of over 95% absorption. The signals generated during analysis were routinely recorded as percent absorption; it was well known that since this scale was logarithmic with respect to concentration, the greatest sensitivity was obtained when the absorption signals were low.

As was previously stated, it was not possible to measure random variability alone for the described technique of hair analysis. It was believed that the majority of variation observed between hair strands from a single individual was due to differences between the hair strands themselves, with some contribution due to random errors in the analysis technique. This belief was reinforced by the fact that the random variability of the quartz "T" atomizer method when used in the analysis of sweat and urine samples (Chapters 2 and 3) was on the order of 22-28%. It would be expected that the reproducibility for hair analysis using the quartz "T" would be further enhanced because of the absence of a filter paper disk blank and the more complete degradation of the sample matrix. Unfortunately, an additional source of error was introduced in hair analysis by the weighing of the sample.
4. Range of Cadmium Concentration in the Hair of Individuals in a Non-Exposed Population

It was stated earlier in this discussion that the average concentration of cadmium found in the hair was greatly dependent on the section of the hair strand taken for analysis. Since most of the common analytical techniques employed for hair analysis used bulk amounts of sample, it was believed that such analyses represented the measurement of cadmium not only excreted from the body but also absorbed from environmental sources.

The data presented in Table 19 have been separated to show the average concentration of cadmium at the root of the hair strand and that at the end for each individual sampled. The data also have been separated into groups by sex because differences in the cadmium content between males and females had been reported in the literature.\textsuperscript{143,144} No significant difference was observed between the two groups in the average cadmium concentration at the root terminus of the hair. Since it was believed that this section of the hair strand was most representative of the cadmium excreted by the body, these results indicated no detectable difference in the excretion of cadmium between the sexes.
The average concentration of cadmium found at the end of the hair strand was higher in females than in males; some of this increase could be attributed to the greater average length of a hair strand from a female. These results suggested that the differences reported in the literature for the cadmium content of hair due to sex could in part be due to the different average lengths of the hair strands, and thus to differing amounts of environmental cadmium. The analytical technique employed in this research eliminated this problem by making possible the analysis of specific segments of a hair strand.

A comparison of the average concentration of cadmium found in hair using the quartz "T" atomizer technique with the concentrations reported by other researchers is listed in Table 23. It can be seen that the average overall concentration of cadmium reported here as the concentration at the root (0.3 ppm) was lower than most of the reported literature values. This result was not unexpected since environmentally-absorbed cadmium may have been a significant factor in many of the techniques reported in the literature. Considering this influence, it was felt that the results obtained in this research compared well with those of other researchers using a wide variety of analytical techniques.
Table 23

COMPARISON OF LITERATURE VALUES FOR CADMIUM IN HAIR

<table>
<thead>
<tr>
<th>Concentration Cd (ppm)</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 (at root)</td>
<td>electrothermal AAS</td>
<td>in press</td>
</tr>
<tr>
<td>1.98 (non-exposed)</td>
<td>dry-ash, flame AAS</td>
<td>142</td>
</tr>
<tr>
<td>2.49 (occupationally exposed)</td>
<td>dry-ash, flame AAS</td>
<td>143</td>
</tr>
<tr>
<td>2.76±0.483 (males)</td>
<td>dry-ash, flame AAS</td>
<td>144</td>
</tr>
<tr>
<td>1.77±0.239 (females)</td>
<td>wet-ash, flame AAS</td>
<td>144</td>
</tr>
<tr>
<td>0.5–2.5</td>
<td>NAA</td>
<td>134</td>
</tr>
<tr>
<td>0.25–2.7 (rural)</td>
<td>NAA</td>
<td>134</td>
</tr>
<tr>
<td>0.26–3.7 (urban)</td>
<td>NAA</td>
<td>134</td>
</tr>
<tr>
<td>0.40–8.8 (near refineries)</td>
<td>NAA</td>
<td>155</td>
</tr>
<tr>
<td>0.61–2.99</td>
<td>NAA</td>
<td>155</td>
</tr>
</tbody>
</table>
Using the average concentration of cadmium found at the root, it was possible to estimate the average amount of cadmium excreted from the body through the hair. The rate of hair growth has been estimated to be approximately 0.1-0.4 mm/day, depending on such factors as race, sex, age, region of the body, season of the year, nutrition, and hormones.\textsuperscript{156} It has also been estimated that there are approximately 100,000 hairs on the scalp alone.\textsuperscript{156} If the growth rate of hair is taken to be 0.4 mm/day, and the number of hairs on the entire body roughly estimated to be 200,000, then it can be calculated that a human being will grow roughly 80 m of hair in a day. The average weight of a centimeter of hair at the root was found to be approximately 70 µg; this same length of hair also contained approximately 0.3 ppm Cd, corresponding to about $2 \times 10^{-5}$ µg of cadmium per cm. If a human grows 80 m of hair per day, therefore, approximately 0.2 µg of cadmium will be excreted daily by this means. This figure, of course, is based on the assumption that the cadmium concentration of hair does not vary for different regions of the body. Although many rough estimates have been made in the calculation of this value, the general statement can be made that cadmium excretion through the hair matrix is less than 0.5 µg/day.
5. The Effect of Washing Procedures on the Concentration of Cadmium in Hair

It was found that rinsing of hair samples in acetone as a representative of an organic solvent did not significantly affect the concentration of cadmium found. This was in agreement with the results of Jervis, et al.\textsuperscript{134} On the other hand, washing the hair with a detergent appeared to result in a higher cadmium content when compared to hair that was simply rinsed in distilled-deionized water. It had been reported by Harrison, et al.,\textsuperscript{150} that detergent washing decreased the concentration of iron and magnesium found in hair, but no data were reported for cadmium. It was possible that detergent washing could contaminate the hair with cadmium because of the fact that many detergents and shampoos were known to contain zinc and selenium compounds. It was very likely that cadmium also was present.

The results of these studies showed that simple rinsing with distilled-deionized water was the best method for eliminating surface contaminants and dust from hair samples without contaminating the hair shaft with cadmium. This was the routine procedure used in the collection of the data reported here. Detergent washing was used occasionally, however, in those cases where the hair appeared dirty or oily. In these cases,
it was felt that initial washing with a detergent would serve to subject the hair to a comparable treatment as would normally be accomplished by routine personal grooming in the case of the other individuals sampled.

6. The Concentration of Cadmium in Shampoo

The indicated contamination of hair samples from washing with detergents suggested that shampoos and other hair treatments might be important sources of the additional cadmium found near the ends of hair strands. This source of what has been described as "environmental" cadmium seemed the most plausible explanation for cadmium adsorbed from outside the body, especially in view of the fact that the concentration of cadmium in air and water is very low. The normal concentration of cadmium in air is on the order of 0.01 μg/m³ and the concentration in tap water has been reported to be approximately 1 ppb.

As reported in the RESULTS section, the cadmium concentration in a representative commercial shampoo was determined to be 0.17 ppm. This was more than 100 times the concentration in water alone, indicating that shampoos were indeed a possible source of cadmium adsorption onto the hair shaft. It was expected that the cadmium concentration in anti-dandruff shampoos would be higher than that determined here due to the use of zinc- and selenium-containing compounds in these
products. Cadmium would be likely to be found in association with these elements.

7. The Cadmium Concentration of Hair as Compared to That of Urine and Sweat

Hair samples were analyzed from the same two individuals who were repeatedly sampled for earlier studies of cadmium in urine and perspiration (referred to as Subjects A and B in Chapters 2-3). The results from these analyses allowed a comparison of the relative cadmium concentration levels in the three tissues. The average cadmium concentrations found in the three samples for Subjects A and B, as well as the overall average values found for the population sampled in each case, are compared in Table 24.

It was found (Chapter 2) that the average concentrations of cadmium in the urine of Subject A and Subject B were 23 ppb and 24 ppb, respectively. These values were very near the overall average concentration of 20 ppb Cd found for the entire population sampled. (None of the individuals sampled were known to be occupationally exposed to cadmium.) The analysis of sweat samples, however, revealed that the average cadmium concentration in the perspiration of Subject A (41 ppb) was lower than the average concentration found for the entire population (84 ppb Cd). At the same time, the average concentration of cadmium found in the sweat
Table 24

COMPARISON OF THE AVERAGE CADMIUM CONCENTRATIONS FOUND
IN URINE, SWEAT, AND HAIR FOR TWO INDIVIDUALS AND FOR
THE POPULATION SAMPLED

<table>
<thead>
<tr>
<th>Sample</th>
<th>Avg. Concentration of Cd (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population Sampled</td>
</tr>
<tr>
<td>Urine</td>
<td>20</td>
</tr>
<tr>
<td>Sweat</td>
<td>84</td>
</tr>
<tr>
<td>Hair (conc. at root)</td>
<td>300</td>
</tr>
</tbody>
</table>
from Subject B (90 ppb) was higher than the overall average (see Chapter 3). These results suggested that there was no correlation between the amount of cadmium excreted in the sweat and in the urine of an individual. Indeed, this observation was made when the cadmium concentrations of these two excretory fluids were compared for the entire population sampled. This comparison was discussed in Chapter 3; the data have been presented in Table 15 and in Figure 20.

Hair samples also were analyzed for Subjects A and B. Among the hair data listed in Table 19, Sample 10 was obtained from Subject A and Sample 17 was collected from Subject B. It can be seen that the cadmium concentration at the root of the hair of Subject A (0.2 ppm, or 200 ppb) was lower than the average for the overall population (0.3 ppm = 300 ppb). However, the concentration found for this section of the hair from Subject B (1.3 ppm = 1300 ppb) was much higher than the overall average.

Thus it was observed that for both sweat and hair samples, the cadmium concentration levels found for Subject A were below the average while those found for Subject B were above the average (Table 24). This indicated that there may be a relationship between the excretion of cadmium through the hair and through the sweat in man.

The cadmium level in hair was noted to be higher than that in either urine or sweat.
E. CONCLUSIONS AND SUMMARY

1. The use of the carbon bed atomizer for atomic absorption spectroscopy was a simple and effective technique for the direct determination of cadmium in hair. Complete decomposition of the sample was possible, eliminating background absorption and allowing the use of aqueous standards for calibration purposes.

2. Cadmium in the hair seemed to result from both excretion from within the body and from absorption from environmental sources. That cadmium detected nearest the root most nearly represented excreted cadmium, while that measured nearest the end of the hair reflected environmental exposure. The cadmium concentration increased with increasing distance from the root.

3. The average concentration of cadmium at the root of the hair was found to be 0.3 ppm for a non-occupationally exposed (university) population. The cadmium concentration at the root was found not to be significantly different between males and females.

4. The increase in cadmium concentration per unit length between the root and the end of the hair strand differed between individuals and, on the average, between males and females. This indicated that the amount of cadmium found at the end of the hair was not dependent solely on the total length of the hair.
strand. The increase in cadmium concentration over the length did not appear to be linear.

5. A possible source of cadmium accumulation from the environment was demonstrated to be due to shampoos. Many other sources of this adsorbed cadmium were possible.

6. The concentration of cadmium at the root of the hair strand could possibly be used to indicate the body burden of cadmium within the individual. In such studies, only the section of the hair nearest the root should be taken for analysis, as this is the region which most nearly represents the direct excretion of elements from the body. A technique such as that described here would be a useful analytical method for the selective determination of the cadmium present at the root of the hair strand.

7. The daily excretion of cadmium through the hair was estimated to be approximately 0.2 μg/day.

8. Hair samples were analyzed from two individuals who had previously donated both urine and sweat samples. Comparison of the data obtained showed no correlation between the concentration of cadmium in the urine and in the sweat. However, the data indicated that a relationship may exist between the amount of cadmium excreted through the sweat and through the hair.

9. The concentration of cadmium in the hair was generally higher than that found in the urine or in the sweat.
CHAPTER 5
THE DETERMINATION OF CADMIUM IN BREATH

A. INTRODUCTION

Breath possesses several advantages as a specimen for clinical chemical analysis. It is known that the lungs are very efficient boundary organs. The numerous alveoli within the lungs possess a total surface area of some 70 m$^2$ in contact with blood capillaries; the average daily respiratory volume has been estimated to be $10^4$ liters.$^{157}$ It seemed, therefore, that breath should reflect the concentration in the blood capillaries of those substances which are capable of transfer across the alveolar-capillary membranes. It is believed that for many substances an equilibrium is established between the pulmonary circulation and alveolar air. Examples of some compounds for which this has been demonstrated are CO$_2$ and blood alcohol.$^{157}$ Many other compounds would be expected to have sufficient vapor pressure and transfer capabilities to allow them to be present in appreciable quantities in breath.

Breath is a very convenient specimen for monitoring purposes. It is a sample that can be collected rapidly, simply, and without inconvenience to the individual sampled. The analysis of breath also can be easily repeated with little risk of major changes in the sample
composition. Since the sample is gaseous, pretreatment is unnecessary for most analytical techniques. Breath sampling easily lends itself to real-time analysis, although the sample can be retained on an adsorbant for later analysis if necessary.

One disadvantage of the use of breath as a clinical specimen is the fact that only those substances which can pass through the circulatory-alveolar boundry can be determined. It is also unfortunate that these components are usually present in very low concentrations. In addition, a certain amount of cooperation is required from the individual sampled for proper specimen collection. Gaseous samples such as breath must be collected under known and controlled conditions of temperature, pressure, and flow rate in order to accurately determine the sample volume and concentration.

It has been suggested that there are several types of breath samples that can be collected and analyzed. The lungs are known to contain some dead-space air that is not in direct contact with the pulmonary capillaries and thus is not in equilibrium with the concentrations of volatile components in the blood. It was suggested by Dubowski that the composition of breath expired was constant after the dead-space air was expelled. This portion of expired air with constant composition was called alveolar
air and occurred after different volumes of exhalation, depending on the individual.


Most of the substances commonly monitored in breath are gaseous compounds such as CO₂, O₂, CO, other volatile organics, and drugs or drug metabolites with high vapor pressures. A comprehensive listing of many common breath analysis techniques has been published by Dubowski.¹⁵⁷ The most common compound determined in breath is alcohol, and a variety of methods and automated analyzers are in use for this purpose.¹⁵⁷ Other compounds that have been determined in breath included acetone,¹⁵⁸,¹⁵⁹ anesthetics,¹⁶⁰,¹⁶¹ carbon dioxide,¹⁶² carbon monoxide,¹⁶³ mercaptans and sulfides,¹⁶⁴ and methane and hydrogen.¹⁶⁵ The methods used for these analyses include techniques such as gas chromatography,¹⁵⁸,¹⁶⁰,¹⁶⁴,¹⁶⁵ infrared absorption,¹⁵⁹,¹⁶¹-¹⁶³ and mass spectrometry.¹⁵⁷,¹⁶⁶,¹⁶⁷

Several techniques also have been published concerning collection and retention of breath samples for analysis at a later time.¹⁵⁷,¹⁶⁸ Most of these methods involve either storage of whole breath in some suitable container (bags, capsules, syringes, etc.) or retention by physical or chemical means on an adsorbant or in a suitable solvent.
2. The Determination of Metals in Breath

Little attention has been paid to the determination of metals in breath. It has long been known that certain metals are excreted in the form of volatile compounds in expired air. As early as 1925, selenium had been observed to cause a distinct garlic odor of the breath. It was later shown that the actual compound exhaled was dimethyl selenide. It was found, through studies with rats, that this compound was excreted in the breath after exposure to either dimethyl selenide itself or to inorganic selenium. Tellurium is also known to be excreted through respiration, apparently by a similar mechanism.

A limited number of analytical techniques have been published for the determination of metals in breath. The methods used for determining selenium in the breath involved absorption of the expired gases in 6% Hg(NO₃)₂ or in saturated HgCl₂. The selenium present was determined by weighing as a mercuric derivative (such as (CH₃)₂Se•HgCl₂ in the latter case).

A method for the determination of expired mercury also has been reported. The researchers measured elemental mercury exhaled after restorative dental treatment with amalgams. The method of analysis had been previously published for the determination of mercury in ambient air and involved passing the
expired air over a silver wool collector preceded by a drying tube. The mercury was released from the collector by heating to 400°C and drawing the free mercury vapors through an atomic absorption spectrophotometer.\textsuperscript{171} Of course, the mercury determined in these studies was not excreted from the body through the lungs but rather was volatilized from within the mouth. Nevertheless, the method reported could be used to discover whether volatile mercury compounds are excreted via respiration.

No reference could be found to a determination of cadmium in expired air. Cadmium was known to be a volatile metal and also to form volatile compounds such as dimethyl cadmium. In fact, studies had shown that a volatile cadmium species, presumably dimethyl cadmium, was formed by bacteria when cultures containing cadmium salts and Vitamin B-12 were incubated.\textsuperscript{173} It seemed possible that such a conversion could occur in the human body, perhaps with subsequent exhalation of dimethyl cadmium.

Another possible source of cadmium in the breath was respiratory perspiration. It was stated in Chapter 3 that a significant loss of water (140-450 g/day) occurs through respiration and that this loss was part of the total insensible perspiration.\textsuperscript{125} It was possible that excretion of cadmium could occur through this
respiratory perspiration, especially in view of the fact that losses of cadmium had been observed in cutaneous sweating.

Analysis of breath could be accomplished using the quartz "T" atomizer. The atomizer had been used extensively by a former member of this research group in ambient air analysis for trace metals. Similar sampling procedures were applied to the determination of metals in breath.

B. EXPERIMENTAL

1. Equipment

The equipment used for the analysis of breath was the same as that previously described for the analysis of other biological materials. All components of the atomic absorption instrument were unchanged.

The top of the quartz "T" atomizer was fitted with a Teflon piece which screwed into the stainless steel head. This adapter narrowed the opening into the atomizer to approximately 0.25 inch and allowed the attachment of a length of Tygon tubing for the purpose of breathing directly into the atomizer.

In order to adsorb any metal components in breath onto the carbon bed, active carbon was necessary. A supply of active National brand carbon was available from previous air sampling studies and was used to
make up the carbon bed for breath analysis.

2. Sampling Procedure

Expired air was collected on the carbon bed by directly breathing into the atomizer. During sampling, the carbon bed was left cold; that is, the radio-frequency (RF) generator which heated the bed was not activated. The bottom of the bed was warm due to the fact that the light path was heated to approximately 900°C.

Before any samples were collected, the carbon bed was heated to about 1500°C to atomize and eliminate any cadmium contamination on the surface of the carbon. During this procedure, air was drawn through the carbon bed at a rate of 200 cc/min. The bed was allowed to remain heated until the signal recorded at the cadmium resonance line (2288Å) returned to baseline, indicating no further cadmium coming from the carbon bed. The RF generator was then turned off and the bed allowed to cool under air for a period of from 2 to 3 minutes. A breath sample was collected following this period by having a subject exhale through Tygon tubing directly into the atomizer, allowing the cell vacuum pump to pull the expired air over the carbon bed at a rate of 200 cc/min. A 5-minute sampling period, therefore, resulted in the collection of one liter of expired air and a 10-minute period collected 2 liters.
After the sample had been collected, 30 seconds were allowed for the breath remaining in the Tygon tubing to be pulled through the bed. Flow of air through the cell was then diverted by means of a bypass valve; after 15 seconds the RF generator was turned on and the bed was heated to atomize and decompose the sample on the bed. A 35-second period was allowed for the heating and atomizing process, after this time flow was again resumed through the cell. The atomized sample thus was drawn from the bed through the lightpath, where atomic absorption was recorded.

All stages of the sample collection procedure were timed using a stopwatch so that successive samples could be collected and analyzed in an identical manner.

A "blank" sample was analyzed to determine if some absorbance could be seen from air alone. In this analysis, all collection steps were repeated and a 1- or 2-liter aliquot of room air was drawn over the bed in place of a breath sample.

C. RESULTS

1. Analysis of Room Air

A volume of room air was drawn over the active carbon bed to detect any cadmium that would be present in the air inhaled or that which would come from the
carbon bed itself. This determination was considered to be a "blank" for subsequent breath samples. Example traces from these blank samples can be found in Figure 28. It can be seen that only a slight deviation from the baseline occurred for both 1-liter and 2-liter samples of room air. This indicated no detectable cadmium in the laboratory air under the sampling conditions used. Previous studies by other members of this research group had found between 0.02-0.1 µg/m³ of cadmium in ambient (outside) air. The sampling procedure used in these studies was identical to that described here except that a 6-liter sample of air was collected.57

2. Analysis of Expired Air

    A 5-minute (1 liter) sample of expired air was initially analyzed using the described procedure. No detectable cadmium signal was recorded; an example trace is illustrated in Figure 29(a).

    It was thought to be possible that the level of cadmium in the breath was low enough such that a 1-liter sample was insufficient to allow its detection. Therefore, a 10-minute (2 liter) sample was analyzed from the same subject. The traces resulting from two such samples are shown in Figure 29(b). It can be seen that, although a signal was detected in one case, this result was not consistent on repeated sampling.
FIGURE 28

This figure shows the absorption traces obtained for 1-liter and 2-liter samples of room air collected on an active carbon bed. These samples served as blank measurements for the subsequent analysis of breath.
FIGURE 29

This figure shows some typical absorption traces obtained for the analysis of 1-liter and 2-liter samples of expired air. The samples represented here were all collected from the same individual.
The breath of several individuals was analyzed; most samples showed no detectable cadmium. Occasionally, a signal was observed, such as that illustrated in Figure 30, but again these results were not found to be reproducible.

The sensitivity of the quartz "T" atomizer for cadmium is approximately $2 \times 10^{-13}$ g. Since cadmium was not detectable in 2 liters of breath, this indicated that the cadmium concentration in breath was less than $10^{-13}$ g/l ($10^{-4}$ μg/m$^3$). The estimated daily respiratory volume is $10^4$ l; therefore, the daily excretion of cadmium through the breath must be less than $10^{-9}$ g (less than 1 ng).

D. DISCUSSION

It appeared that the concentration of cadmium in the breath was too low to be detected under the conditions used in this study. Based on the sensitivity of the technique for cadmium, this meant that the concentration of cadmium in breath was less than $10^{-4}$ μg/m$^3$. It was also observed in this study that no detectable cadmium signal was recorded for a 2-liter sample of room air. Therefore, the concentration of cadmium in the laboratory air was also less than $10^{-4}$ μg/m$^3$. This was considerably less than the published value for the cadmium concentration in the ambient atmosphere (0.01-0.05 μg/m$^3$). It was also less than the concentration
FIGURE 30

This figure shows an absorption trace obtained on one occasion for a 2-liter sample of expired air. Additional samples collected from the same individual did not exhibit this same high absorption.
of cadmium found in ambient (outside) air by other members of this research group (0.02-0.1 μg/m³).\textsuperscript{57}

The technique employed in breath and air analysis resulted in a rather broad atomic absorption signal. One reason for this broad signal was the time required to physically displace the cadmium that had been adsorbed and atomized in the carbon bed. The approximate volume of the carbon bed was 35 cm³. During atomization, there was no air flow through the bed. Therefore, when flow was resumed in order to draw the atomized sample from the bed through the light path, a finite period of time was necessary to completely displace the air within the bed. At a flow rate of 200 cm³/min, it would take 10.5 seconds to displace the volume of the carbon bed, assuming perfect piston displacement. This is the minimum time possible for all the cadmium to enter the light path and exhibit absorption. Of course, ideal piston displacement did not occur in this case; the cadmium signals observed in practice usually required between 50 and 85 seconds to be recorded. Thus the average width of the cadmium signal was 7-18 mm. This can be compared to the fact that under conditions of perfect piston displacement, the signal would be expected to be approximately 1.5 mm in width.
A second possible contribution to the broad signals observed during breath analysis may be that atomization was incomplete at the time that flow was resumed through the bed. The atomizer was heated from ambient temperature to 1500°C; at the same time the sample was being atomized. Even though stop-flow methods were used, the carbon bed did not reach 1500°C before a signal began to be recorded. Flow was resumed through the cell after a 35-second period primarily because atomized cadmium began to diffuse into the lightpath and cause absorption at this time. However, the bed temperature after 35 seconds was approximately 1000-1100°C and it was possible that portions of the bed were even cooler. Therefore, it was possible that not all the cadmium was atomized at this time. Some metal may be released and its absorbance recorded at a later time, when the atomizer has reached upper temperatures. A broader, less easily detected signal would result.

In order to detect cadmium in breath it would be necessary to take a much larger sample, perhaps 10 liters or more. This amount of sample was very difficult to collect using the described procedure. At a flow rate of 200 cm³/min, 50 minutes of breath would be required to collect a 10-liter sample. The collection of such a sample would be inconvenient and uncomfortable
for the individual being tested. It was considered that an increase in the sample flow rate through the atomizer would not be beneficial because this would decrease the probability of all components of the sample being adsorbed onto the active carbon.

E. CONCLUSIONS AND SUMMARY

1. The concentration of cadmium both in the laboratory air and in exhaled breath was too low to be detected in a 2-liter sample using the quartz "T" atomizer. The cadmium concentration of breath therefore was less than $10^{-4}$ μg/m$^3$. The average daily excretion of cadmium through the breath must be less than 1 ng.

2. The results of this study by no means disproved the excretion of cadmium in the breath. Volatile cadmium compounds may be present at concentrations less than $10^{-4}$ μg/m$^3$. A method of greater sensitivity, or some means of conveniently collecting larger samples, would be necessary to determine whether cadmium was indeed exhaled in the breath.
PART II

METAL SPECIATION BY

DIFFERENTIAL ATOMIZATION
A. INTRODUCTION

It has become increasingly evident in recent years that the toxicity of a metal is dependent not only on its concentration but also upon the particular chemical form. The ion or compound form of an element in the environment will often determine its toxic effects in man and animals; the form of a metal in the body will determine its metabolic effects. Speciation of heavy metals has also become important in the consideration of synergistic effects of groups of elements in the biosphere.

There have been many examples of the importance of chemical form in determining heavy metal toxicity. It has been known, for instance, that hexavalent chromium is more toxic than trivalent chromium. Nickel carbonyl is known to be much more dangerous in industrial environments than metallic nickel or its other compounds. Lead toxicity also depends on the chemical form; the carbonate, monoxide and sulfate are known to be more toxic than metallic lead. Alkyl lead compounds are even more toxic than the oxy-anion species.
Some of the differences in toxicity for various metal species are due to differences in the efficiency of absorption into the body or into the blood cells. It also has been known that variations exist in the metabolic pathways followed by different chemical forms within the body, causing some compounds to be more easily detoxified than others. Geological and environmental studies have shown that the metal form affects the interaction of trace metals with biological organisms, sediments, suspended particles, and water.\textsuperscript{175} One such study showed the varying toxicity of copper compounds in natural waters. Anionic hydroxy-copper complexes were found to cause 15-18\% of the observed toxicity, while free copper and neutral or cationic hydroxy complexes were responsible for 60-70\% of the copper toxicity seen in the study.\textsuperscript{176}

1. Current Methods for Metal Speciation Analysis

In view of this evidence, it is apparent that analytical techniques are becoming increasingly necessary to provide not only total metal analysis but also to differentiate between metal compounds. Unfortunately, only a limited number of techniques are currently available to accomplish this task. Many procedures designed for metal speciation involve first a separation step, using a chromatographic technique or perhaps sequential chemical extractions,\textsuperscript{177} followed by determination of
the metal content of each fraction. Needless to say, these methods are time-consuming, inconvenient, and subject to many sources of error. Alternatively, some researchers have used anodic stripping voltammetry or polarography for differentiating metal species. A comprehensive discussion of such methods as applied to zinc analysis in water samples can be found in a review by Florence.

The majority of metal speciation techniques have involved coupling a gas or liquid chromatograph to a metal specific detector. The chromatographic stage separates various metal compounds by retention time. The conventional chromatographic detector is replaced by one which will respond only to the metal-containing compounds. Techniques such as atomic absorption, atomic fluorescence, or emission spectroscopy detect the metals present, while the chromatograph separates the various chemical forms. Such a coupled system does not detect non-metallic compounds present in the solvent or in the sample matrix, and therefore the spectrum produced is greatly simplified.

A review of the methods for metal speciation by coupling chromatography to atomic spectrometry has been published to Van Loon. The earliest attempts at such coupled systems employed gas chromatography with emission detectors such as microwave plasma emission
(McCormack, et al., 1965) and flame photometry (Juvet and Durbin, 1966, and Zado and Juvet, 1966). Plasma emission detection systems were plagued by the problem of too much solvent extinguishing the plasma. Flame photometric detectors had the disadvantage that only those elements which had strong emission in a flame could be determined.

Most commonly, atomic absorption spectrometry (AAS) has been used as a metal-specific detector for chromatographic effluents. Atomic absorption is well-known and widely-used technique in most analytical laboratories; it is also relatively free from spectral interference problems. These advantages stimulated the development of several chromatographic-AAS systems. A comprehensive review of the reported speciation methods using atomic absorption has been published by Fernandez.

2. Coupled Methods for Metal Speciation Using Atomic Absorption Spectroscopy

Atomic absorption was first used as a metal-specific detector for gas chromatography (GC) by Kolb, et al., in 1966. He used his system to separate the lead alkyls in gasoline. Gonzalez and Ross and Longbottom used GC-AAS to separate mercury compounds; the GC effluent was burned and the resulting mercury vapors were detected by pulling them through a quartz tube in the light path. Gas chromatography also has
been coupled to graphite furnace atomic absorption by
Robinson, et al., for the determination of lead alkyls
in gasoline.\textsuperscript{188} A similar system has been described by
Segar.\textsuperscript{189}

In addition to GC, liquid chromatography has been
linked to atomic absorption for metal speciation studies.
Several applications utilizing this type of system have
been described by Van Loon, et al.\textsuperscript{190} Manahan and Jones
reported the use of ion exchange coupled to AAS for
the determination of chelating agents.\textsuperscript{191} The compounds
were separated as copper complexes. Ion exchange-AAS
also has been used to separate metal compounds from
other matrix components in biological samples,\textsuperscript{184} and
to speciate arsenic and mercury at the ng/ml level in
water.\textsuperscript{192} Yoza and Ohashi described the separation
of magnesium and potassium by gel chromatography-AAS.\textsuperscript{193}
This system also was used for the separation of phosphate
anions as magnesium complexes.\textsuperscript{194}

Atomic absorption has been coupled to high performance
liquid chromatography (HPLC) for the determination of
organometallic chromium compounds.\textsuperscript{195} Van Loon, et al.,
used HPLC-AAS for speciation of copper-aminio acid
complexes and zinc compounds in lubricating oils.\textsuperscript{190}
Systems also have been described which couple HPLC to
a graphite furnace atomizer. Cantillo and Segar developed
a system which interrupted the effluent flow to allow
a sample to be injected into a commercial, temperature-programmed graphite tube atomizer.\textsuperscript{196} A similar system was recently used to determine organic lead compounds in automotive oils.\textsuperscript{197} Van Loon, \textit{et al.}, described the coupling of HPLC to non-dispersive atomic fluorescence spectroscopy for metal speciation, but the technique has not been widely used.\textsuperscript{198}

A quite different method for distinguishing between complexed and ionic metallic species using electrodeposition has recently been reported.\textsuperscript{199} It was found that complexed metals were not removed from aqueous solutions when passed through a stripping electrode and thus could be separated from ionic species.

3. Need for an Improved Analytical Technique

All of the above mentioned techniques accomplished speciation of metals by two analytical methods that were either applied consecutively or coupled together. While many of these methods were successful, the fact that two techniques were being employed caused additional margin for error, time of analysis, and expense in equipment. In addition, these techniques were limited to those samples for which an appropriate gas or liquid chromatography column could be found. Many biological samples were unsuitable for chromatographic analysis without previous breakdown of the matrix.

The development of a spectroscopic method which could
accomplish both separation and detection of metal compounds would offer distinct advantages in speciation studies. It was believed possible to modify an atomic absorption spectrometer to separate various metallic compounds.

It has long been known that atomic absorption spectroscopy is subject to chemical interferences. These interferences were caused by differing combined forms of the element of interest volatilizing and atomizing at different rates in the atomizer. It has long been the goal of spectroscopists to eliminate these interferences, either by complexing the metal so that all atoms are in the same chemical form, or by adjusting the atomizer design or conditions such that all species would be atomized with equal efficiency. It seemed, however, that the existence of these chemical interferences could provide a means for identifying various chemical forms by taking advantage of differences in atomization efficiency.

4. Atom-Formation Processes in Graphite Furnace

Atomic Absorption

A study of atom-formation processes in carbon atomizers has been published by Campbell and Ottaway. This study revealed that most oxy-anion salts of metals decompose to the corresponding metal oxide upon heating. Metal atoms, then, have been proposed to result from the
reduction of the metal oxide by carbon, yielding carbon monoxide and free gaseous metal atoms (Equation 4).^{200}

\[ \text{MO(s)} + \text{C(s)} \rightarrow \text{CO(g)} + \text{M(g)} \]  

(4)

The free energy change of this reaction can be calculated, depending on the temperature, and a prediction can be made regarding the temperature at which this reaction becomes thermodynamically favorable. This is the temperature at which atomization would begin to occur. This temperature has been found to vary widely with the metal oxide involved.^{200}

It was also noticed during the studies described above that significant loss of metal could occur at temperatures below the so-called appearance temperature. It was suggested that this phenomenon resulted from an alternate reaction taking place (Equation 5).^{200}

\[ \text{MO(s)} + \text{C(s)} \rightarrow \text{CO(g)} + \text{M(s)} \]  

(5)

The free energy changes for the conversion of metal oxides into solid metals become favorable at much lower temperatures.^{200} The losses of metal therefore would depend on the extent to which this reaction takes place and the vapor pressure of the resulting M(s) at the temperature in question. The occurrence of the reaction described by Equation 5 could account for losses of metals during ashing stages in commercial
graphite furnaces.

Metal compounds which did not decompose to the oxide in the atomizer would be expected to atomize by different mechanisms, and therefore at different temperatures, than those proposed for oxy-salts. It has been suggested that chloride solutions would be less desirable for use in graphite furnace AAS because they give a higher degree of molecular volatilization.\textsuperscript{200}

All of these considerations reinforced the possibility that selective volatilization and atomization of various metal species could be accomplished with AAS. Such a system called for a dual-stage atomizer. The first stage should be variable-temperature controlled for the purpose of volatilizing metal compounds at their corresponding vaporization temperatures. The second stage would be kept at a temperature sufficient for atomization of any compound volatilized from the initial section. It had been noticed previously that some metal compounds vaporized at low temperatures at which atomization was extremely inefficient. This design avoided the possibility of any species vaporizing in the atomizer at low temperature and reaching the lightpath without being completely atomized.

For the initial experimentation with such a technique, it was decided to use a simple wire loop as the initial stage of the atomizer. Such an apparatus
could be easily and simply constructed and attached to the top of the quartz "T" atomizer, which would serve as the second atomization stage. A heat stable and inert metal was necessary for fabrication of the wire loop. Platinum was the material chosen.

5. The Use of Wire Loops in Atomic Absorption Spectroscopy

Wire loops have been used as atomizers for atomic absorption by several researchers. For example, tantalum ribbons have been used, but they were found to become brittle after repeated heating cycles.\textsuperscript{201} Wire loops made of tungsten and platinum\textsuperscript{202} or of a platinum-rhodium alloy\textsuperscript{203} were reported as atomizers for atomic fluorescence studies. Platinum filaments also have been used to preconcentrate cadmium from urine samples by electrodeposition.\textsuperscript{108} Afterwards, the loop was inserted into the light path of an atomic absorption spectrophotometer and the cadmium absorbance monitored as a voltage was applied through the wire.

Extensive studies using a wire loop atomizer made from a tungsten-rhenium alloy have been published by West, \textit{et al.}\textsuperscript{204} These workers reported sensitivities in the part-per-billion range using a 5-\(\mu\)l sample.

Platinum loops were investigated as a means of sample introduction to the carbon bed atomizer by a former member of this research group.\textsuperscript{73} Unfortunately,
problems were encountered in reproducibility using this sampling procedure. Manning, et al., also reported the use of a wire loop for the introduction of samples into a heated graphite furnace atomizer.\textsuperscript{205} In these cases, the wire filament was not used as an atomizer, but simply served to introduce a sample aliquot into the carbon furnace or bed.

In the studies described in this dissertation, a platinum loop has been used both for sample introduction and for a primary atomization or volatilization stage. The apparatus used was quite unrefined and was designed simply to demonstrate the potential of the dual atomization technique in distinguishing between various metal species. These studies thus were designed for qualitative rather than for quantitative purposes, and would serve as an initial indication of the type of information which could be obtained by such a technique.

B. EXPERIMENTAL

1. Equipment

The components of the atomic absorption spectrometer were the same as previously described for the determination of cadmium in biological materials. The top of the quartz "T" atomizer was fitted for these studies with the platinum loop apparatus as shown in
Figure 31. A loop of platinum wire (0.005 in.) was spot-welded to nichrome wire leads which had been threaded through a ceramic tube for insulation purposes. This assembly was held approximately 3 cm above the surface of the carbon bed by means of a rubber stopper which was fitted into the opening of the stainless steel atomizer head. The stopper also held a quartz capillary tube which extended to the level of the carbon bed surface and an additional inlet tube which extended only about 0.7 cm through the stopper. The shorter inlet was connected to a source of scrubbed nitrogen gas and provided an inert atmosphere around the wire loop. The longer capillary inlet was open to the air and served to introduce oxygen at the level of the carbon bed; this oxygen was necessary for efficient atomization in the carbon bed.

Additional equipment necessary for the platinum loop apparatus was as follows.

a. Variable transformer: Variac Type V5
b. Filament transformer: Stancor No. P-6432, 5 volt step-down
c. Stopwatch

Other equipment utilized in these studies has been previously described in Chapter 1. A schematic diagram of the wire loop circuit can be found in Figure 32.
FIGURE 32

Schematic Diagram of the Wire Loop Circuit

120V AC

Variable transformer

Filament transformer

Pt loop
2. Chemicals

Aqueous stock solutions of 1000 ppm concentration were prepared of the following lead and cadmium salts. These solutions were diluted to obtain standards of the desired concentrations for various studies.

a. CdCl₂
b. CdI₂
c. Cd(NO₃)₂: prepared by dissolution of Cd metal in HNO₃
d. CdSO₄
e. Cd(C₂H₃O₂)₂
f. PbCl₂
g. Pb(NO₃)₂
h. Pb(C₂H₃O₂)₂

Saturated solutions of the following insoluble lead and cadmium compounds were prepared by placing 0.5 g of the compound into 100 ml of water and allowing the solutions to stand at least 24 hours at room temperature.
i. CdS
j. CdCO₃
k. CdO
l. PbSO₄
m. PbCO₃
n. PbO
o. PbO₂
p. Pb₃O₄
In addition, a 1000 ppm solution of EDTA was prepared from the disodium salt.

3. Sampling Procedure

Prior to analysis using this system both the carbon bed and the platinum loop were cleaned of surface metal contamination by heating to 1500°C under air. Heating was continued until the signal recorded at the appropriate resonance line returned to baseline. The bed was allowed to remain hot while the loop was cooled to receive a sample.

Samples were placed on the platinum loop by simply dipping the loop into the solution to be analyzed. The surface tension of the liquid samples was sufficient to hold approximately 1 to 2 \( \mu l \) within the turns of the loop. The loop apparatus containing the sample was then placed into the top of the atomizer over the hot carbon bed. The loop was gradually heated by manually controlling a Variac, thereby increasing the voltage passed through the filament. The heating rate was controlled by timing the intervals of increase in voltage. The heating rate commonly used was achieved by turning the Variac two units (where 0-100 units represented full scale) every 5 seconds. At this rate, the platinum loop increased in temperature from ambient conditions to 1500°C in approximately 4 minutes. The Variac was increased from a setting of
zero to about 90 units during this heating process.

During heating of the platinum wire, a flow of nitrogen in excess of 200 cc/min was maintained through an inlet which terminated near the loop (Figure 31). This maintained an inert atmosphere around the loop which preserved the chemical form of the metals in the sample during the heating process. The effect of heating in the presence of air also was investigated; the traces obtained were quite different from those observed under the nitrogen purge.

For all analyses, air was allowed to enter the atomizer at the level of the carbon bed by means of a quartz capillary tube (Figure 31). This arrangement provided the oxygen necessary for complete atomization to take place in the bed.

After each sample run was completed and the loop was still heated, the nitrogen flow was diverted and air was pulled throughout the atomizer. This practice assured the complete breakdown and atomization of all components of the sample and prevented any memory effects from influencing subsequent samples.

Successive samples could be analyzed by this technique by simply cooling the wire loop, then removing the loop apparatus to apply another sample. It should be pointed out that while positioned in the top of the atomizer, the platinum wire was exposed to the heat of
the carbon bed below. Thus, the loop was partially heated by its proximity to the carbon bed even with no application of voltage.

The absorption spectra obtained using this technique may be best described as absorption traces. Different forms of the metal of interest theoretically would vaporize off the platinum loop at different temperatures. This would be expected to yield different traces for each metal compound, with peaks occurring at temperatures characteristic for that compound.

Absorption spectra were recorded using the resonance lines of 2288Å for cadmium and 2833Å for lead. Molecular background was monitored at the non-resonance lines of 2266Å for cadmium and 2805Å for lead.

The sensitivity of the analysis of lead and cadmium salts was found to be poor; 10 to 100 ppm solutions were necessary to produce a detectable signal. Because of this, it was of interest to find if any cadmium remained on the platinum loop after several analyses of cadmium solutions. In order to investigate this possibility, a scan of the platinum wire was obtained using Electron Spectroscopy for Chemical Analysis (ESCA).
C. RESULTS

1. Lead and Cadmium Inorganic Compounds

Absorption traces were obtained using the described technique for all the previously listed lead and cadmium compounds. The soluble salt solutions analyzed were of 10 ppm concentration for the cadmium compounds and 100 ppm for the lead compounds. In addition, saturated solutions of several insoluble cadmium and lead compounds were analyzed. Example traces for these solutions, together with their respective background absorption traces, are illustrated in the next several Figures (Figures 33-48). It could be seen that the traces differed for the different salts.

2. Effect of EDTA

Mixtures were made of aqueous cadmium and lead solutions with EDTA in order to investigate the effect of complexing on the observed absorption traces. Example traces are presented for CdCl$_2$ plus EDTA in both 1:1/2 and 1:4 molar ratios (Figures 49 and 50) as well as for Pb(NO$_3$)$_2$ plus EDTA in a 1:1 molar ratio (Figure 51). It was observed that these traces differed from those of their respective pure salts.

3. Biological Samples

Whole blood samples were analyzed for both cadmium and lead using the platinum loop technique.
FIGURE 33
CdCl$_2$, 10 ppm

This figure shows the absorption traces obtained for a 10 ppm solution of CdCl$_2$ using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
FIGURE 34
CdI₂, 10 ppm

This figure shows the absorption traces obtained for a 10 ppm solution of CdI₂ using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
FIGURE 35
Cd(NO₃)₂, 10 ppm

This figure shows the absorption traces obtained for a 10 ppm solution of Cd(NO₃)₂ using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
FIGURE 36
CdSO₄, 10 ppm

This figure shows the absorption traces obtained for a 10 ppm solution of CdSO₄ using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
This figure shows the absorption traces obtained for a 10 ppm solution of Cd(C$_2$H$_3$O$_2$)$_2$ using the platinum loop apparatus.

(a) Resonance absorption (2283 Å).
(b) Background (2266 Å).
FIGURE 38
CdCO$_3$, saturated

This figure shows the absorption traces obtained for a saturated solution of CdCO$_3$ using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
FIGURE 39

Cd S, saturated

This figure shows the absorption traces obtained for a saturated solution of CdS using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
FIGURE 40
CdO, saturated

This figure shows the absorption traces obtained for a saturated solution of CdO using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
FIGURE 41

PbCl₂, 100 ppm

This figure shows the absorption traces obtained for a 100 ppm solution of PbCl₂ using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 42

Pb(NO₃)₂, 100 ppm

This figure shows the absorption traces obtained for a 100 ppm solution of Pb(NO₃)₂ using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 43

Pb(C₂H₃O₂)₂, 100 ppm

This figure shows the absorption traces obtained for a 100 ppm solution of Pb(C₂H₃O₂)₂ using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 44

PbSO$_4$, saturated

This figure shows the absorption traces obtained for a saturated solution of PbSO$_4$ using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 45

PbCO₃, saturated

This figure shows the absorption traces obtained for a saturated solution of PbCO₃ using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 46
PbO, saturated

This figure shows the absorption traces obtained for a saturated solution of PbO using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 47

PbO$_2$, saturated

This figure shows the absorption traces obtained for a saturated solution of PbO$_2$ using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 48

Pb$_3$O$_4$, saturated

This figure shows the absorption traces obtained for a saturated solution of Pb$_3$O$_4$ using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
FIGURE 49

10 ppm CdCl₂⁺ EDTA (1:½ molar ratio)

This figure shows the absorption traces obtained for a mixture of 10 ppm CdCl₂ and EDTA in a 1:½ molar ratio using the platinum loop apparatus.

(a) Resonance absorption (2288 Å).
(b) Background (2266 Å).
FIGURE 50

10 ppm \( \text{CdCl}_2^+ \) EDTA (1:4 molar ratio)

This figure shows the absorption traces obtained for a mixture of 10 ppm CdCl\(_2\) and EDTA in a 1:4 molar ratio using the platinum loop apparatus.

(a) Resonance absorption (2283 Å).
(b) Background (2266 Å).
FIGURE 51

100 ppm Pb(NO₃)₂⁺ EDTA (1:1 molar ratio)

This figure shows the absorption traces obtained for a mixture of 100 ppm Pb(NO₃)₂ and EDTA in a 1:1 molar ratio using the platinum loop apparatus.

(a) Resonance absorption (2833 Å).
(b) Background (2805 Å).
The resulting spectral traces, both resonance and background, can be found in Figures 52 and 53. Urine and sweat samples were also analyzed for cadmium, but no detectable absorption was recorded for either type of sample.

4. ESCA Studies

An ESCA spectrum was obtained on the platinum wire after several aqueous cadmium solutions had been heated using the loop. It was hoped that these studies would show whether any cadmium remained on the wire after the heating cycle was completed.

To provide a "known" sample, a piece of platinum wire was soaked in a 5M solution of CdCl₂ for approximately 60 hours. The wire was then removed from the solution and the solution was allowed to dry on the surface. The ESCA spectrum obtained on this sample is shown in Figure 54. It can be seen that both the platinum and the cadmium peaks were of unexpectedly low intensity.

The ESCA spectrum obtained on the platinum loop from the atomizer is shown in Figure 55. A scale-expanded spectrum of the region between 400-450 eV is shown in Figure 56. The platinum signal again was small, and no cadmium signal was observed.
This figure shows the absorption traces obtained for cadmium in whole blood using the platinum loop apparatus.
FIGURE 53
WHOLE BLOOD

This figure shows the absorption traces obtained for lead in whole blood using the platinum loop apparatus.
This figure shows the ESCA spectrum obtained for a platinum wire coated with 5M CdCl₂.
FIGURE 55

This figure shows the ESCA spectrum obtained for the platinum loop from the atomizer apparatus.
FIGURE 56

This figure shows a scale expansion of the ESCA spectrum obtained for the platinum loop from the atomizer in the region of cadmium emission.
This figure shows a scale expansion of the ESCA spectrum obtained for the platinum loop from the atomizer in the region of cadmium emission.
D. DISCUSSION

1. General Observations Concerning the Platinum Loop Method

   a. Absorption Traces

      The first and most important observation was that the absorption traces of solutions of different lead and cadmium compounds were of different shapes. This indeed indicated that a distinction was made between different chemical forms of an element using this technique. The results suggested that the different forms of the metal vaporized off the platinum loop at temperatures characteristic of the particular compound. The vaporous metallic compound then passed through the hot carbon bed, where it was atomized, and into the lightpath, where absorption occurred. An absorption peak was thus recorded at a time corresponding to that at which a particular form of the metal was volatilized from the platinum wire. The temperatures at which these peaks occurred were found to be characteristic of the individual salts analyzed.

      The platinum loop atomizer, unfortunately, possessed several inherent difficulties which must be considered shortcomings of the technique. These observations are discussed in the next several sections.

   b. Loop Temperature

      It was difficult to know the exact tempera-
ture of the platinum loop at any one point during the heating cycle. An attempt was made to monitor the temperature of the wire as a function of the Variac setting using an optical pyrometer. The resulting heating curve is presented in Figure 57. The exact temperature of the loop at any one Variac setting was expected to vary somewhat from one scan to another. One cause of this variation may be a change in the conductivity of the platinum wire after successive heating cycles. The platinum seemed to physically change after several heating cycles had been applied; it was found to be pliable and to easily lose its shape when heated. This effect could be partially counteracted by boiling the wire loop in water after several scans had been completed. This practice seemed to lengthen the lifetime of the platinum loop. The change in character of the wire upon heating, however, would cause variations in its heating response during an analysis cycle.

An exact correspondence between loop temperature and Variac setting also was difficult to obtain due to the margin of error in measuring temperature using the optical pyrometer. It was found to be difficult to focus the pyrometer on the small platinum loop. In addition, the temperature range of the pyrometer (750°-3500°C) made it impossible to measure the temperature
This figure shows the estimated temperature of the platinum loop as a function of the Variac reading during a heating cycle. These temperatures were obtained using an optical pyrometer.
of the loop until it was at least red-hot. This tempera-
ture was not achieved until more than half-way through
the heating cycle, at a Variac reading of approximately
55 units. As a result, the temperature of occurrence
of those peaks which appeared before this point in the
heating cycle could only be estimated from an extra-
polation of the curve in Figure 57.

An additional problem arose when measuring tem-
peratures within the range of 1100°-1300°C. These
temperatures were on the outer edges of both the low
and high temperature scales of the optical pyrometer.
When the loop was heated to a temperature within this
range, different temperature measurements were obtained
using each scale, and the measurements differed by as
much as 140°. Therefore, it was difficult to obtain
an accurate temperature reading within these limits.

A final contribution to variation in loop tempera-
ture resulted from the fact that the Variac was manually
controlled rather than being motor-driven.

Because of the difficulties in temperature
measurement, it was considered that the position of a
given peak could vary by 2 to 4 units (using the setting
of the Variac transformer as a measure of peak position).
Such variation may not indicate a different vaporiza-
tion temperature nor a different chemical form being
detected.
c. Sample Size

The size of the sample picked up by the platinum loop during successive analyses was not found to be reproducible. This problem may be related to the previously mentioned change in the integrity of the wire itself. Upon repeated heating cycles, the platinum became softer and more pliable and held its shape less readily. Any change in the precise size or shape of the loop would alter the amount of sample that would be held within the loop by surface tension.

The variation in the amount of sample picked up by the loop was a major factor precluding the use of the platinum loop atomizer for quantitative analyses. This problem with sample size could be minimized by measuring an aliquot of the solution to be analyzed and placing it in the wire loop using a syringe. For qualitative purposes, however, sampling by means of dipping the loop into the solution was considered to be satisfactory.

d. Sensitivity

It was observed that the sensitivity of the dual-stage atomizer system was significantly decreased from that of the carbon bed atomizer alone. The aqueous solutions analyzed using the platinum loop were of 10 and 100 ppm concentrations for cadmium and lead, respectively. These solutions caused signals of about 50% absorption. If it was assumed that 1 μl
of sample was contained in the loop, the amount of metal present was approximately $1 \times 10^{-8}$ g of cadmium or $1 \times 10^{-7}$ g of lead. The sensitivity of the loop atomizer was therefore two to three orders of magnitude less than that reported for the quartz "$T$" atomizer.²

Part of this decrease in sensitivity could be attributed to the fact that the absorption signal was spread over a period of about 4 minutes during an analysis cycle. Absorption traces were recorded against time, and such a widening of the signal meant that a greater total amount of metal was necessary to produce an easily detected absorption.

It also was possible that some of the cadmium or lead in an aqueous solution may remain on the loop after analysis. If cadmium or lead formed a stable platinum compound or complex, it was possible that part of the metal atoms introduced onto the platinum loop could combine with or adhere irreversibly to the surface of the wire. This would prevent them from being detected. Although no evidence was found in the literature suggesting such compounds, a study was done of the surface of the platinum wire, using ESCA, to discover whether any cadmium was present which had not been removed during resistance heating. This study will be discussed in a later section.
e. Nitrogen Purge

It was observed that the absorption traces recorded for various lead and cadmium solutions differed when nitrogen was and was not present in the vicinity of the platinum loop. If air alone was pulled through the dual-stage atomizer, it was found that the characteristic absorption traces for many inorganic salts disappeared. Instead, the traces for many of the solutions looked alike. It was assumed that this result was caused by the formation of the metal oxide on the platinum loop early in the heating cycle. Volatilization and detection of this oxide then became common to several metal salts; speciation capability was lost. Maintenance of an inert atmosphere around the platinum loop prevented the formation of a common oxide.

f. Evaporation Peaks

Introduction of the wire loop containing a sample into the top of the atomizer inevitably caused an absorption peak prior to the start of the heating program. It was believed that this peak was the result of the evaporation of the solvent due to the heat from the bed below. This initial peak did not occur when monitoring absorption of a non-resonance wavelength; therefore, it represented atomic absorption due to cadmium or lead. The peak was usually small and similar
between compounds; however, occasionally this evaporation signal was large or oddly-shaped. It was believed that the shape and size of this initial peak was also characteristic of the particular metal species present. However, since the peak was caused by inductive heating from the bed, it was difficult to control and rarely reproducible. Primary attention, therefore, was paid to the absorption peaks which occurred during the controlled heating of the wire loop. It was possible that this spontaneous peak resulted from the rapid evaporation of the solvent from the sample, perhaps sputtering some of the metal components off the loop as vaporization occurred.

2. Absorption Traces for Lead and Cadmium Compounds

The absorption traces for various lead and cadmium compounds were found to differ when aqueous solutions were analyzed using the platinum loop apparatus. Table 25 summarizes the spectral data for these various compounds as well as information on their melting and boiling points. The temperatures corresponding to absorption peaks, as listed in Table 25, have been estimated from the Variac setting at which they occurred using the heating curve presented in Figure 57.

It was noticed that, among the cadmium salts, the absorption traces for Cd(NO$_3$)$_2$, CdCO$_3$ and CdS looked very similar, as did the traces for CdSO$_4$, Cd(C$_2$H$_3$O$_2$)$_2$
Table 25
SUMMARY OF PLATINUM LOOP AND PHASE CHANGE DATA
FOR SOME COMPOUNDS OF CADMIUM AND LEAD

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transition Temperature (°C)</th>
<th>Temperatures Corresponding to Absorption Peaks (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd°</td>
<td>b.p. 765</td>
<td></td>
</tr>
<tr>
<td>Cd(NO₃)₂</td>
<td>m.p. 350</td>
<td>850</td>
</tr>
<tr>
<td>CdCO₃</td>
<td>d. &lt; 500</td>
<td>900</td>
</tr>
<tr>
<td>CdS</td>
<td>subl. 980</td>
<td>800</td>
</tr>
<tr>
<td>CdSO₄</td>
<td>m.p. 1000</td>
<td>700,800</td>
</tr>
<tr>
<td>Cd(C₂H₃O₂)₂</td>
<td>m.p. 256, d.</td>
<td>700,850,1000</td>
</tr>
<tr>
<td>CdO</td>
<td>d. 900-1000</td>
<td>600,800,950</td>
</tr>
<tr>
<td></td>
<td>subl. 1559</td>
<td></td>
</tr>
<tr>
<td>CdCl₂</td>
<td>b.p. 960</td>
<td>600,800-900</td>
</tr>
<tr>
<td>CdI₂</td>
<td>b.p. 796</td>
<td>450,950</td>
</tr>
<tr>
<td>Pb°</td>
<td>b.p. 1740</td>
<td></td>
</tr>
<tr>
<td>Pb(NO₃)₂</td>
<td>d. 470</td>
<td>1100</td>
</tr>
<tr>
<td>Pb(C₂H₃O₂)₂</td>
<td>m.p. 280</td>
<td>1100</td>
</tr>
<tr>
<td>PbO</td>
<td>m.p. 886</td>
<td>baseline shift, &gt;900</td>
</tr>
<tr>
<td>PbO₂</td>
<td>d. 290</td>
<td>baseline shift, &gt;900</td>
</tr>
<tr>
<td>Pb₃O₄</td>
<td>d. 500</td>
<td>baseline shift, &gt;900</td>
</tr>
<tr>
<td>PbCO₃</td>
<td>d. 135</td>
<td>none detected</td>
</tr>
<tr>
<td>PbSO₄</td>
<td>m.p. 1170</td>
<td>none detected</td>
</tr>
<tr>
<td>PbCl₂</td>
<td>b.p. 950</td>
<td>650,1100</td>
</tr>
</tbody>
</table>
and CdO. This indicated formation of a common compound on the platinum loop for each of these groups of salts, with subsequent atomization of that compound. Among the lead compounds, spectral similarities were noticed between Pb(NO₃)₂ and Pb(C₂H₃O₂)₂ as well as for the three lead oxides analyzed (PbO, PbO₂, and Pb₃O₄). Again, this indicated volatilization of some common species for each group of compounds.

In contrast, the chloride salt for both lead and cadmium, as well as the iodide of cadmium, appeared markedly different from all other absorption traces. In each case, the spectrum showed two distinct peaks, one at low temperature and one at higher temperature. The shapes of the spectra recorded for PbCl₂ and CdCl₂ were similar; it can be seen from the data in Table 25 that their boiling points were also very close in value. The boiling point of CdI₂, however, was found to be approximately 150°C below those for the chloride compounds. Correspondingly, it was observed that the low temperature absorption peak for CdI₂ occurred earlier than those for the chlorides.

No absorption signals were recorded for PbCO₃ or PbSO₄. This indicated that the amount of the salt in solution was below the detection limit of the platinum loop technique. Although, as was previously discussed, the sensitivity of this technique was much below that
for carbon bed atomic absorption, it was significant that signals were obtained for saturated solutions of several insoluble salts. Absorption was detected for saturated solutions of CdCO₃, CdS, and CdO. The concentrations of these compounds in solution could be estimated to be between 1 and 10 ppm Cd.

There were several similarities between the selective volatilization technique using the platinum loop and the techniques of thermal analysis. In each of these techniques, a sample is subjected to a constant rate of heating. In thermal analysis, of course, the sample is exposed to a constant increase in temperature and any transition which absorbed or released heat would be detected. The platinum loop method, in contrast, detected only volatilization of the metal species of interest. The platinum loop also was used in the analysis of solutions while thermal analysis is commonly applied to solid samples. However, both techniques are capable of detecting vaporization or decomposition of the sample.

DTA thermograms were obtained for four of the metal salts analyzed. Unfortunately, it was difficult to make direct comparison between the two types of spectra for several reasons.

First of all, phase changes were detectable by DTA but not by the metal-specific technique of
platinum loop-AAS. This resulted in a much more complex trace from DTA analysis. Secondly, a DTA thermogram, in most cases, recorded changes in the sample up to approximately 800°C. Many of the absorption peaks obtained using the platinum loop technique occurred at temperatures over this value. Thirdly, the platinum loop was heated such that there was a constant increase in the voltage passing through the platinum filament. This did not necessarily correspond to a constant increase in the temperature of the loop. The spectrum recorded, therefore, would be expected to differ from that recorded by DTA.

It appeared that although similarities could be drawn between the techniques of dual-atomizer AAS and DTA, important differences in the detection and analysis conditions of the two processes made inter-comparison of spectral data difficult.

3. Effect of EDTA

EDTA was added to solutions of CdCl₂ and Pb(NO₃)₂ to observe the effect of this complexing agent on the absorption traces. In comparing the traces of CdCl₂ plus EDTA (Figures 49 and 50) to that of pure CdCl₂ (Figure 33), it was evident that the initial, low temperature peak which occurred at approximately 600°C was decreased in size compared to that which occurred at a higher temperature range (800°-900°C). This
effect also was magnified when the molar ratio of Cd:EDTA was changed from 1:1/2 to 1:4. This indicated that some species of cadmium present in the chloride solution was removed by the complexing agent. It was significant that the overall shape of the absorption trace was unchanged after the addition of EDTA.

The traces of Pb(NO₃)₂ plus EDTA (Figure 51) and of pure Pb(NO₃)₂ (Figure 42) had the same appearance except that the absorption peak occurred at a lower voltage for the solution containing EDTA. It was not certain whether this peak shift was due to the complexing agent or was the result of a change in other atomizer conditions.

4. Biological Samples

Samples of whole blood containing EDTA as an anti-coagulant were analyzed using the platinum loop apparatus. It was hoped that the use of such a technique would indicate whether more than one form of a metal, such as cadmium or lead, existed in the bloodstream. The spectra obtained (Figures 52 and 53) indicated that more than one form of lead and cadmium may be present, but that one type of compound dominates for each metal. It also appeared that the dominant peaks occurred at the same positions in the spectrum as did the low temperature peaks for PbCl₂ and CdCl₂.
Although this data could not be presented as conclusive evidence that the majority of lead and cadmium in the bloodstream exists in the chloride form, this suggestion did not seem illogical. Chloride is by far the predominant anion in the blood plasma (Table 1).

The possible existence of other forms of cadmium and lead in whole blood were indicated by other, smaller peaks in both spectra; these peaks occurred at higher temperature than did the major peak.

A rather large peak occurred at the end of the analysis of whole blood for lead when air was drawn through the atomizer to complete oxidation of the sample (Figure 53). This may indicate a form of lead present in blood which did not vaporize from the platinum loop under nitrogen.

A background scan for each metal was performed on whole blood samples using a non-resonance line emitted by the appropriate hollow cathode lamp. In the case of both cadmium and lead, little background molecular absorption was observed. This proved that the signals recorded at the resonance wavelengths were indeed due to metallic species present in the sample.

The recording of such high signals for cadmium and lead in whole blood was unexpected. In the analysis of aqueous cadmium and lead salts, solutions of con-
centrations of 10 and 100 ppm were necessary to yield signals of approximately 50% absorption. In contrast, the levels of these metals in whole blood were known to be in the ppb range, three orders of magnitude below the aqueous standards used. This indicated that a different vaporization mechanism was being observed for the two types of samples.

It was possible that some component in the blood matrix greatly increased the efficiency of vaporization and atomization of cadmium and lead from the platinum loop. It also was possible that vaporization and atomization of aqueous lead and cadmium from the platinum loop was made extremely inefficient by some interaction between the platinum and the metal being determined. To investigate this possibility, an ESCA spectrum was obtained of the surface of the platinum wire in order to discover if any metal had adhered to the surface of the loop. This study will be further discussed in the next section.

Urine and sweat samples also were analyzed by the platinum loop technique. Unfortunately, no absorption was recorded due to cadmium for either specimen. The level of cadmium in urine and sweat was known to be between 10 and 100 ppb, which was not significantly different than that in whole blood. Yet there was a great difference in the level of absorption due to
cadmium between these samples using the platinum loop. Obviously, the effect which caused an increased efficiency in the detection of cadmium in whole blood was not observed in the analysis of urine or sweat samples, nor in the analysis of aqueous cadmium solutions.

5. ESCA Studies

The results of the ESCA studies of the platinum loop from the dual-stage atomizer were very disappointing. The signals obtained, even for elemental platinum, were of unexpectedly low intensity, indicating poor sensitivity for this type of sample.

The fact that no signal was observed for cadmium in this scan of the platinum loop did not prove nor disprove the presence of cadmium on the loop. The reason for the poor sensitivity in this study was not immediately apparent.

6. Future Directions

Several improvements could be made on the dual-atomizer system for metal speciation using atomic absorption. The most obvious of these improvements would be to devise a sampling procedure which would lend itself to quantitative applications. This could be accomplished, as mentioned earlier, by applying the sample to the initial atomization stage in measured aliquots using a syringe. An alternate metal which would maintain its integrity after several heating cycles would also be
advantageous in order to minimize differences in heating efficiency between sample runs.

A method of automatic temperature programming would increase the reproducibility of the technique. Development of optical temperature control so that a constant rate of temperature increase could be maintained would be of benefit. A temperature program of this type would enable a direct correlation between time (measured from the start of the heating program) and temperature; interpretation of spectra would be greatly simplified.

Increasing the sensitivity of the technique also would be highly desirable. This improvement may be best accomplished by redesign of the first atomization (or vaporization) stage of the dual atomizer. An alternate design in which both stages of the atomizer were fabricated from carbon rods has been reported by another member of this research group.\textsuperscript{57} This design overcomes both the problem of inadequate sensitivity and that of an atomization temperature limit. The application of the platinum loop-carbon bed dual stage atomizer was limited both by the melting point of the quartz atomizer cell (1650°C) and the melting point of the platinum wire (1750°C). An atomizer made of carbon or a more heat-stable metal, such as molybdenum, would allow heating to temperatures of 2200°-2300°C, thereby expanding the number of heavy metals which could be
determined by this technique.

E. CONCLUSIONS AND SUMMARY

1. The dual-stage atomization technique exhibited much promise in the speciation of heavy metals by atomic absorption spectroscopy. Differences between heavy metal compounds were observed. Whole blood samples were analyzed; the spectra indicated one predominant form of lead and of cadmium in the blood.

2. Further refinement of the platinum loop technique could lead to quantitative studies whereby the proportion of various compounds in a sample could be determined. Increased reproducibility of the technique could result in the ability to identify metal species by the characteristic absorption trace. This would be of great value in the study of biological samples because of the fact that the relative benefit or toxicity of metals in the body is known to be dependent on their chemical form. The use of atomic absorption for the identification and quantification of such species in body fluids would be a simple, rapid, and dependable means of obtaining much valuable clinical information.
GENERAL CONCLUSIONS

In summary, the following general conclusions can be made.

1. Atomic absorption spectroscopy using the carbon bed atomizer was shown to be a successful technique for the direct determination of cadmium in biological materials. The technique was used successfully in the direct analysis of whole blood, urine, perspiration, hair, and breath samples. The liquid samples (blood, urine, and perspiration) were introduced into the carbon bed on 6-mm filter paper disks. Hair and breath samples required no sample introduction vehicle.

Use of the carbon bed atomizer together with sample introduction on a filter paper disk had several advantages. There was a more complete degradation of the organic matrix in biological samples; this successfully reduced molecular background. The technique also was sufficiently sensitive so that cadmium concentrations of the order of 10 ppb could be detected using only 1 μl of sample. The described method, therefore, avoided many of the errors associated with sample pretreatment or preconcentration steps, which were common in other analytical procedures. No reagents were added to the sample, which prevented positive errors due to contamination. In addition, no sample was lost due to transfer or due to heating in dry-ashing stages.
These sources of negative errors thus were avoided. The technique was simple and analysis rapid.

2. The carbon bed-filter paper disk technique can be applied to other metals which are volatile at temperatures below 1550°C.

3. The concentration of cadmium in the whole blood of a group of individuals not occupationally exposed to the metal was found to be between 1 and 25 ppb, with a mean concentration of 10 ppb. The average concentration of cadmium in the blood of smokers was higher than the average concentration for nonsmokers.

4. The average cadmium concentration in the urine of a group of individuals not occupationally exposed was found to be approximately 20 ppb.

5. The average concentration of cadmium in the perspiration of the same group of non-exposed individuals was approximately 84 ppb.

6. The concentration of cadmium in both the urine and the sweat of an individual was found to vary on a day-to-day basis.

7. The matrix effects of urine and sweat samples were observed to be similar. This observation was based on the fact that standard addition calibration curves for the two types of samples, collected from one subject, were nearly parallel. This indicated that the cadmium excreted in these fluids may be in the same chemical form.
In addition, it was observed in general that an increase or decrease in the cadmium concentration in one fluid (urine or sweat) was reflected in a corresponding change in the other fluid. This indicated that an increase or decrease in total cadmium excretion was reflected in both of these excretory pathways. 

8. Cadmium in the hair seemed to result both from excretion from within the body and from adsorption from environmental sources. Cadmium found in the hair segments nearest the root most nearly represented excreted cadmium, while that measured near the end of the hair strand reflected environmentally-contributed cadmium. The cadmium concentration increased with increasing distance from the root.

9. The average concentration of cadmium at the root of the hair was found to be 0.3 ppm for a group of individuals not occupationally exposed to cadmium. This was considered to be a measure of the excretion of cadmium from the body through the hair. The concentration of cadmium at the root was not significantly different between males and females.

10. The increase in cadmium concentration per unit length along the hair strand differed between individuals. On the average, the increase per unit length for males was less than that for females. This indicated that the amount of cadmium found at the end of the hair strand
was not dependent solely on the total length of the strand. The increase in cadmium concentration over the length of the hair did not appear to be linear.

11. No concentration was observed between the concentration of cadmium in the urine and that in the sweat. However, the data obtained for two subjects indicated that a correlation may exist between the excretion of cadmium through the sweat and through the hair in man.

12. The concentration of cadmium in the breath was too low to be detected in a 2-liter sample using the quartz "T" atomizer. The concentration was therefore less than $10^{-4}$ μg/m$^3$.

13. Using the data accumulated in this research and that found in the literature, the average daily excretion of cadmium through body tissues and fluids could be estimated. The following daily excretions were estimated based on the studies reported in this dissertation:

- urine 30 μg/day
- sweat 120 μg/day
- hair 0.2 μg/day

The daily excretion of cadmium through the feces has been estimated to be 31 μg/day$^{206}$ and 42 μg/day$^{207}$. Fecal tissue was not analyzed in this research. This tissue is extremely complex in its content. It is well known that the excretion of many substances in the feces is highly variable and very dependent on the intake of
that substance. Much of the cadmium that enters the body is believed to be excreted through the feces without absorption.\textsuperscript{19} Therefore, the concentration of cadmium in the feces may not reflect cadmium excreted from within the body, but rather that cadmium which passed through the gastrointestinal tract without being absorbed. Nevertheless, the cadmium content of feces must be included in any consideration of the balance between daily intake and excretion of the metal.

There are other possible means of loss of cadmium from the body; for example, through skin loss, saliva, nails, and mucous.\textsuperscript{51} These modes of loss, however, were considered to be minor compared to losses through urine, sweat, and feces. Indeed, the excretion of cadmium through the hair (0.2 µg/day) was found to be insignificant when compared to these other possible modes of loss.

Therefore, the total daily excretion of cadmium from the body can be estimated to be about 180 µg/day.

There has been much conflicting data concerning the average daily intake of cadmium; published estimates included 90 µg/day,\textsuperscript{208} 170 µg/day,\textsuperscript{207} and 200-500 µg/day.\textsuperscript{209} It was expected that daily intake would vary widely, depending primarily on the diet. The accumulated data indicated that, on the average, an individual who is
not occupationally exposed to cadmium will excrete each day an amount of the metal which is roughly equivalent to the average daily intake. Of course, many factors can influence both daily intake and excretion of cadmium. However, this data indicated that the half-life of cadmium in the human body is considerably less than 20-40 years, as reported in the literature.¹⁹

It is possible that an equilibrium exists between the body burden of cadmium and the daily excretion; this equilibrium is likely to differ between individuals.

14. The platinum loop-carbon bed dual stage atomizer exhibited promise as a means of speciation of heavy metals by atomic absorption spectroscopy. Differences were observed between the absorption traces of solutions of different lead and cadmium compounds. In addition, whole blood could be analyzed using this apparatus; the spectra indicated one predominant form of lead and of cadmium in the blood.
BIBLIOGRAPHY


64. Kahn, H. L., Manning, D. C., Amer. Lab., 4, 51 (1972).


104. Garcia, R. I., "Studies of Purification Processes by Atomic Absorption and Studies of the Sputtering


134. Jervis, R. E., Tiefenbach, B., Chattopadhyay, A., *J.*


165. Tadesse, K., Smith, A., Brydon, W. G., Eastwood, M.,
166. Lovett, A. M., Reid, N. M., Buckley, J. A., French, J. B.,
168. Gearhart, H. L., Pierce, S. K., Payne-Bose, D., J.
169. McConnell, K. P., Portman, O. W., J. Biol. Chem., 195,
   277 (1952).
171. Reinhardt, J. W., Boyer, D. B., Chy, D. D., Cox, R.,
   45, 2227 (1973).
173. Huey, C. W., Brinckman, F. E., Iverson, W. P., Grim, S. O.,
   Int. Conf. on Heavy Metals in the Environment, Toronto,
   Canada (1975).
176. Magnuson, V. R., Harriss, D. K., Sun, M. S., Taylor, D. K.,
51, 844 (1979).


VITA

Susan Deborah Weiss was born in Milwaukee, Wisconsin, on February 16, 1955. She attended school in Greendale, Wisconsin, until December, 1970, when her family was transferred to Jackson, Mississippi. She was graduated from Callaway High School in Jackson in June, 1973. She entered Mississippi University for Women in August of that year and received her Bachelor of Science degree with honors in May, 1976. In August, 1976, she began graduate studies in the Department of Chemistry at Louisiana State University, where she is currently a candidate for the degree of Doctor of Philosophy. Upon graduation, she will begin employment as a Project Chemist at the Celanese Chemical Company Technical Center in Corpus Christi, Texas.
EXAMINATION AND THESIS REPORT

Candidate: Susan Deborah Weiss

Major Field: Analytical Chemistry

Title of Thesis: TECHNIQUES OF ATOMIC ABSORPTION: DIRECT DETERMINATION OF CADMIUM IN BIOLOGICAL MATERIALS AND METAL SPECIATION BY DIFFERENTIAL ATOMIZATION

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signature]
R. D. Gandom

[Signature]
Neil A. Koster

[Signature]
M. B. Hause

[Signature]
E. S. Younathan

Date of Examination:

July 23, 1980