THE POTENTIAL USE OF RADIOIODINATED FATTY ACIDS AS MYOCARDIAL IMAGING AGENTS

BY

Connie Joan Chung

B.Sc. (Pharm.) University of British Columbia, 1974

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
Division of Pharmaceutical Chemistry

in

The Faculty of Pharmaceutical Sciences

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
March, 1979

© Connie Joan Chung, 1979
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of **Pharmaceutical Sciences**

The University of British Columbia  
2075 Wesbrook Place  
Vancouver, Canada  
V6T 1W5

Date **Mar 28/79**
ABSTRACT

The potential use of four radioiodinated fatty acids as myocardial imaging agents were evaluated. Preliminary distribution studies revealed that the terminal labeled fatty acids demonstrated higher myocardial uptake. Thus, 10-Iodocapric acid (10-iododecanoic) and 12-Iodolauric acid were subjected to further investigation.

Comprehensive tissue distribution studies involving both radioiodinated fatty acids in mice indicated that the highest accumulation of the total injected activity occurred in the muscle and the blood. Other organs investigated included the heart, liver, lung, kidneys, spleen, stomach, intestines, bone and adrenals. The heart exhibited the highest concentration of the radioiodinated fatty acids for the relative accumulation of activity per unit weight. From the tissue distribution studies, the optimum scanning time was found to be immediately following injection of the radiopharmaceutical.

Toxicity studies were performed in mice after intravenous administration of 10-Bromocapric acid and 12-Bromolauric acid. The LD₅₀ of Sodium Bromolaurate in 10% Human Serum Albumin was found to be 210 mg/kg (194 mg/kg - 228 mg/kg). The stability problem encountered with 10-Bromocapric acid necessitated the use of a different solvent system. The LD₅₀ obtained after intravenous injection was found to be 86.1 mg/kg (83.0 mg/kg - 89.3 mg/kg). However, this observed toxicity may not necessarily reflect the toxicity of the Bromocapric acid solely.
Whole body excretion studies were performed in mice and revealed a triexponential excretion curve. For 10-Iodocapric acid, the effective half-lives were .90 hours (36.7%), 3.91 hours (61.6%) and 74.9 hours (14.5%). For 12-Iodolauric acid, the effective half-lives were 1.67 hours (46.6%), 7.68 hours (38.4%), and 71.6 hours (17.8%). For both 10-Iodocapric acid and 12-Iodolauric acid, the first as well as the second component of the excretion curve presumably represented a decrease in the whole body activity due mainly to urinary excretion. The third component appeared to represent activity which was tightly bound and slowly released. The third component presumably represented elimination by fecal excretion. The excretion of the injected activity was primarily in the urine, although some activity was recovered in the feces. For 10-Iodocapric acid, 82.4% of the injected activity had been recovered in the urine within the first 24 hours and 8.88% had been recovered in the feces. For 12-Iodolauric acid, 78.9% of the injected dose was recovered in the urine at 24 hours and 9.4% in the feces. From the urine results, the effective half-life of the radioiodinated fatty acids in the kidneys was found to be 4.8 hours.

Myocardial scans were done on rabbits using $^{131}$I-capric acid, $^{131}$I-lauric acid, NaI-$^{131}$ (6% Human Serum Albumin), and Thallium-201 at specified time intervals after injection. Iodine-123, a radionuclide possessing more favorable imaging properties, was not readily available due to production problems at the time of scanning.

The mean absorbed dose to the whole body, the liver, the kidneys, the muscle, and the heart were computed based on the results from the
distribution and excretion studies. The dosimetry calculations were done using Iodine-123 as the radionuclide. For $^{123}\text{I}$-capric acid, the radiation doses were calculated as 34.76 mrad/2 mCi for the whole body, 136.3 mrad/2 mCi for the kidneys, 86.6 mrad/2 mCi for the liver, 38.5 mrad/2 mCi for the muscle, and 25.89 mrad/2 mCi for the heart. For $^{123}\text{I}$-lauric acid, the radiation doses were 41.73 mrad/2 mCi for the whole body, 199.8 mrad/2 mCi for the kidneys, 185.9 mrad/2 mCi for the liver, 52.07 mrad/2 mCi for the muscle, and 46.39 mrad/2 mCi for the heart.

Supervisor
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>v</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xi</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xiii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xvi</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. LITERATURE SURVEY</td>
<td>8</td>
</tr>
<tr>
<td>1. Myocardial Energetics</td>
<td>8</td>
</tr>
<tr>
<td>a) Acute Response to Ischemia and Infarction</td>
<td>8</td>
</tr>
<tr>
<td>b) Morphological Features of Acute Myocardial Infarction</td>
<td>9</td>
</tr>
<tr>
<td>c) Mitochondrial Changes</td>
<td>10</td>
</tr>
<tr>
<td>2. Positive Infarct-Imaging Agents</td>
<td>11</td>
</tr>
<tr>
<td>a) Gallium-67</td>
<td>12</td>
</tr>
<tr>
<td>b) Mercury-203</td>
<td>13</td>
</tr>
<tr>
<td>c) Technetium-99m Chelates</td>
<td>14</td>
</tr>
<tr>
<td>(i) 99mTc-tetracycline</td>
<td>14</td>
</tr>
<tr>
<td>(ii) 99mTc-glucoheptonate</td>
<td>17</td>
</tr>
<tr>
<td>(iii) 99mTc-pyrophosphate</td>
<td>18</td>
</tr>
<tr>
<td>3. Negative Infarct-Imaging Agents</td>
<td>20</td>
</tr>
<tr>
<td>a) Potassium</td>
<td>21</td>
</tr>
<tr>
<td>b) Rubidium</td>
<td>24</td>
</tr>
<tr>
<td>c) Cesium</td>
<td>26</td>
</tr>
<tr>
<td>d) Thallium</td>
<td>28</td>
</tr>
<tr>
<td>e) Radioiodinated Fatty acids</td>
<td>32</td>
</tr>
<tr>
<td>f) Future Trends</td>
<td>32</td>
</tr>
<tr>
<td>(i) 13N-ammonia</td>
<td>32</td>
</tr>
<tr>
<td>(ii) 11C-palmitate and 11C-octanoate</td>
<td>34</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (Contd.)

4. Fatty Acids .................................................. 34
   a) Myocardial Metabolism ................................. 36
      (i) Penetration of Plasma Free Fatty Acids into Cells .... 37
   b) Myocardial Extraction ................................. 38
      (i) The Circulating FFA Concentration ................ 39
      (ii) The Molar Ratio of FFA/Albumin .................. 39
      (iii) Chain Length and the Degree of Unsaturation ...... 40
   c) The Utilization of Intracellular Free Fatty Acid ......... 43
   d) Carnitine .............................................. 44
   e) Oxidation of Fatty Acids .............................. 45
      (i) Beta Oxidation ...................................... 46
      (ii) Alpha Oxidation .................................... 46
      (iii) Omega Oxidation ................................... 48
   f) Myocardial Metabolism in Acute Myocardial Infarction .... 48

5. Iodine-123 .................................................. 49

6. Radioiodinated Fatty Acids ............................... 50

III. EXPERIMENTAL METHODS AND MATERIALS ................. 57

1. Apparatus .................................................. 57
   a) Reflux Assembly ....................................... 57
   b) Automated Gamma Counter-1185 ........................ 57
   c) Tubor #8725 ........................................... 58
   d) Uni-melt Capillary Melting Point Apparatus .......... 58
   e) Chromatography Tank ................................... 58
   f) Gamma Camera .......................................... 59
TABLE OF CONTENTS (Contd.)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Chemicals and Reagents</td>
<td>59</td>
</tr>
<tr>
<td>3. Fatty Acid Analysis</td>
<td>62</td>
</tr>
<tr>
<td>a) Purity of the Brominated Fatty Acid Analogs.</td>
<td>62</td>
</tr>
<tr>
<td>(i) Melting Points</td>
<td>62</td>
</tr>
<tr>
<td>(ii) Thin Layer Chromatography (TLC)</td>
<td>62</td>
</tr>
<tr>
<td>b) Preparation of Radioiodinated Fatty Acids</td>
<td>63</td>
</tr>
<tr>
<td>(i) 9-Iodotetradecanoic Acid</td>
<td>63</td>
</tr>
<tr>
<td>(ii) 2-Iodotetradecanoic Acid, 10-Iodocapric Acid, and 12-Iodolauric Acid</td>
<td>63</td>
</tr>
<tr>
<td>c) Analysis of Radiochemical Yield</td>
<td>64</td>
</tr>
<tr>
<td>d) Preparation of the Radioiodinated Fatty Acid-Albumin Solution</td>
<td>64</td>
</tr>
<tr>
<td>e) Removal of Free Iodide-131</td>
<td>65</td>
</tr>
<tr>
<td>f) Analysis of the Stability of the Radioiodinated Fatty Acid in 6% Human Serum Albumin</td>
<td>65</td>
</tr>
<tr>
<td>4. Animals</td>
<td>65</td>
</tr>
<tr>
<td>a) Mice</td>
<td>65</td>
</tr>
<tr>
<td>b) Rabbits</td>
<td>66</td>
</tr>
<tr>
<td>5. Tissue Distribution Studies</td>
<td>66</td>
</tr>
<tr>
<td>6. Toxicity Studies</td>
<td>68</td>
</tr>
<tr>
<td>a) Preparation of Sodium Bromolaurate</td>
<td>68</td>
</tr>
<tr>
<td>b) Preparation of Bromocapric Acid Solution</td>
<td>69</td>
</tr>
<tr>
<td>7. Whole Body Excretion</td>
<td>69</td>
</tr>
<tr>
<td>8. Compartmental Analysis</td>
<td>70</td>
</tr>
<tr>
<td>9. Excretion Analysis</td>
<td>70</td>
</tr>
<tr>
<td>10. The Radionuclide Scan</td>
<td>72</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (Contd.)

IV. RESULTS AND DISCUSSIONS ........................................ 73

1. Analysis of the Brominated Fatty Acid Analogs. .... 73
   a) Melting Points of the Brominated Fatty Acid Analogs ........ 73
   b) Chromatography of the Brominated Fatty Acid Analogs and the Physiologic Fatty Acids .... 74

2. The Radioiodination Procedures .................................. 76
   a) 9-Iodotetradecanoic Acid ....................................... 76
   b) 2-Iodotetradecanoic Acid, 10-Iodocapric Acid, and 12-Iodolauric Acid .......... 76
   c) Optimization of the Radioiodination Procedures .................. 77
      (i) Radiochemical Yields of Radioiodinated Fatty Acids in the Absence of Heat .. 77
      (ii) Radiochemical Yields of Radioiodinated Fatty Acids in the Presence of Heat . . 77
      (iii) Radiochemical Yields of Radioiodinated Fatty Acids Using Variable Quantities of Brominated Fatty Acids and NaI-131. . 79
   d) Analysis of the Stability of the Radioiodinated Fatty Acids in 6% Human Serum Albumin ........................................ 80

3. Preliminary Distribution Studies of 10-Iodo-capric, 12-Iodolauric, and 2-Iodotetradecanoic Acids ................................. 82
   a) Preliminary Radionuclide Scan ................................. 84

4. Tissue Distribution .................................................. 87
   a) 131I-Capric Acid ............................................... 88
   b) 131I-Lauric Acid ............................................... 102
   c) NaI-131 (6% HSA) .............................................. 111
TABLE OF CONTENTS (Contd.)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>d) Comparative Myocardial Uptake of 131I-Capric Acid, 131I-Lauric Acid, and NaI-131 (6% HSA)</td>
<td>119</td>
</tr>
<tr>
<td>5. Toxicity Studies</td>
<td>124</td>
</tr>
<tr>
<td>a) 12-Bromolauric Acid</td>
<td>124</td>
</tr>
<tr>
<td>b) 10-Bromocapric Acid</td>
<td>129</td>
</tr>
<tr>
<td>6. Excretion Studies</td>
<td>132</td>
</tr>
<tr>
<td>a) 131I-Capric Acid</td>
<td>134</td>
</tr>
<tr>
<td>(i) Compartmental Analysis</td>
<td>134</td>
</tr>
<tr>
<td>(ii) Urinary Analysis</td>
<td>137</td>
</tr>
<tr>
<td>b) 131I-Lauric Acid</td>
<td>139</td>
</tr>
<tr>
<td>(i) Compartmental Analysis</td>
<td>139</td>
</tr>
<tr>
<td>(ii) Urinary Analysis</td>
<td>141</td>
</tr>
<tr>
<td>c) NaI-131 (6% HSA)</td>
<td>142</td>
</tr>
<tr>
<td>(i) Compartmental Analysis</td>
<td>142</td>
</tr>
<tr>
<td>d) Calculation of the Renal Half-life</td>
<td>146</td>
</tr>
<tr>
<td>7. The Radionuclide Scans Using the Searle Gamma Camera</td>
<td>147</td>
</tr>
<tr>
<td>a) 131I-Capric Acid</td>
<td>147</td>
</tr>
<tr>
<td>b) 131I-Lauric Acid</td>
<td>149</td>
</tr>
<tr>
<td>c) NaI-131 (6% HSA)</td>
<td>149</td>
</tr>
<tr>
<td>d) Thallium-201</td>
<td>152</td>
</tr>
<tr>
<td>e) Comparison of Radionuclide Scans</td>
<td>152</td>
</tr>
<tr>
<td>8. Dosimetry</td>
<td>155</td>
</tr>
<tr>
<td>a) Theoretical Absorbed Dose from 123I-Capric or 123I-Lauric Acids</td>
<td>157</td>
</tr>
<tr>
<td>(i) Whole Body Dosimetry</td>
<td>157</td>
</tr>
<tr>
<td>(1) 131I-Capric Acid</td>
<td>159</td>
</tr>
<tr>
<td>(2) 131I-Lauric Acid</td>
<td>160</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>(ii) The Cumulated Activity</td>
<td>162</td>
</tr>
<tr>
<td>(1) $^{123}$I-Capric Acid</td>
<td>162</td>
</tr>
<tr>
<td>(2) $^{123}$I-Lauric Acid</td>
<td>163</td>
</tr>
<tr>
<td>(iii) The Mean Dose</td>
<td>164</td>
</tr>
<tr>
<td>(1) $^{123}$I-Capric Acid</td>
<td>164</td>
</tr>
<tr>
<td>(2) $^{123}$I-Lauric Acid</td>
<td>165</td>
</tr>
<tr>
<td>V.</td>
<td>SUMMARY AND CONCLUSIONS</td>
</tr>
<tr>
<td>VI.</td>
<td>BIBLIOGRAPHY</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Radioactivity Injected for Myocardial Scans</td>
<td>72</td>
</tr>
<tr>
<td>II</td>
<td>Melting Points of Brominated Fatty Acid Analogs</td>
<td>73</td>
</tr>
<tr>
<td>III</td>
<td>$R_f$ Values of Fatty Acids and Their Analogs Using Various Detecting Reagents</td>
<td>74</td>
</tr>
<tr>
<td>IV</td>
<td>$R_f$ Values of Fatty Acids and Their Analogs Using Various Developing Solvents</td>
<td>75</td>
</tr>
<tr>
<td>V</td>
<td>The Effect of Time on the Radiochemical Yields of Radioiodinated Fatty Acids (No Refluxing)</td>
<td>77</td>
</tr>
<tr>
<td>VI</td>
<td>The Effect of Variable Volumes of NaI-131 on the Radiochemical Yields of Radioiodinated Fatty Acids</td>
<td>80</td>
</tr>
<tr>
<td>VII</td>
<td>Total Uptake of $^{131}$I-Capric Acid by the Spleen, Stomach, Large and Small Intestines, Muscle, and Bone of Mice</td>
<td>89</td>
</tr>
<tr>
<td>VIII</td>
<td>Relative Organ Uptake of $^{131}$I-Capric Acid in Mice</td>
<td>96</td>
</tr>
<tr>
<td>IX</td>
<td>Organ/Blood Distribution of $^{131}$I-Capric Acid in Mice</td>
<td>99</td>
</tr>
<tr>
<td>X</td>
<td>Total Uptake of $^{131}$I-Lauric Acid by the Spleen, Stomach, Intestines, Muscle, and Bone of Mice</td>
<td>103</td>
</tr>
<tr>
<td>XI</td>
<td>Relative Organ Uptake of $^{131}$I-Lauric Acid in Mice</td>
<td>107</td>
</tr>
<tr>
<td>XII</td>
<td>Organ/Blood Distribution of $^{131}$I-Lauric Acid in Mice</td>
<td>109</td>
</tr>
<tr>
<td>XIII</td>
<td>Total Uptake of NaI-131 (6% HSA) by the Spleen, Stomach, Intestines, Muscle, and Bone of Mice</td>
<td>112</td>
</tr>
<tr>
<td>XIV</td>
<td>Relative Organ Uptake of NaI-131 (6% HSA) in Mice</td>
<td>115</td>
</tr>
<tr>
<td>XV</td>
<td>Organ/Blood Distribution of NaI-131 (6% HSA) in Mice</td>
<td>117</td>
</tr>
<tr>
<td>XVI</td>
<td>Determination of the LD$_{50}$ for Na-Bromolaurate in 10% Human Serum Albumin</td>
<td>126</td>
</tr>
<tr>
<td>XVII</td>
<td>Determination of the Intravenous LD$_{50}$ for 10-Bromocapric Acid Solution in Mice</td>
<td>130</td>
</tr>
<tr>
<td>XVIII</td>
<td>The Effect of Shelf Levels and Axial Direction on Count Rates Using the Tubor</td>
<td>133</td>
</tr>
</tbody>
</table>
### LIST OF TABLES (Contd.)

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>XIX</td>
<td>Excretion of $^{131}$I-Capric Acid in Urine and Feces of Mice</td>
<td>138</td>
</tr>
<tr>
<td>XX</td>
<td>Excretion of $^{131}$I-Lauric Acid in Urine and Feces of Mice</td>
<td>141</td>
</tr>
<tr>
<td>XXI</td>
<td>Comparison of Half-lives for $^{131}$I-Capric Acid, $^{131}$I-Lauric Acid, and NaI-131 (6% HSA)</td>
<td>143</td>
</tr>
<tr>
<td>XXII</td>
<td>Comparison of the Effective Half-Lives and the Percentages Eliminated by the Three Components of the Elimination Curves</td>
<td>143</td>
</tr>
<tr>
<td>XXIII</td>
<td>S, Absorbed Dose Unit Cumulated Activity</td>
<td>162</td>
</tr>
<tr>
<td>XXIV</td>
<td>The Mean Absorbed Dose to the Whole Body, Kidneys, Liver, and Muscle Using $^{123}$I-Capric and $^{123}$I-Lauric Acids</td>
<td>166</td>
</tr>
<tr>
<td>XXV</td>
<td>Absorbed Dose Fractions for the Heart</td>
<td>168</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Schematic Outline of the Beta Oxidation Cycle of Fatty Acids with an Even Number of Carbon Atoms</td>
<td>47</td>
</tr>
<tr>
<td>2. Radiochemical Yields of Radioiodinated Fatty Acids by Bromine Replacement</td>
<td>78</td>
</tr>
<tr>
<td>3. Stability of Radioiodinated Fatty Acids in 6% Human Serum Albumin</td>
<td>81</td>
</tr>
<tr>
<td>4. Total Myocardial Uptake of 2-Iodotetradecanoic Acid, 10-Iododecanoic Acid, and 12-Iodolauric Acid in Mice</td>
<td>83</td>
</tr>
<tr>
<td>5. Relative Myocardial Uptake of 2-Iodotetradecanoic Acid, 10-Iododecanoic Acid, and 12-Iodolauric Acid in Mice</td>
<td>85</td>
</tr>
<tr>
<td>6. Myocardial/Blood Distribution of 2-Iodotetradecanoic Acid, 10-Iododecanoic Acid, and 12-Iodolauric Acid in Mice</td>
<td>86</td>
</tr>
<tr>
<td>7. Total Organ Uptake of 10-Iodocapric Acid by Liver, Heart, Lung, Kidneys, and Blood in Mice</td>
<td>90</td>
</tr>
<tr>
<td>8. Relative Uptake of 10-Iodocapric Acid by Liver, Heart, Lung, Kidneys, and Blood in Mice</td>
<td>97</td>
</tr>
<tr>
<td>9. Organ/Blood Distribution of 10-Iodocapric Acid by Liver, Heart, Lung, and Kidneys in Mice</td>
<td>100</td>
</tr>
<tr>
<td>10. Total Organ Uptake of 12-Iodolauric Acid by Liver, Heart, Lung, Kidneys, and Blood in Mice</td>
<td>104</td>
</tr>
<tr>
<td>11. Relative Uptake of 12-Iodolauric Acid by Liver, Heart, Lung, Kidneys, and Blood in Mice</td>
<td>108</td>
</tr>
<tr>
<td>12. Organ/Blood Distribution of 12-Iodolauric Acid by Liver, Heart, Lung, and Kidneys in Mice</td>
<td>110</td>
</tr>
<tr>
<td>13. Total Organ Uptake of NaI-131 (HSA) by Liver, Heart, Lung, Kidneys, and Blood in Mice</td>
<td>113</td>
</tr>
<tr>
<td>14. Relative Uptake of NaI-131 (HSA) by Liver, Heart, Lung, Kidneys, and Blood in Mice</td>
<td>116</td>
</tr>
<tr>
<td>15. Organ/Blood Distribution of NaI-131 (HSA) by Liver, Heart, Lung, and Kidneys in Mice</td>
<td>118</td>
</tr>
<tr>
<td>FIGURES</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>16.</td>
<td>Total Myocardial Uptake of 10-Iododecanoic Acid, 12-Iodolauric Acid, and NaI-131 (HSA) in Mice</td>
</tr>
<tr>
<td>17.</td>
<td>Relative Myocardial Uptake of 10-Iododecanoic Acid, 12-Iodolauric Acid, and NaI-131 (HSA) in Mice</td>
</tr>
<tr>
<td>18.</td>
<td>Myocardial/Blood Distribution of 10-Iododecanoic Acid, 12-Iodolauric Acid, and NaI-131 (HSA) in Mice</td>
</tr>
<tr>
<td>19.</td>
<td>Whole-body Excretion of 10-Iododecanoic Acid After Intravenous Injection in Mice</td>
</tr>
<tr>
<td>20.</td>
<td>Whole-Body Excretion of 12-Iodolauric Acid After Intravenous Injection in Mice</td>
</tr>
<tr>
<td>21.</td>
<td>Whole-Body Excretion of NaI-131 (HSA) After Intravenous Injection in Mice</td>
</tr>
<tr>
<td>22.</td>
<td>Serial Scans of $^{131}$I-Capric Acid (Anterior Projection in Rabbit)</td>
</tr>
<tr>
<td>23.</td>
<td>Serial Scans of $^{131}$I-Lauric Acid (Anterior Projection in Rabbit)</td>
</tr>
<tr>
<td>24.</td>
<td>Serial Scans of NaI-131 (6% HSA) (Anterior Projection in Rabbit)</td>
</tr>
<tr>
<td>25.</td>
<td>Thallium-201 Scan in Rabbit (Anterior Projection in Rabbit at 15 minutes)</td>
</tr>
<tr>
<td>26.</td>
<td>Decay Scheme of $^{123}$Iodine</td>
</tr>
</tbody>
</table>
TO MY PARENTS
I would like to acknowledge the assistance from Dr. D.M. Lyster throughout this study.

I wish also to thank Laura Ford, Y.K. Tam, Helen Burt, R. Venkataraman, Pat Toy, and R. Chu for their encouragement and support. Special thanks to R. Burton for his assistance in the computer analysis.

In addition, I would like to thank the members of my committee for their comments and suggestions.

The financial assistance from the Stanley Drug-Novogroup Scholarship and the University of British Columbia Fellowship is gratefully acknowledged.
I INTRODUCTION
The word infarction comes from the Latin term "infarcire", which means to stuff in (Wartman, 1963). It has been used in various contexts and, in the 19th century, was used to describe a morbid condition of the tissues resulting from obstruction of the circulation, as by embolus or thrombus (Wartman, 1963). At the present time, an area of cell death secondary to ischemia would be termed a myocardial infarction. Ischemia occurs when the arterial supply becomes insufficient to allow normal delivery of exogenous substrates and removal of cellular metabolites (Jennings, 1970; Jennings et al., 1975). Consequently, intra-cellular respiration switches from aerobic oxidation to anaerobic glycolysis and glycogenolysis.

**Incidence of Acute Myocardial Infarctions**

Diseases of the heart are the main cause of deaths in British Columbia (MacDonald, 1976). Acute myocardial infarction (AMI) heads the list of heart diseases and result in high morbidity and mortality. Of the total deaths recorded in British Columbia in 1974, AMI was the causal factor in 19% (MacDonald, 1976).

It is estimated that 25% of AMI patients die within the first three hours of the first attack and an additional 10% die within the first week (Silber and Katz, 1975) In view of this high mortality rate, it is crucial to diagnose the myocardial damage within a few hours of the infarct, as the clinical progress of the patient depends directly on the quantity of residual, viable myocardial cells. Early estimation of infarct size could therefore have important implications regarding the prognosis, the selection of treatment regimen, the screening of patients for surgical revascularization, as well as evaluating physiologic...
and pharmacologic therapy for reducing infarct size (Harper et al., 1971). By limiting tissue damage following coronary occlusion, it may be possible to prevent complications such as pump failure and cardiogenic shock.

The Importance of Early Diagnosis of AMI

In experimental animal studies, myocardial tissues supplied by an occluded vessel did not show an essentially homogeneous area of necrosis (Braunwald, 1976). In 1975, Cox et al. identified three different concentric zones of myocardial injury after coronary ligation in dogs. These zones included a) a central area of necrosis, b) an intermediate zone of ischemia, and c) a zone of normal tissue. It appeared that this "intermediate zone" was ischemic but still viable. However, it was gradually being infiltrated by the central necrotic zone.

The fate of the intermediate zone depended on the myocardial oxygen supply-demand relationship, the availability of nutrients, as well as the inflammatory reaction itself (Weiss et al., 1976; Cox et al., 1975). Interventions which modify these factors may therefore limit further ischemic injury, and consequently affect the ultimate size of the infarct (Harrison et al., 1976; Ross and Franklin, 1976).

Diagnosis of Acute Myocardial Infarctions

Physical symptoms are highly variable in AMI and the diagnosis is commonly based on 3 principles: the history, the electrocardiogram (ECG), and the serum enzymes (Fowler, 1976).

1. History: If the patient is conscious and lucid, he is able to supply
pertinent details regarding the onset of pain. However, this is not possible if he has suffered a silent infarct or if he is comatose.

2. Electrocardiograms: Electrocardiogram findings can be diagnostic if serial changes are observed. ECG results are of value when the patient is very ill or if the history is atypical of AMI. In the absence of serial changes, the diagnosis may be equivocal as pathologic Q wave changes may be associated with disorders other than AMI (Fowler, 1976). Although a change in ECG may be observed, it is not diagnostic in 40-50% of the patients (Fowler, 1976).

The timing of electrocardiograms is very important as early myocardial infarction features may differ from the latter pattern. If a record is made early, 1-2 hours after the onset of pain, the characteristic Q waves are absent (Fowler, 1976). There may be prominent T waves with an elevation of the S-T segment indicating the area of electrical involvement. Changes in the Q patterns are usually not observed until several hours to one day after the AMI.

3. Serum Enzymes: There are many enzymes which catalyse biochemical reactions in the heart. The serum levels of various enzymes may be increased in AMI as well as in other diseases. The results of the enzyme test should be integrated with the ECG findings and the patient's history in order to assess the degree of myocardial necrosis (Fowler, 1976; Silber and Katz, 1975). The magnitude and the duration of elevation of the serum enzymes are roughly proportional to the mass of the irreversibly injured myocardial cells and could be used in prognosis (Rao and Mueller, 1976; Shell et al., 1976; Shell et al., 1973). Enzymes used in the diagnosis
of AMI include SGOT, LDH, and CPK. It is customary to assay at least two of the enzymes to exclude non-coronary conditions.

a) **SGOT** -- Serum glutamic oxaloacetic transaminase levels peak in 24-48 hours after AMI. True positive values are reported in 97-98% of AMI (Fowler, 1976; Silber and Katz, 1975). Elevated SGOT levels alone are not diagnostic as levels may be increased in liver disease, hepatic congestion, congestive heart failure, shock, and other skeletal muscle disorders (Fowler, 1976; Silber and Katz, 1975). In addition, SGOT values may be normal in patients with subendocardial infarctions.

b) **LDH** -- Lactic dehydrogenase is found in the serum as well as in other organs including the heart, skeletal muscle, liver, kidney, pancreas, spleen, brain and lungs. Due to its wide distribution throughout the body, elevation in LDH levels is not specific for AMI and diagnostic accuracy is less than with SGOT. The true positive elevations of LDH have been reported in 85-86% of infarct patients (Fowler, 1976). LDH activity exceeds normal levels within 24-48 hours, peaks in 3-6 days, and returns to normal by 8-14 days.

c) **CPK** -- Creatine phosphokinase is more distinctive for myocardial infarctions and is usually a sensitive indicator (Fowler, 1976). Serum CPK activity typically peaks in 24 hours after AMI. The height and duration of serial serum CPK changes correlate well with the size of the infarct and represents a prognostic guide to AMI.
MYOCARDIAL IMAGING

The conventional diagnostic methods such as serum enzymes and ECG are rather limited for the early diagnosis of myocardial lesions. Myocardial scans, however, are a highly sensitive and non-invasive method of correlating the size, site, and extent of necrotic injury. This early diagnosis permits cardiac assistance or surgical treatment to be instituted before blood flow conditions deteriorate (Cox et al., 1975).

Myocardial imaging is particularly useful when the patient has a suggestive history, but has equivocal electrocardiograms or is in a coma (Zaret et al., 1974). ECG studies are limited in that intraventricular defects could mask findings on an ECG. Enzyme studies may not be used in the early course of AMI as the levels may still be normal. In addition, the possibilities of physiological conditions other than AMI causing the elevation of serum enzymes must first be eliminated. Even though serial serum enzyme levels may provide evidence of AMI, there is no indication of the exact location of the infarct.

The use of myocardial scans has been limited due mainly to the high cost. However, in a recent study by Gorten (1977), the conventional diagnostic methods were found to be of comparable costs to myocardial scans. This was attributed to the fact that serial ECG and serial serum enzyme studies had to be performed to positively identify AMI. Abnormal first scans had a sensitivity of 86%, contrasted to 83% sensitivity for serial ECG, and 67% sensitivity for serum enzymes. In view of the greater sensitivity of myocardial scans at a shorter interval after the acute attack, myocardial scans are valuable diagnostic aids.
There are two approaches to myocardial imaging. These are imaging agents which localize selectively in necrotic tissues, and imaging agents which estimate the regional coronary flow in intact, oxygenated myocardial cells.

Radiopharmaceuticals used to detect recent acute myocardial infarctions are selectively concentrated by damaged cells and are indicated as 'hot spots' on myocardial scans. Although the exact mechanism of localization may differ, the concentration of these agents depend on some structural modification in the necrotic cells.

Radiopharmaceuticals used for estimation of regional coronary flow localize in normal, functional myocardial cells and show up as a 'cold spot' or an area of decreased uptake in areas of necrosis (Cooper, 1974). Agents of this group are usually analogs of intracellular cations, of which potassium is the prototype. Biochemical substrates such as glucose as well as fatty acids have also been used.

Radioiodinated Fatty Acids

Long-chain fatty acids are an important source of fuel for the heart. Terminal radioiodination of the omega-carbon has been successfully carried out by Robinson and Lee (1975). The resultant omega-radioiodinated fatty acid has been found to behave similarly to the physiologic fatty acid molecule. Iodine-123, a mono-energetic gamma emitter with a 13.0 hours half-life and almost ideal imaging properties has been used to label 16-hexadecenoic acid. High quality scans were obtained which indicated the clinical usefulness of these fatty acid analogs (Robinson and Lee, 1975). The rapid myocardial clearance of these agents reflects the functional
state of the myocardial cells as well as allowing repeated imaging if necessary (Poe, 1977).

The cyclotron facilities at TRIUMF promise to be a convenient source of I-123. With this in mind, we carried out a search for alternative fatty acids to radioiodinate. The preferred fatty acid is a mono-unsaturated odd-numbered carbon fatty acid of medium to long chain length. Since synthesis of omega-brominated fatty acids is particularly lengthy and of low yields, it was decided to obtain omega-brominated fatty acids from commercially available sources.

Objectives

The purpose of this study was to investigate the possible use of 9-iodotetradecanoic acid, 2-iodotetradecanoic acid, 12-iodolauric acid, and 10-iodocapric acid as myocardial imaging agents. Investigation was to include their distribution, excretion, and toxicity in mice as well as a scan in a rabbit. Answers to the following questions were sought.
1) Where do the radioiodinated fatty acids localize?
2) What are the target/non-target ratios?
3) What is the major route of excretion?
4) How toxic are these compounds and their metabolites?
5) What is the radiation exposure to the heart, to other organs, as well as to the whole animal?
6) Are these agents better than those currently available for myocardial imaging?
II LITERATURE SURVEY
1. **Myocardial Energetics**

In order to successfully image any organ, it is essential to understand the basic physiology of the tissues involved (Hildner, 1976). The heart pumps blood throughout the body, delivering nutrients, hormones and other regulatory substances and removing metabolic wastes from various regions (Katz, 1977). The cardiac muscle cells contain a complex control system which is capable of modulating the pumping action of the heart to meet the changing demands of the body. The heart therefore requires adequate perfusion at all times to steadily generate metabolic energy at a high rate (Silber and Katz, 1975).

Metabolic energy, in the form of adenosine triphosphate (ATP) can be generated in the heart by oxidative phosphorylation or by anaerobic glycolysis. In the healthy heart, aerobic oxidation prevails but during periods of ischemia, anaerobic glycolysis occurs. However, energy derived from anaerobic glycolysis is only about 5% of the energy generated by oxidative metabolism (Katz, 1977). This low level of energy is inadequate for useful cardiac work.

When insufficient energy is generated, metabolic changes occur, both on a microscopic and a macroscopic scale. These changes influence the distribution of the radiopharmaceuticals within the heart, thus allowing visual differentiation of normal from necrotic myocardium in myocardial scans.

a) **Acute Response to Myocardial Ischemia and Infarction**

Ischemia is the primary event which leads to regional hypoxia and cellular damage. Cellular damage becomes aggravated with time and results in infarction (Lefer, 1975). No structural damage occurs if the
the period of ischemia is less than 20 minutes. If the duration of ischemia is from 30-60 minutes, the cells suffer partially reversible ischemic damage with the result that some cells die. Longer durations of ischemia results in the death of the ischemic cells, and the spread of damage to the surviving cells (Lefer, 1975). This is recognized as the beginning of the infarct. If ischemia persists, cellular necrosis is accompanied by inflammation, and the myocardial infarction spreads.

Cell injury in marginally ischemic areas may occur by 2 mechanisms which involve loss of membrane integrity (Hoffstein et al., 1976): (i) Early events lead to loss of sarcolemmal integrity which allows an uncontrolled entry of ions and macromolecules into the cells and a concomitant loss of cellular cytoplasmic constituents, (ii) The early release of intracellular components (may be lysosomes) may exert glycogenolytic and proteolytic effects. If the sarcolemma is intact, it may lead to increased osmotic pressure and consequent intracellular edema.

b) Morphological Features of Acute Myocardial Infarction

Ischemia results in early macroscopic changes such as pallor and cyanosis, followed by swelling in the area supplied by the affected blood vessel (Silber and Katz, 1975). Discoloration occurs within 36-48 hours and the infarcted tissues turn purplish as if it were the site of hemorrhage (Silber and Katz, 1975). The infarcted region turns gray after the third day, and becomes streaked with yellow. These serial changes in gross appearance result from cellular infiltration by polymorphonuclear leukocytes. The degeneration of muscle fiber is followed by removal of the necrotic cells, which is replaced by granulation cells and
scar tissue (Silber and Katz, 1975). The length of healing depends on the size and the extent of the infarction.

Within 5-10 minutes, microscopic examination reveals clumping of nucleoplasm, and cytoplasmic distortion with vacuoles (Hildner, 1976). Fat droplets are deposited inside the cell membrane. Capillary dilatation, congestion, and eosinophilic changes in the muscle fibres occur from 12-18 hours. Approximately 24 hours following the infarction, leukocytic infiltration takes place. The muscles appear fragmented as cellular infiltration begins and the necrotic tissue is removed. Dead muscle is replaced by granulation tissue and fibroblasts lay down collagen from the second week onwards (Silber and Katz, 1975). After a month, the morphologic age of the infarct is assessed by the density and cellularity of the collagen in relation to the mass of the dead muscle.

c) Mitochondrial Changes

The oxidative metabolism of myocardial cells occur within the mitochondria. These structures are numerous and are located within the cells (Hildner, 1976). Myocardial cells which have been ischemic, whether reversibly or irreversibly, all show swollen mitochondria and relaxation or stretching of the myofibrils (Jennings, 1970). It appears that the increased permeability of the mitochondrial membrane is a fatal event, possibly promoting proton leakage and obliterating oxidative phosphorylation (Trump et al., 1976).

The ability of the cardiac cells to function adequately if there is restoration of blood flow depends on the extent of the damage to the mitochondrial components and enzyme systems (Trump et al., 1976). In irreversibly damaged cells, the mitochondria is extremely fragile,
and there is a loss of matrix density along with the appearance of intramitochondrial granules (Sobel, 1974).

2. Positive Infarct-Imaging Agents

Radiopharmaceuticals which are incorporated into myocardial infarctions provide a method to detect, to localize, and to quantitate the area of necrotic damage. These agents are administered intravenously, and after subsequent localization, the acutely damaged myocardial tissues can be externally visualized as a 'hot spot' or an area of increased uptake with a gamma camera. Positive infarct-imaging agents include Gallium-67, (Kramer et al., 1974; Kramer et al., 1973); Mercury-203 compounds, (Hanson et al., 1977; Ramanathan et al., 1971; Hubner, 1970; Gorten et al., 1966; Carr et al., 1962a), and Technetium-99m chelates, such as $^{99m}$Tc-pyrophosphate, (Klein et al., 1977; Holman et al., 1976a; Buja et al., 1975; Lowenthal et al., 1975; Bonte et al., 1974); $^{99m}$Tc-glucoheptonate, (Holman et al., 1976b; Rossman et al., 1975a; Fink/Bennett et al., 1974); and $^{99m}$Tc-tetracycline, (Holman et al., 1975; Cook et al., 1974; Dewanjee and Prince, 1973).

Although the exact mechanism of binding may differ, each radiopharmaceutical has definite affinity for infarcted tissues. It is generally recognized that incorporation of infarct-avid radiotracers are related to the alterations in the cell membranes associated with ischemia. It has been hypothesized that these agents may bind either 1) directly to the sulphydryl groups of proteins 2) to damaged proteins, or 3) to intracellular calcium ions (Poe, 1977). In order for the radiotracers to reach and become fixed to damaged cells, there must be
some residual blood flow to the infarcted region (Pitt and Strauss, 1976a; Rossman et al., 1975a). Subsequently, the radiopharmaceutical must be cleared from the blood and surrounding organs before adequate images could be obtained. This often depends on the renal function of the patient (Pitt and Strauss, 1976b).

a) Gallium-67

As previously mentioned, a consequence of AMI is the inflammatory process, whereby there is a cellular infiltration by polymorphs and monocytes to the site of the injured tissue (Kramer et al., 1974). It is known that Gallium-67 (Ga-67) accumulates in inflammatory as well as neoplastic cells and therefore its role as an infarct-imaging agent has been investigated (Kramer et al., 1973; Wiseman et al., 1973; Higasi et al., 1972; Edwards and Hayes, 1970.) Using $^{67}$Ga-citrate, myocardial scans obtained 24-48 hours after coronary ligation of dogs showed that the intensity of the Gallium-67 uptake correlated well with the severity of ischemic damage and the degree of polymorphonuclear leukocyte infiltration (Kramer et al., 1974). Good correlations were also obtained between histologic sections and serum CPK levels with the Ga-67 concentration within the infarct (Kramer et al., 1973). There was no Gallium-67 uptake by normal functioning myocardial cells (Wiseman et al., 1973).

The disadvantage of Ga-67 lies mainly in the high accumulation of the radionuclide by the reticuloendothelial elements of the liver and bone marrow (Kramer et al., 1974). Therefore, the inferior borders of the heart are obscured in scans (Kramer et al., 1974). In addition, the
uptake of the radionuclide by infarcted myocardium appeared inconsistent, as only five out of eight hearts with documented AMI showed positive $^{67}$Ga uptake (Kramer et al., 1974). It would appear that the uptake of $^{67}$Ga by the infarcted myocardium was a secondary phenomena, being dependent on the degree of the inflammatory response.

b) Mercury-203 Compounds

The successful visualization of primary brain tumors with $^{203}$Hg- Neohydrin suggested its possible use as a myocardial infarct-imaging agent (Carr et al., 1962a; Blau and Bender, 1960). Initial canine studies by Carr et al. in 1962 showed promising results and good correlation between histologic samples, intact scans, and scans of excised hearts. However, human trials were disappointing in that only one out of thirteen patients with documented AMI had a positive scan (Carr et al., 1962a).

Further studies by Gorten et al. (1965, 1966) demonstrated the selective uptake of $^{203}$Hg-chlormerodrin by damaged tissue as definite areas of increased radioactivity identifiable on myocardial scans. There appeared to be good agreement between 'hot' areas and the site of gross anatomic and histologic evidence of infarction (Gorten et al., 1965). Optimum results, however, were not obtained until 3-5 days post-infarction (Gorten et al., 1965). The studies in living subjects were thought to be hindered by the movement of the heart, the radiopharmaceutical concentration in the lungs, and the scatter caused by the thoracic wall (Gorten et al., 1966). Gorten felt that accumulation of the radiopharmaceutical was probably due to mercury bound to the
sulfhydryl group of the cell proteins. It must be emphasized that the preliminary trials of Gorten's utilized very large doses which would not be permissible in humans.

Other mercurial compounds were investigated for infarct-imaging. Hg-203 or Hg-197 were tagged to hydroxy-mercury derivatives of fluorescein (Malek et al., 1967a; Malek et al., 1967b). These compounds were also selectively taken up by the infarct. Malek found brommecurascan-203 and mecurascan-203 to be superior to neohydrin for imaging (Malek et al., 1967b). Injections were made 24 hours after coronary ligation and scans performed 24, 48 and 72 hours later. Although there was good correlation of AMI and the scan, there was appreciable accumulation of Hg-203 in the liver and gall bladder (Malek et al., 1967b).

Additional research in this area was carried out by Ramanathan et al. (1971) and Spar et al. (1970) with $^{203}\text{Hg}$-fluorescein derivatives which showed promising results in animal models. However, in view of the sub-optimal decay characteristics, the high radiation dose to the liver and kidney, and the difficulty in preparation of pure $^{203}\text{Hg}$-labeled fluorescein (Davis et al., 1976), $^{203}\text{Hg}$-derivatives have fallen into disuse as radiopharmaceuticals for myocardial infarct-imaging.

c) Technetium-99m Chelates

(i) $^{99}\text{Tc}$-tetracycline

Tetracycline has been shown to localize in pathologically altered tissues (Rall et al., 1957). Malek et al. (1963) suggested the possible clinical use of coupling tetracycline with a gamma-emitting radionuclide for external detection of myocardial infarcts.
$^{99m}$Tc-tetracycline was prepared and injected into ligated canine hearts. The accumulation of the radiopharmaceutical in the infarcted tissues was characteristic and reproducible. It was found that the $^{99m}$Tc-tetracycline was bound to the muscle proteins which were biologically altered by ischemia.

A stable complex of $^{99m}$Tc-tetracycline was synthesized by Dewanjee et al. in 1973 using the stannous chloride reduction method. The selective uptake of $^{99m}$Tc-tetracycline by dead cells as opposed to viable cells was shown by Dewanjee and Prince (1973) in tissue cultures of human liver.

Holman et al. (1973a) evaluated the usefulness of $^{99m}$Tc-tetracycline in dogs which had been subjected to coronary ligation. Initially, there was high uptake in the liver, the gall bladder, and the kidneys. The optimal scan time was found to be 24 hours, at which time there was maximal concentrations in necrotic tissues.

Further experimental studies were undertaken to determine the accuracy of $^{99m}$Tc-tetracycline in defining the extent of infarctions (Cook et al., 1974). Experimental myocardial infarcts were produced in dogs and after a lapse of 24 hours, $^{99m}$Tc-tetracycline was injected. Epicardial electrocardiography was obtained at 15 sites and monophasic action potentials recorded from the same sites. The animals were sacrificed, the hearts excised, and biopsy specimens taken at the 15 sites where electrodes had been placed in order to determine the concentration of $^{99m}$Tc-tetracycline. Good correlation was found in the size and the location of the infarction measured by MAP (Monophasic
Action Potentials), epicardial ECGs, and the $^{99m}$Tc-tetracycline concentrations in 17 out of 18 animals.

Additional experiments to elucidate the time course of $^{99m}$Tc-tetracycline were performed by Holman and Zweiman (1975). Histologic study of biopsy samples showed the optimal imaging time to be 48 hours after the infarction.

In uncomplicated infarcts, imaging would not be definitive if the infarcts were older than 1 week (Wynne et al., 1978). Abnormal scans obtained more than one week after the onset of infarction would suggest reinfarction.

In view of the promising preliminary results obtained with $^{99m}$Tc-tetracycline in the canine model, a pilot study was performed to assess the utility of detecting and sizing AMI in humans (Holman et al., 1974). In this clinical trial, 14 abnormal scans were obtained from 14 patients who had sustained AMI. Scintigraphic and ECG localization correlated well. The size of the infarct from the scans correlated well with peak creatine phosphokinase levels.

In additional studies, Holman and Zweiman (1975) demonstrated that the greatest concentration of $^{99m}$Tc-tetracycline was in the portion of the heart which had the highest degree of necrosis. These results were substantiated by Zweiman et al. (1975), who showed that the $^{99m}$Tc-tetracycline only concentrated in acutely infarcted tissues but not in ischemic tissues.

Although $^{99m}$Tc-tetracycline has been shown to concentrate in necrotic cells, the clinical usefulness is impeded by the slow blood
clearance of the antibiotic. Adequate scans cannot be obtained until 24 hours after injection of the radiopharmaceutical, by which time the Tc-99m has decayed through four physical half-lives (Wynne et al., 1978). In addition, there is high uptake of $^{99m}$Tc-tetracycline in the liver which obscures infarcts in the apex of the heart.

(ii) $^{99m}$Tc-glucoheptonate

$^{99m}$Tc-stannous glucoheptonate has been evaluated as a myocardial imaging agent by various investigators (Holman et al., 1976b; Rossman et al., 1975a; Fink/Bennett et al., 1974). Initial studies by Fink/Bennett et al. (1974) were encouraging and showed a maximum ratio of 20:1 of infarcted/normal myocardium at 24 hours.

Further studies performed in dogs subjected to coronary ligation showed areas of increased uptake in scintigrams done as early as 6 hours post-infarction, although the best resolution was obtained at 24 hours (Rossman et al., 1975a).

Limited clinical trials have been done using $^{99m}$Tc-glucoheptonate. In patients with clinically documented AMI, Rossman et al. (1975b) found that 80% had positive scans. Sizing of the infarcts based on the CPK levels demonstrated a general agreement with the scintigraphic appearance (Rossman et al., 1975b). The rapid clearance of $^{99m}$Tc-glucoheptonate from the blood allowed identification of positive scans as early as 5 hours after the onset of chest pain (Rossman et al., 1975b). However, the contrast between infarct/normal background activity was often too low for definitive identification.
Additional clinical assessment of $^{99m}$Tc-glucoheptonate by Holman et al. (1976b) showed poor results. Only 3 out of 13 patients with clinical evidence of infarcts had true positive scans.

Due to the variable results and the low quality scans obtained using $^{99m}$Tc-glucoheptonate, it is not generally accepted for use as an infarct-avid radiopharmaceutical.

(iii) $^{99m}$Tc-pyrophosphate

$^{99m}$Tc-pyrophosphate, a bone scanning agent, is currently the most satisfactory of the available positive infarct-imaging agents (Davis et al., 1976; Bonte et al., 1975). Bonte et al. (1974) first postulated the use of $^{99m}$Tc-pyrophosphate for myocardial infarct imaging. This was based on the observations of D'Agnostino and Chiga (1972) that in necrotic cells, the calcium seemed to be incorporated into a crystalline structure which was the hydroxyapatite. The studies of Shen and Jennings (1972) confirmed these observations.

The initial results obtained by Bonte et al. (1974) were encouraging in that favorable imaging ratios of 10:1 were obtained for infarct/normal myocardium, with little uptake by blood, lung, or liver. However, accumulation of the tracer was not detectable until 12-24 hours after infarction.

Further studies with $^{99m}$Tc-pyrophosphate clarified its infarct labeling ability (Buja et al., 1976). Electron microscope studies demonstrated consistent alterations in the mitochondria of infarcted areas. Electron dense spicules were observed which were thought to be apatite crystals.
Documented clinical trials of Willerson et al. (1975) and Parkey et al. (1974) showed positive scans in most AMI patients and negative scans in the control patients. There appeared to be good agreement in the patients with positive scans and the ECG and enzyme evidence of AMI. The maximal myocardial infarct/normal ratio was 10.9:1. However, there was noticeable uptake by the bones. The results of Coleman et al. (1976) were also encouraging. Abnormal scans were obtained in 23 out of 25 patients with definite confirmed myocardial infarcts while the remaining 2 scans were equivocal.

Parkey et al. (1976) evaluated AMI imaging using both thallium-201 as well as $^{99m}$Tc-pyrophosphate. In 24 documented cases of AMI, 22 had positive $^{99m}$Tc-pyrophosphate scans.

More recent studies by Prasquier et al. (1977) revealed discrete myocardial uptake in 25/26 patients with transmural infarctions. Berman et al. (1977) demonstrated 76 abnormal scans out of 81 patients with acute transmural infarcts. The remaining 5 scans were equivocal.

$^{99m}$Tc-pyrophosphate is the most promising of the $^{99m}$Tc-chelates for positive infarct-imaging. Imaging can be started about 1-2 hours after the injection of the radiotracer (Poe, 1977). However, infarcts must be at least 12 hours old to be consistently detectable (Poe, 1977), and the optimum time for scanning is 24-48 hours after the infarction (Parkey et al., 1976).

Using $^{99m}$Tc-pyrophosphate, there is about a 6% false negative and a 17% false positive rate (Wynne et al., 1978). Due to the avidity of $^{99m}$Tc-pyrophosphate for bone, there is significant uptake in the
ribs or in any injured area of the skeletal system. Similarly, breast
tumors, functional breast tissue in pre-menopausal woman, and damage
to the chest wall may lead to positive scans (Parkey et al., 1974).
More important are the possible cardiac causes of positive scans other
than AMI. Two cancer patients without evidence of AMI had localized
areas of uptake in the heart (Soins et al., 1975). Positive scans have
also been reported in patients with unstable angina, cardiac contusions,
aneurysms, and cardiac tumors (Ahmad et al., 1976; Perez et al., 1976;
Grames and Jansen, 1973).

Controversy has arisen over the uptake of $^{99m}$Tc-pyrophosphate
by myocardium which is not necrotic but merely ischemic. The positive
scans obtained in patients with angina have added to this controversy
and it would appear that $^{99m}$Tc-pyrophosphate may not be specific for
necrotic cells.

3. **Negative Infarct-Imaging Agents**

The second class of myocardial imaging agents is distributed
in proportion to blood flow and is incorporated by intact, viable
myocardial cells. These agents show up as areas of decreased uptake
or 'cold spots' in ischemic lesions. The distribution of these agents
is based on the Sapirstein indicator fractionation principle, which
predicts that after intravenous administration, the uptake of
radiotracers by the heart is equal to the fraction of the cardiac
output perfusing the heart (Sapirstein, 1956). There is sufficient
uptake by the myocardium to permit external imaging, since approximately
5-8% of the cardiac output supplies the myocardium at rest (Holman et al., 1973b).

a) Potassium

Potassium is a primary intracellular cation whose concentration is dependent upon active transport (Prokop et al., 1974). Active transport is oxygen dependent and any periods of ischemia will lead to a decrease in the intracellular potassium levels (Gerlings et al., 1969). Potassium is a highly extractable, cell dependent, non-metabolizable tracer and thus fulfills Sapirstein's criteria for measuring myocardial blood flow (Poe, 1975; Sapirstein, 1956).

Potassium ions are not specific for the myocardium, which only receives 5-8% of the injected dose, while the remaining potassium ions are distributed throughout the body (Poe, 1975). On a single passage through the heart, however, over 70% of the available potassium from the plasma will enter the oxygenated myocardial cells (Poe, 1977; Poe, 1972; Love et al., 1968).

Love et al. (1954) were the first to study the myocardial accumulation of potassium and its analogs. The rate of radionuclide uptake by the myocardium was found to be dependent on the coronary blood flow as well as the rate of exchange of the elements between the circulatory system and the myocardium.

Hurley et al. (1971) introduced $^{43}$KCl for clinical myocardial imaging in view of its favorable half-life and gamma photons. K-43 has a half-life of 22.4 hours and gamma photons of 373 and 619 KeV. Scans were performed on normal men, patients with AMI, as well as dogs.
Normal scans demonstrated well visualized ventricular muscle and a central 'cold area' corresponding to the ventricular cavity. In myocardial infarcts, additional cold spots were observed, which confirmed that K-43 appeared only in viable myocardial cells.

Subsequent distribution analysis showed that the maximal uptake of potassium by the myocardium was between 5-20 minutes after intravenous administration (Poe, 1972). This would be the optimal scan time as the potassium is rapidly concentrated by the myocardium and also rapidly cleared from the blood.

Gorten (1972) further evaluated the clinical usefulness of K-43 for myocardial scanning. Administration of .9-1.5 mCi of $^{43}$KCl in 20 AMI patients was followed by scans. There were definite scan abnormalities found in 19 out of the 20 patients. Good agreement was found between ECG and enzyme changes compared to the infarct size and location obtained from myocardial scans.

The correlation of myocardial K-42 uptake with coronary arteriography was compared by Bennett et al. (1972) in 12 patients. Bennett et al. utilized K-42 in view of the fact that K-43 was not readily available. There was good correlation in patients with lesions of the anterior descending artery using both methods. It appeared that it was possible to externally detect areas of ischemic myocardium by decreased tracer uptake. The decrease in the uptake of K-42 represented inadequate capillary blood flow and/or replacement of normal myocardium with inactive necrotic cells. However, K-42 was unfavorable due to its large radiation dose to the patient.
Zaret et al. (1973a, 1973b) administered $^{43}KCl$ intravenously to delineate areas of myocardial infarction as well as transiently ischemic tissues in human subjects. These studies of regional myocardial perfusion were assessed at rest, with exercise, and during angiography. Working in collaboration with Zaret et al., Strauss et al. (1973) also evaluated K-43 in rest and exercise myocardial perfusion studies. The rationale of this testing was that in exercise, there is an increase in coronary blood flow which is marked in regions supplied by normal coronary arteries but greatly reduced in occluded arteries (Strauss et al., 1973). Exercise appeared to maximize the difference between normal and abnormal coronary vascular beds. Therefore, by combining rest and exercise scans, the site and the extent of transient myocardial ischemia could be evaluated more accurately.

Good correlations were found between myocardial perfusion using K-43 and microspheres (Prokop et al., 1974). In subsequent studies, gated cardiac blood pool scanning was combined with myocardial imaging using K-43. The akinetic areas of a gated scan corresponded to a decrease in K-43 concentration (Rigo et al., 1975). The correlation of the size of the akinetic zone and the decrease in uptake of tracer K-43 was used to assess the extent of left ventricular wall involvement. However, K-43 ions also tended to concentrate in the gastric muscosa, thus obscuring the inferior wall of the myocardium (Rigo et al., 1975).

Gorten (1977) performed an extensive clinical evaluation of radiopotassium and its analogs. Seventy out of the eighty patients with myocardial infarcts demonstrated abnormal K-43 scans, which
represented a sensitivity of 87.5%. Of the 50 normal patients not suffering from myocardial infarcts, 2 had abnormal scans due to chest wall trauma. K-43 cannot differentiate recent from old infarcts. This may be clinically useful in cases of inconclusive histories or ECG regarding therapy or rehabilitation. However, the inability of K-43 to be singularly indicative of recent infarcts rules out the precision of abnormal scans.

While the overall results with K-43 have been promising, the use of the radionuclide is limited by its relatively high cost and lack of availability; by beta-emission resulting in increased radiation dose; by the 373 gamma photopeak, which is not ideal for scintillation cameras; as well as by the 619 KeV photopeak, which requires extra shielding and contrast enhancement to minimize scatter, septal penetration, and penetration of the collimator (Holman, 1974; Poe, 1977).

The analogs of potassium are mono-valent, alkaline metal ions as Rubidium (Rb) and Cesium (Cs), which are concentrated and maintained in the cell by the same mechanism as potassium. However, the extraction efficiency appears to be inversely proportional to the size of the crystal radius of the ion (Poe, 1977).

b) Rubidium

Rubidium has a crystal radius of intermediate size and its uptake and clearance closely resembles that of potassium (Poe, 1975; Pauling, 1969; Love et al., 1968). In as early as 1954, the initial tissue distribution and rate of redistribution of K-42 were found to be parallel to that of Rb-86 (Love et al., 1954). In 1958, Nolting et al.
measured the effect of coronary blood flow on Rb-86 extracted by the heart. It was found that as coronary blood flow decreased, the percentage myocardial extraction of Rb-86 increased. It was thought that the Rb-86 diffused into a large miscible pool. Myocardial extraction, measured by coronary sinus catheterization was found to be only about 43% (Nolting et al., 1958). However, later studies of myocardial extraction of Rb-86 showed values of 65%, and these values have now been accepted as the myocardial extraction efficiency of rubidium (Love et al., 1968).

Rubidium-86 was first investigated for myocardial imaging by Carr et al. (1962). It has a 18.7 days half-life and a photopeak at 1.1 MeV. The primary organs of uptake appeared to be the liver and the heart (Carr et al., 1962b). However, it required special collimators to reduce interference from organs adjacent to the heart, especially the liver. In addition, the whole body radiation dose was very high. In 1969, Love, Smith and Pulley verified the similar biological behavior of Rb and K in the myocardium by mapping myocardial mass and regional coronary blood flow. However, the scans were of inferior quality and showed a variety of shapes of the myocardium.

Rb-81 is the only radionuclide of rubidium which has been effectively imaged (Martin et al., 1974). Rubidium-81 has more favorable photon energies (511 KeV), a shorter half-life (4.6 hours), and can be economically produced (Dwyer, 1975; Martin et al., 1974). It decays by electron capture to Krypton-81m, which has a 13 seconds half-life, and emits a 190 KeV photon, which is good for the present imaging systems available. With these more favorable photon energies,
there is less lateral collimator penetration and better resolution (Martin et al., 1974). The short half-life allows studies to be accomplished at shorter intervals without degradation of images due to excessive background activity (Martin et al., 1974). Rb-81 can be used for both rest and exercise myocardial imaging. Clinical studies, even though promising, are limited due to the short half-life and the need for daily supplies from a reactor.

c) Cesium

Cesium has the largest crystal radius of the potassium analogs (Pauling, 1969; Love et al., 1968). It required a longer time for cesium to reach peak levels in the myocardium and, accordingly, it was cleared more slowly from the myocardium as well as the blood (Poe, 1972). At one hour post-injection of cesium, approximately 5% of the injected dose was concentrated in the heart, 9% in the kidneys, and 28% in the GIT, while 2% remained in the bloodstream (McAfee and Subramanian, 1975; Matthews et al., 1969).

The functional differences between cesium and potassium were attributed to the larger crystal radius of cesium, which presumably interfered with the transfer of the ion across the cell membrane (Poe, 1972; Pauling, 1969). This accounted for the low myocardial extraction of only 22% after a single circulation through the heart (Love et al., 1968).

Carr et al. (1963) have shown myocardial infarcts as imaging defects after intravenous administration of Cs-131 in dogs. Scans were performed 1-3 hours after injection of the radionuclide. The
animals were sacrificed, the hearts excised, washed free of blood, and rescanned. It was found that the myocardial Cs-131 to blood ratios increased linearly with time and approached 20:1 by 2 hours (Carr et al., 1963). There was a pronounced decrease of Cs-131 in the infarct.

In view of the success of the animal studies, clinical trials were initiated by Carr et al. (1964). Anterior myocardial infarcts were demonstrated as imaging defects after the intravenous administration of 2.5 mCi of Cs-131. However, the principal emissions of the radionuclide (30 KeV Xenon-X-rays) were so weak that posterior myocardial infarcts could not be detected. The hepatic uptake as well as the uptake by the bony thorax obscured the anterior portions of the myocardium.

Further clinical trials by McGeeham et al. (1968) showed 8 out of 9 imaging defects which correlated well with the locations of the infarcts as indicated by ECG and coronary arteriograms. However, the energy of Cs-131, along with the long half-life of nine days, makes this radionuclide unsuitable for external imaging.

Use of other radioisotopes of cesium have also been reported. Yano et al. (1970) utilized the improved imaging characteristics of Cs-129. It has a half-life of 32 hours and decays 100% by electron capture. The photopeaks are from 375-416 KeV. In preliminary studies in dogs, major organs of uptake included the heart, liver, GIT, kidney, lungs, and the spleen. The highest concentration was in the GIT while the greatest radiation exposure was to the kidneys. The optimal myocardial scan time was found to be 40-90 minutes post-injection and
showed an absence of radionuclide in regions of infarctions (Yano et al., 1970).

Clinical evaluation by Romilt et al. (1973) exhibited visual defects in scans of patients with myocardial infarcts. Cs-129 remained in the myocardium longer than the other potassium analogs and thus allowed serial imaging to be performed. Because of its lower energy, there is better resolution with Cs-129 than with K-43.

Despite these results, cesium-129 is not widely used due to the low extraction by the heart, the high uptake by the GIT, the high exposure to the kidneys, and the lack of a commercially available source.

d) Thallium

Thallium-201 is currently the radionuclide of choice for myocardial perfusion imaging (Poe, 1977; Strauss and Pitt, 1977). Although thallium (Tl) is not chemically related to potassium, it is similar to potassium in terms of distribution and neurophysiologic function (Gehring and Hammond 1967; Mullins and Moore, 1960). The explanation for the biological similarity of thallium to potassium is based on the ionic radius of these elements. The hydrated ionic radius of thallium is between that of potassium and rubidium, and this radius has been suggested as the property which determines penetration through a membrane (Britten and Blank, 1968).

Thallium-201 decays by electron capture and has a half-life of 73 hours (Lebowitz et al., 1975). It emits mercury X-rays of 69-83 KeV in 98% abundance plus gamma rays of 135 and 167 KeV in 10%
abundance (Lebowitz et al., 1975). The low energy 80-KeV rays are suboptimal as there is some loss of spatial resolution due to incomplete elimination of scattered radiation from the primary photopeak by the pulse height analyser. However, Tl-201 is available from several commercial sources and can be successfully visualized with the presently available nuclear instruments.

After IV administration, Tl-201 is distributed throughout the whole body where it is extracted by various organs (Lebowitz et al., 1975; Strauss et al., 1975). About 2-5% of the injected dose is concentrated in the heart (Strauss and Pitt, 1977). Thallium has the highest heart/blood and heart/liver ratios compared to the other potassium analogs (Janowitz et al., 1977; Strauss et al., 1975). The blood circulation decreases in an exponential manner to 2-3% of the injected dose by 8-10 minutes (Strauss et al., 1975).

A comparison of the plasma disappearance of Tl-201 and K-43 suggests the ionic movements are related (Gehring and Hammond, 1967). However, once inside the cell, thallium is less readily released than potassium (Gehring and Hammond, 1967). Thallium is probably extracted by the Na\(^+\)-K\(^+\) ATPase system by binding on 2 sites of the enzyme in contrast to the single binding site by potassium (Britten and Blank, 1968). Although thallium can cause toxic effects, the administered dose for imaging is less than 10,000 times the minimal toxic dose (Janowitz et al., 1977; Lund, 1956). There is no evidence of toxicity with the currently used dosages of thallium.
Kawana et al. (1970) suggested the use of Tl-199 as a potassium analog for myocardial scanning. However, the energy of Tl-199 was unsuitable for external imaging.

Different radioisotopes of thallium were evaluated by Lebowitz et al. (1975) and the best spectral properties were found to belong to Tl-201. Strauss et al. (1975) assessed regional myocardial distribution in dogs comparing Tl-201 to microspheres under conditions of partial occlusion and reactive hyperemia. The correlation was good although thallium required a short but finite time to clear from the blood and enter into the tissues.

In the animal studies by Jambroes et al. (1975) it appeared that myocardial infarcts should involve 10% of the myocardium in order to be visualized in the beating heart. Bradley-Moore et al. (1975) did comprehensive studies on the biological behavior of Tl-201 in goats with coronary infarctions. They found the half-time in the heart to consist of 2 components.

Documented clinical trials were initially done by Wackers et al. (1975). These trials showed Tl-201 to be of diagnostic value for identification of acute myocardial infarcts. All the patients with myocardial infarcts had myocardial scans with diminished radioactivity at the area corresponding to the electrocardiographic localization of the infarct.

Due to the success of the initial clinical trials, more extensive clinical trials were undertaken to evaluate the value and limitations of thallium-201 in myocardial scanning (Wackers et al., 1976). Of the 200 patients studied, defective scans were found in 165 patients.
However, the diagnostic accuracy was higher, 94% in those patients who were scanned within 24 hours after the onset of pain. The advantage of TI-201 included the early and precise anatomic localization of the infarct. TI-201 is hindered, however, by its inability to differentiate between recent and old infarcts (Wackers et al., 1976).

In an effort to improve the quality of the TI-201 scans, Cook et al. (1976) imaged healthy patients after tracer administration, both at rest and at maximal stress. The rest scans showed TI-201 uptake by the left ventricle, liver, and spleen while the exercise scans showed a more homogeneous left ventricle and better definition of the right ventricle.

A method to augment myocardial TI-201 uptake using sodium bicarbonate has been devised (Hetzel et al., 1977). In vivo imaging of dogs showed a 1.5-2 times increase of thallium in the heart and a three times enhancement of heart to liver uptake. The exact method by which bicarbonate altered the distribution of thallium has not been fully elucidated.

Recently, Watson et al. (1978) derived a multi-compartment model for the in vivo distribution of thallium. The model indicated a three phase thallium uptake.

The usefulness of TI-201 in stress scintigraphy to clarify equivocal electrocardiograms when chest pain was present was demonstrated by Botvinick et al. (1978). Stress scans identified the coronary causes of chest pain to be due to left bundle branch block.
The sensitivity of myocardial imaging with Tl-201 for reproducible image interpretation was tested among 4 experienced readers at 2 different institutions (Trobaugh et al., 1978). Of 100 scans of myocardial infarction or ischemia, there was complete agreement in 79 scans, minor disagreement in 8 scans, and major disagreement in 13 scans. Thus, the interobserver disagreement found suggest variability in reading and interpretation of unprocessed scans.

e) Radioiodinated Fatty Acids

Biochemical substrates such as fatty acids have proven to be useful for myocardial imaging. These will be discussed in a later section.

f) Future Trends

Short-lived positron emitters such as Nitrogen - 13 and Carbon-11 have also been investigated for myocardial imaging. However, the advantages, as well as the disadvantages of these agents are based mainly on their extremely short half-lives.

(i) $^{13}$N-ammonia

$^{13}$N-ammonia was introduced as a physiologic radiotracer for nuclear medicine by Hunter and Monahan (1971). N-13 has a half-life of 9.96 minutes and decays by positron emission (Hunter and Monahan, 1971). Initial studies using $^{13}$N-ammonia demonstrated good quality myocardial scans in patients with malignant neoplasms. Subsequent clinical trials by Harper et al. (1972) demonstrated the feasibility of myocardial imaging with $^{13}$NH$_3$. In these human studies, the brain, liver, kidney and bladder were also imaged (Hoop et al., 1973; Harper et al., 1972).
The myocardial uptake of $^{13}\text{NH}_3$ was rapid following direct arterial injection, and greater than 90% extraction was demonstrated after a single passage through the dog heart (Harper et al., 1973). Only 15% of the tracer remained in the blood pool after the first minute (Harper et al., 1972). About 15% of the injected dose was concentrated in the liver. Pulmonary uptake was early and transient, except in chronic smokers (Harper et al., 1973).

In clinical trials of 22 infarct patients studied by Harper et al. (1973), successful scans definitely indicative of myocardial infarcts were obtained in 17 patients, while 2 scans showed possible infarct and 3 scans were negative.

Walsh and Resnekov (1976) evaluated myocardial perfusion in 112 patients with $^{13}\text{NH}_3$. Successful imaging was obtained as the blood clearance of the tracer was rapid. About 2-4% of the injected dose was taken up by the myocardium where it remained for up to 30 minutes (Walsh and Resnekov, 1976). The ammonia was mainly in the ionized cationic form in physiologic pH and was therefore impermeable to cell membranes (Walsh et al., 1977; Walsh and Resnekov, 1976). The mechanism of uptake of the ammonium ion has not been elucidated but the rapid blood clearance suggested a different method from that of the potassium analogs. Once incorporated into the myocardial cells, the ammonium ion was metabolized via the glutamine synthetase pathway to glutamine, which then entered the amino acid pool (Walsh et al., 1977). Subsequent studies at short intervals after AMI showed good correlation between the ECG site of infarct and the perfusion defect on the $^{13}\text{NH}_3$ scan (Walsh et al., 1977).
The limitations of $^{13}$NH$_3$ include the necessity of an on-site cyclotron and a positron camera. Generally, in the $^{13}$NH$_3$ scans, overlapping regions of the heart could not be separated and activity in the more distant regions was not readily detected (Walsh et al., 1977).

(ii) $^{11}$C-palmitate and $^{11}$C-octanoate

Carbon-11 has a half-life of 20.3 minutes and decays by positron emission (Weiss et al., 1976). It has been used to label various fatty acids for myocardial studies. Weiss et al. (1976) scanned dogs with and without induced ischemia after the intravenous administration of $^{11}$C-palmitate using ECG gated positron-emission transaxial tomography. There was diminished $^{11}$C-palmitate uptake in zones of ischemia.

Subsequently Weiss et al. (1977) quantified cross sections of canine myocardium in vivo using $^{11}$C-palmitate with positron transaxial tomography and compared the results with regional myocardial CPK depletion. The canine studies showed CPK activity to parallel the $^{11}$C-palmitate decrease in the ischemic zone. The percentage infarction in the tomographic cross sections correlated well with infarction in the corresponding cross sections from the same heart estimated morphometrically and by CPK depletion (Weiss et al., 1977). The studies of Hoffman et al. (1977) showed similar tomographic images.

In spite of these promising results, the major drawback is that Carbon-11 will never be generally available.

4. Fatty Acids

The naturally occurring fatty acids are usually straight-chained,
even-numbered, mono-carboxylic acids with the general formula: \( \text{CH}_3\text{(CH}_2\text{)}_n\text{COOH} \) (Sackheim and Schultz, 1977; Routh et al., 1973). These organic acids may be saturated or unsaturated and are a constituent of the daily dietary fats.

Ingested fats are emulsified in the stomach, followed by digestion by bile salts, pancreatic juices, and chyme in the small intestines (Sackheim and Schultz, 1977; Gunstone, 1967). Digestion involves hydrolysis of fats to fatty acids and glycerols. Most of the glycerol and any short chain acids pass to the portal blood while monoglycerides and longer chain fatty acids pass through the lacteals of the villi into the lymphatics, where they appear as resynthesized fats. These then pass into the general circulation via the thoracic duct and are transported by the blood stream to the liver (30%), fat depots (30%) musculature and other organs (40%) (Gunstone, 1967).

Blood lipid levels may fluctuate reflecting the dynamic processes involved. Circulating lipid levels are increased by intestinal absorption, by synthesis, or by release from adipose tissue, while they are alternatively decreased by storage in fat depots, oxidation, and synthesis of structural components of cells (Fredrickson and Gordon, 1958).

The blood lipids consists of triglycerides, phospholipids as well as cholesterol which are combined as lipoprotein complexes. Small amounts of free fatty acids are also present in the blood and exist mainly as albumin-bound free fatty acids (FFA) (Goodman, 1958a; Gordon et al., 1957; Gordon and Cherkes, 1956; Dole, 1956). This albumin-bound FFA is the principal form by which depot fat is transported to various tissues for oxidation. It is essential for fatty acids to be in the free or unesterified
form in order to be oxidized. (Spector, 1968; Wasserman and Meyerson, 1951).

Oxidative degradation of fatty acids is a universal biochemical capacity among living organisms (Chapman, 1969). In mammals, such oxidation occurs in the liver, the kidneys, and the heart. Intracellularly, fatty acid oxidation occurs principally in the mitochondria.

Fatty acids are the most active form of lipids involved in metabolism (Sackheim and Schultz, 1977). Fatty acids contain more stored food energy per carbon atom than any other biological fuel (Fredrickson and Gordon, 1958). They are degraded by enzymatic processes to end products of carbon dioxide, water and energy stored as adenosine triphosphate (ATP) (Routh et al., 1973). It has been estimated that 88% of the released energy is trapped by the complete oxidation of one mole of palmitic acid (Gunstone, 1967).

a) **Myocardial Metabolism**

Two thirds of the total daily myocardial consumption has been attributed to the oxidation of plasma free fatty acids (Oliver, 1976; Spitzer and Spitzer, 1971; Rothlin and Bing, 1961). Other substrates utilized by the heart include glucose, 15%, lactate and pyruvate, 12%, amino acids, 5%, and the balance consisting of ketone bodies, triglycerides, and glycerols (Oliver, 1976). A direct correlation has been observed between the myocardial oxygen consumption and free fatty acid oxidation (Spitzer and Spitzer, 1971; Cowley et al., 1969; Carlsten et al., 1963).

Plasma free fatty acid concentration has been found to be in the range of .37 to 1.23mM in healthy humans (Rothlin and Bing, 1961; Ballard et al., 1960; Gordon and Cherkes, 1956). Under physiologic
conditions, the molar ratio of FFA/albumin was found to vary from .3 to 1.8 (Gilbertson, 1977; Rothlin and Bing, 1961). Fatty acids found in human plasma consisted of saturated acids from 10 to 18 carbons as well as oleic, palmitoleic, linoleic, and longer chain polyethenoic acids (Willebrand, 1964; Fredrickson and Gordon, 1958). Analysis of plasma FFA by arteriovenous differences by Rothlin and Bing (1961) demonstrated oleic acid (50%) to be the largest component, followed by palmitic acid (28.3%).

An estimated turn-over rate of 20-40% per minute was calculated for plasma FFA (Dole and Rizack, 1961). This rapid turn-over has been attributed primarily to the avid extraction of FFA by the working tissues. Plasma free fatty acids are derived almost exclusively from adipose tissues (Evans, 1964). Mobilization of fatty acids from the adipose tissue was found to be regulated by dietary, hormonal, and nervous stimuli (Spector, 1968; Evans, 1964). Hormones such as epinephrine, norepinephrine, and ACTH increased mobilization while glucose and insulin decreased mobilization (Evans, 1964; Gordon et al., 1957).

(i) Penetration of Plasma Free Fatty Acids into Cells

Under physiologic concentrations and pH, almost all of the plasma FFA is ionized and bound to albumin (Fredrickson and Gordon, 1958). However, the bound and unbound forms exist in an equilibrium and circumstantial evidence suggests that it is the unbound FFA which gains entry into the cells by simple diffusion (Kurein and Oliver, 1971; Spitzer and Spitzer, 1971; Spector et al., 1965a). The magnitude of FFA uptake has been shown to be 100% in excess of the uptake of albumin (Wasserman and
Meyerson, 1951). It has been postulated that albumin facilitates FFA uptake by some interaction with the cell membrane although FFA uptake was observed in the absence of albumin in lower yet similar linear relationships (Spector et al., 1965a). As the unbound FFA is taken up by the cell, free fatty acid dissociates from the albumin-complex to maintain the equilibrium between the bound and unbound forms.

Free fatty acids diffuse into the utilizing cells due to a concentration gradient maintained by the rapid metabolism of the FFA (Spector, 1968). This energy-independent transport of FFA into the cells was demonstrated by the fact that metabolic inhibitors such as cyanide and 2-4 dinitrophenol did not decrease cellular incorporation of $^{14}$C-palmitate (Spector and Steinberg, 1965b). The FFA was located within the sarcoplasmic reticulum as well as the mitochondria.

The diffusion process was, in addition to being energy independent, reversible. Spector et al. (1965a) demonstrated the reversibility by incubation of cells in a labeled FFA solution, washing, and reincubation in a second medium with albumin. Release of labeled FFA was found to be dependent on the albumin concentration of the medium, varying from 45-70% of the incorporated FFA. This exchangeable labeled FFA was located near the cell membrane. Release of fatty acid was also observed during incubation with metabolic inhibitors as well as incubation at low temperatures (Spector, 1971).

b) Myocardial Extraction

The myocardial extraction of FFA was found to be dependent on (i) the circulating FFA concentration, (ii) the FFA/albumin molar ratio,
and (iii) the chain length and degree of unsaturation of the fatty acid 
(Evans, 1964).

(i) The Circulating FFA concentration

In initial studies of FFA utilization, arterial-venous differences 
indicated that uptake of FFA was dependent on plasma concentrations of 
the fatty acids (Gilberton, 1977; Carlsten et al., 1961; Ballard et al., 
1960): Palmitic and oleic acids were taken up to a greater extent than 
stearic and linoleic acids due to their higher arterial concentrations. 
However, the fractional uptake of the fatty acids was essentially the 
same, thus indicating that preferential uptake did not occur (Miller 
et al., 1962). Gold and Spitzer evaluated arterial-venous differences 
for individual fatty acids using titration and liquid scintillation and 
found that the net uptake of plasma FFA occurred in the heart(1964). 

There appeared to be a FFA threshold of about .350mM arterial 
FFA, below which there was no evidence of myocardial extraction by the 
human heart (Carlsten et al., 1963). Studies by Gordon and Cherkes 
(1956), Scott et al.(1962), and Ballard et al.(1960) also indicated the 
existance of a FFA threshold, although the threshold was variable.

(ii) The Molar Ratio of FFA/Albumin

Utilization of $^{14}$C-palmitate by various cultured cell 
preparations including skeletal muscle, erythrocytes, and heart muscle 
were shown to be inversely related to the albumin present (Reshef and 
Shapiro, 1962; Fritz and Kaplan, 1960; Fritz, 1959). In isolated heart 
preparations, the uptake of $^{14}$C-palmitate increased as the molar ratio 
of FFA/albumin increased (Evans et al., 1963a). When the molar ratio
was decreased by maintaining a constant FFA concentration while increasing the albumin concentration, the FFA uptake decreased.

Similar results were obtained in a series of experiments by Spector et al. in 1965. An increase in the molar ratio of FFA/albumin was accompanied by an exponential increase in uptake. The uptake capacity of FFA did not appear saturated even at high molar ratios, thus indicating that the rate limiting step in FFA oxidation was not uptake, but rather a saturation of the tricarboxylic acid cycle (Spector, 1968).

It must be kept in mind that the FFA/albumin ratios under consideration in most experiments were unphysiologically high, and that under normal physiologic conditions, blood albumin has sufficient first class binding sites for the FFA present in the circulation (Goodman, 1958a).

(iii) Chain Length and the Degree of Unsaturation

The molecular structure was found to affect the cellular transport and utilization of fatty acids. The pKa's of fatty acids are between 4.7 and 5.0. Thus, at physiologic pH, fatty acids exist as anions (Goodman, 1958a). The existence of these fatty acid anions appeared to be essential for binding to albumin (Spector, 1968). Fatty acid anions of low and medium length increased the electrophoretic mobility of serum albumin, thereby suggesting the formation of a fatty-acid albumin complex (Ballou et al., 1945). In Plasma, over 99% of the free fatty acids exist as a bound complex to albumin (Teresi and Luck, 1952).
Teresi and Luck studied quantitatively the combination of seven short-chain fatty acids with bovine serum albumin using equilibrium dialysis in 1952. They found that binding increased as the chain length increased from anions of acetate through caprylate and indicated the existence of at least 2 classes of binding sites, each having a characteristic association constant. However, their experiments were limited in that the initial fatty acid content of the bovine albumin used was unknown.

In subsequent experiments, Goodman (1958a; 1958b) analyzed the interaction of human albumin with 6 different long-chain fatty acids by phase partition analysis. There appeared to be 3 classes of binding sites on the human albumin molecule: the first with a high association constant for 2 moles of fatty acid, the second with a lower association constant for 5 moles of fatty acid, while the third had a much lower association constant for about 20 moles of fatty acids. From these studies, there appeared to be 2 binding sites in the first class which were specifically constructed to bind 16 or 18 carbon fatty acids much more avidly than fatty acids of lower carbon chains (Goodman, 1958a). The second class of binding sites contained less structural specificity than the first class. It is conceivable that the first class binding sites were already occupied and therefore were not observable in the studies by Teresi and Luck (1952).

The analysis of Goodman was based on the assumption that each albumin molecule has a discrete number of binding sites for fatty acids and that these sites are independent of one another (Goodman, 1958a). It was hypothesized that interaction of a fatty acid with any
one site was a simple association-dissociation equilibrium which obeyed the law of mass action and could be described quantitatively by an association constant (Fredrickson and Gordon, 1958; Goodman, 1958a).

The affinity of FFA for albumin depended on the length of the hydrocarbon chain as well as the degree of unsaturation (Goodman, 1958a). Studies on isolated rat hearts showed a consistent relationship between myocardial uptake and molecular structure (Evans, 1964). In a series of saturated fatty acids, uptake decreased with increasing chain length (Evans, 1964; Willebrand, 1964), which is consistent with the association constants derived by Goodman (1958a). As the chain lengths increased, FFA associated more tightly to the albumin and thus did not dissociate as readily for cellular uptake. The myocardial extraction appeared to be inversely proportional to the chain length of the saturated fatty acids (Evans et al., 1963b; Carlsten et al., 1961).

Monoenoic acids such as palmitoleic and oleic acids were taken up in preference to saturated or dienoic species of equal chain length (Willebrand, 1964; Evans, 1964). The preferential uptake of monoenoic acids was attributed in part to rapid intracellular utilization since in studies with $^{14}$C-labeled fatty acids, oleic acid was oxidized more readily than either stearic or linoleic acid (Evans, 1964).

Modifications in the structure of FFA altered the strength of binding to bovine serum albumin (BSA) (Spector, Johns and Fletcher 1969). Binding of the FFA was greatly affected if the carboxylic group was modified or removed (Spector et al., 1969).

It would appear that fatty acid binding to albumin was
dependent on 1) electrostatic attraction of the carboxylic group of the free fatty acid to the protein cation site, 2) hydrophobic interactions between the non-polar hydrocarbon chain and the non-polar side chains of the albumin (Spector et al., 1969; Goodman, 1958a). The effect of chain length and unsaturation on the cellular transport of individual fatty acids was attributed to differences in solubility in aqueous media, strength of binding to albumin, affinity to tissue binding sites, and rate of intracellular utilization (Evans, 1964).

c) The Utilization of Intracellular Free Fatty Acid

The fate of incorporated fatty acids was determined in Ehrlich cells loaded with radioactive FFA which were then reincubated in a second medium (Spector et al., 1965a). A large portion of the $^{14}$C-palmitate initially incorporated into the cell was subsequently metabolized during reincubation and the label was recovered almost quantitatively as carbon dioxide and lipid esters (Spector et al., 1965a; Spector, 1968). At low cell FFA concentrations, the rate of $^{14}$CO$_2$ production was proportional to the initial amount of radioactive FFA in the cell. However, as the cellular FFA level increased, the system appeared to approach saturation. Two factors appeared to regulate FFA utilization: 1) FFA content of the cell 2) the turn-over rate of the cellular FFA pool (Evans, 1964).

The existence of two pools of intracellular free fatty acids was shown by Shohet et al. in 1968 using human erythrocytes. There appeared to be a superficial membrane pool to which the FFA was reversibly bound as well as a 'deeper' membrane pool with irreversible
The passage of FFA into the superficial membrane pool was energy-independent while passage into the deeper membrane pool required metabolic energy (Spector, 1971). Both of these pools were calculated to be of equal size although uptake of FFA into the superficial membrane pool was twenty times faster than movement into the 'deeper' membrane pool (Spector, 1971).

Oxidation of fatty acids was found to occur in the mitochondria, in which metabolism of medium chain fatty acids differed from that of long chain fatty acids. Medium chain fatty acids such as octanoic acid can penetrate the inner mitochondrial membrane whereas long chain fatty acids required an initial activation prior to participation in any metabolic pathways (Spector, 1971).

**d) Carnitine** (Beta-hydroxy gamma-trimethyl ammonium butyrate)

Although the transfer of fatty acid between the plasma and the binding sites on the myocardial cell membranes is not energy-dependent; long-chain fatty acids must undergo activation from these sites prior to active cellular metabolism (Evans, 1964). The presence of carnitine was found to be essential for the oxidation of long chain FFA by heart mitochondria (Bode and Klingenberg, 1964).

High carnitine concentrations were found in tissues which were dependent on fatty acids as the major fuel for respiration, primarily the heart and the skeletal muscles (Marquis and Fritz, 1954). The activity of carnitine was found to be dependent on structural components and cofactors of the cell and directed towards the fatty acid oxidase system (Evans, 1964). The independent studies of Fritz
and Yue (1963) and Bremer (1963) demonstrated the catalytic role of carnitine as a carrier of long chain fatty acids from the extramitochondrial acyl-coenzyme A pool to the intracellular fatty acid oxidase system. This was thought to be mediated by carnitine acyl-transferase, which transferred acyl-CoA as a permeable carnitine intermediate across the inner mitochondrial membrane (Fritz and Yue, 1964). Within the mitochondrial matrix, acyl-carnitine was converted back to acyl-CoA, thus confirming the shuttle capacity of carnitine for the acyl moiety across the inner mitochondrial membrane (Bremer and Wojtczak, 1972; Hoppel and Tomec, 1972). Thus, carnitine plays a crucial role in the transport of activated long chain fatty acyl groups from extramitochondrial sites of activation to sites of beta oxidation within the inner membrane matrix of the mitochondria.

e) Oxidation of Fatty Acids

Oxidative degradation of fatty acids is a universal biochemical capacity among living organisms (Chapman, 1969). In mammals, such oxidation occurs in the liver, kidneys, and heart. Intracellularly, fatty acid oxidation occurs principally in the mitochondria. Oxidation of fatty acids to acetyl-CoA involves three enzyme systems; the acyl-CoA synthetases; the carnitine acyl transferase; as well as enzymes of the beta-oxidation system (Spector, 1971). All of these enzyme systems are located in the outer membrane subfraction of isolated mitochondria (Spector, 1968).

Beta-oxidation catalyses the stepwise conversion of long chain acyl-CoA's to a number of acetyl-CoA moieties (Gilbertson, 1977). Although alpha, beta, and omega-oxidation of long chain fatty acids are known to
occur, beta oxidation is the only one of any significance.

(i) **Beta Oxidation**

In beta oxidation, the parent fatty acid is activated by reaction with Coenzyme-A to form fatty acyl-S CoA, which is subsequently oxidized to an alpha-beta-unsaturated compound. It is then hydrated, further oxidized to the beta-keto derivative, and finally subjected to a thiolytic cleavage yielding acetyl-S CoA and the fatty acyl-S CoA containing 2 less carbon atoms (Sackheim and Schultz, 1977). This same series of reactions is repeated, reducing the carbon chain by 2 carbons each time. The by-products as well as the end product of beta-oxidation of an even chain fatty acid are acetyl CoA moieties which enter the Krebs or Tricarboxylic acid cycle. The final products of the Krebs cycle are carbon dioxide, water, and energy (ATP) (Sackheim and Schultz, 1977).

Fatty acids which contain an odd number of carbon atoms are metabolized in the same way with the exception that the final products are acetyl CoA and propionyl CoA (Sackheim and Schultz, 1977). Through a series of steps, propionyl CoA is changed to succinyl CoA, which enters the Krebs cycle, as does the acetyl CoA.

Unsaturated fatty acids are metabolized slowly as they must be reduced by dehydrogenases first. They are then oxidized in the same manner as the saturated fatty acids.

(ii) **Alpha Oxidation**

Enzymes for alpha oxidation have been reported in the liver and brain microsomes (Chapman, 1969; Levis and Mead, 1964). Alpha oxidation is not a major pathway for fatty acid catabolism but is essential for
Figure 1: Schematic Outline of the Beta Oxidation Cycle of Fatty Acids with an Even Number of Carbon Atoms (Sackheim and Schultz, 1977.)
degradation of certain branched chain fatty acids (Anthony and Landau, 1968). During alpha oxidation, an hydroxyl group is inserted on the second or alpha carbon of the fatty acid, forming an alpha-hydroxy acid. This alpha-hydroxy acid forms an intermediate alpha-keto acid, and from subsequent oxidative decarboxylations, yields an odd-numbered long chain fatty acid (Chapman, 1969).

(iii) Omega-Oxidation

Omega oxidation may accompany beta-oxidation and includes an oxidative attack on the terminal carbon of the hydrocarbon chain. The 2-step pathway includes formation of an omega-hydroxy fatty acid. Oxidation of this hydroxy-acid requires cofactors and enzymes (Den et al, 1959). Once the dicarboxylic acid is formed, beta oxidation can occur at both positions. Formation of the dicarboxylic acid is most prominent in acids containing 8-12 carbons (Chapman, 1969). Omega-oxidation is not an important pathway for fatty acid degradation under normal circumstances.

f) Myocardial Metabolism in Acute Myocardial Infarction

Myocardial ischemia is reflected by an alteration in metabolism, depending on the severity of oxygen deprivation. In studies of rat hearts undergoing increasing degrees of oxygen deprivation, there was a progressive decrease in the rate of FFA uptake (Gilbertson, 1977). In addition, the metabolic fate of the fatty acids which were taken up by the heart also varied with the availability of oxygen. In infarcts, the fatty acids were not oxidized for energy, but accumulated within the
myocardial cells as fat droplets (Bilheimer et al., 1978).

The basis of using radioiodinated fatty acids for myocardial scanning is based on the decreased uptake in myocardial cells deprived of oxygen. This decreased uptake can be visualized on a myocardial scan as a 'cold spot'.

5. Iodine-123

Iodine-123 fulfils the criteria of the ideal radionuclide for in situ and in vivo diagnostic procedures. I-123 emits 159 KeV gamma rays in 84% abundance (Poe, 1975; Myers, 1966). This energy is within the ideal energy range and can be efficiently collimated with the cameras presently available. The short 13.0 hours half-life is desirable in that large doses can be administered and serial administrations can be made within short time intervals. Iodine-123 decays by electron capture and the lack of any alpha or beta emissions reduces the radiation exposure to the patient. Iodine-123 provides improved counting statistics over the other isotopes of iodine available (Poe, 1975).

Iodine-123 can be produced by various direct or indirect methods. The indirect methods involve the initial production of $^{123}$Xe, which then decays to $^{123}$I. The advantage of the indirect method of production is the lack of radioiodide contaminants (Sodd, 1975). By first producing xenon-123, there is no problem in the separation of the xenon-123 from the iodine-123. The decay of $^{123}$Xe results in monoatomic $^{123}$I atoms which are chemically reactive and can be utilized for direct labeling reactions in the future (Sodd, 1975).
There have been many methods reported for the production of $^{123}$Xe. The $^{127}$I (p, 5n)$^{123}$Xe reaction is one of the methods of choice. The starting material is readily available and inexpensive. The disadvantage of this reaction is that only a limited number of accelerators are capable of producing photons with energies around 50 MeV.

The spallation process is limited in that impurities in the form of neighboring radioisotopes and radioelements are produced in quantities comparable to the desired radioisotope.

Iodine-123 can be produced directly from bombardment of enriched Tellurium targets. Direct methods of production are limited by the cost as well as the purity of the isotopic enrichment of the Tellurium targets commercially available. In addition, there is always some contamination by Iodine-124, which is impossible to separate from Iodine-123.

It can be seen that Iodine-123 has great potential in nuclear medicine. Problems with contaminants may be reduced or eliminated by alterations in the production methods. However, because of the short half-life and the necessity of direct cyclotron production, its routine acceptance in nuclear medicine will be limited until more cyclotrons are made available for its production.

6. **Radioiodinated Fatty Acids**

As discussed, fatty acids are known to be a major source of fuel for myocardial metabolism, and are distributed according to blood flow to oxygenated, functional myocardial cells. In as early as 1962,
Evans et al. were investigating the use of radioiodinated oleic acid for photoscans of the heart. The radioiodinated oleic acid was bound to albumin and injected into dogs in which myocardial infarctions had been produced. In these early scans, definition between normal myocardium and degrees of infarction was only marginal. In addition, free radioiodide concentrated in the gastric mucosa, thus obscuring the lower border of the heart. Results in human subjects with myocardial infarctions showed similar distribution to the canine studies. The limited success of these early scans were attributed in part to the low specific-activity label produced. Furthermore, the imaging properties of Iodine-131 as well as the radiation exposure to the patients were unfavorable.

Subsequent clinical trials carried out by Gunton et al. (1965) demonstrated sufficient incorporation of $^{131}$I-oleic acid for myocardial scans in which definite areas of decreased radioactivity corresponded to the location of the infarction. The radioiodinated fatty acid was found to be incorporated into tissue triglycerides and phospholipids. This incorporation was enhanced by the simultaneous administration of glucose and insulin. However, the scans obtained were not definitive due to radioactivity present in the thoracic skeletal muscle, lungs, and fundus of the stomach. The quality of the scans was poor and scans of diagnostic quality were obtained in only 50% of the studies (Gunton et al., 1965).

Until 1973, only I-131 labeled oleic, linoleic, and linolenic acids had been investigated. Although the synthesis of high-specific
activity compounds had been achieved, the imaging properties of Iodine-131 were still suboptimal for the gamma cameras available (Anghileri, 1965; Poe et al., 1973). Thus, new radioisotopes were sought to label long chain fatty acids.

Due to the proven usefulness of Technetium-99m as a radioisotope, an attempt was made to label long chain fatty acids by Bonte et al. (1973). 99mTc-oleic acid was injected intravenously into dogs with artificially induced ischemic areas. The resultant scans, however, were of poor resolution and showed high background activity.

Carbon-11 and Fluorine-18 were also used to label various carboxylic acids by Poe et al. (1973). The myocardial extraction capacity for 11C-oleate, 18F-hexanoate, and 18F-tetradecanoate were compared (Poe et al., 1973). The 11C-oleate was almost identical in its extraction pattern by the myocardium to K-43 while the fluorine-18 analogs demonstrated about one-half of the extraction capacity of K-43. It is doubtful whether these radionuclides will be readily available as on-site facilities are essential for their production.

With the production of Iodine-123, a radionuclide with almost ideal imaging properties, attention was again focused on radioiodination of fatty acids. Previous to 1974, the majority of the myocardial scans were done using 131I-oleic acid. This was probably due to the availability, the low cost, and the ease of radioiodination across the double bond. However, by 1974, it was recognized by Poe et al. that fatty acids radioiodinated across a double bond exhibited different biological behavior to their non-radioiodinated counterparts. The radioiodination
appeared to alter the shape of the fatty acid molecule and thus affect the myocardial extraction (Poe et al., 1974). This hypothesis was subsequently confirmed by the studies of Beierwaltes et al. (1975).

Consideration of this problem led to the evaluation of 16-123I-hexadecenoic acid for myocardial scanning (Robinson et al., 1974; Poe et al., 1974). In 16-iodohexadecenoic acid, the I-123 label was on the omega carbon, thereby having minimal stearic interference and not altering the shape of the molecule. Preliminary studies indicated that terminal radioiodinated compounds were metabolized in a fashion similar to that of the parent compound (Poe et al., 1974). From data on intracoronary administration, the extraction and blood clearance of 16-iodohexadecenoic acid was found to approach that of Potassium-43 (Robinson et al., 1974).

By 1975, Robinson and Lee had developed a method for the preparation of high specific activity terminally radioiodinated fatty acids. This labeling procedure was of short enough duration to permit the usage of Iodine-123. The myocardial uptake of 6-iodohexanoic and 11-iodoundecanoic acids was inferior to that of 16-iodohexadecenoic acid (Robinson and Lee, 1975). In addition, hydrolytic liberation of radioiodide from terminally labeled fatty acids was found to be inversely related to carbon chain length. It was postulated that in terminally labeled fatty acids, the iodine atom maintained a configuration similar to the methyl group. Thus, the resultant molecule behaved similarly to the next higher carbon fatty acid (Poe et al., 1975). This appears quite probable due to the similar atomic radius of the iodide
atom to a methyl group (Korolkovas, 1970). The high myocardial extraction of the terminal radioiodinated carboxylic acids could be attributed to their structural resemblance to the true 'physiologic' unsaturated fatty acids (Poe et al., 1975; 1976).

Eckelman et al. (1975) attempted to synthesize fatty acids and long chain hydrocarbon analogs with strong chelating groups to transport metallic isotopes to the myocardium. However, inconsistent production and variable biological behavior rendered these compounds to be of limited use.

Additional experiments were done on the biological behavior of 16-iodohexadecenoic acid by Poe et al. (1976b, 1977). Analysis of canine blood samples 10 minutes after injection revealed over 75% of the activity present was free iodide-131 (Poe et al., 1976b). The free iodide-131 was attributed to the complete metabolism of the original fatty acid molecule; with hydrolysis of iodoacetate, the end product of beta-oxidation (Poe et al., 1976b, Robinson and Lee, 1975). The bound component in the blood represented a mixture of residual labeled hexadecenoic acid, iodinated breakdown products, and released iodine bound to plasma proteins (Poe et al., 1976b). In the heart, 16-iodohexadecenoic acid was found to have a myocardial clearance half-time of 20 minutes. The distribution patterns found were similar to that of K-43 in animals with acute or chronic infarctions (Poe et al., 1976b). Good quality images were attainable within 2-3 minutes.

In recent studies, Machulla et al. (1978) evaluated fatty acids labeled with Carbon-11, Chlorine-34m, Bromine-77, and Iodine-123
for metabolic studies of the myocardium. Comparative kinetic studies in mice indicated extraction of the omega-fatty acids to be more efficient than the alpha-halofatty acids. Of the omega-halofatty acids, 17-iodoheptadecenoic acid showed the highest uptake, which was comparable to $^{11}$C-palmitic acid.

The behavior of alpha-halofatty acids was explained by stearic hindrance and/or inductive effects that influenced esterification with Coenzyme A and carnitine and thus affected passage through the mitochondrial membrane (Machulla et al., 1978). This was reflected in the blood activity. One minute after injection of alpha-bromostearic and alpha-iodostearic acids, 43% and 57% of the total blood activity was as free halide respectively. In contrast, by 1 minute post-injection of the omega-fatty acid, 100% of the total blood activity was free halide (Machulla et al., 1978).

Numerous in vitro and in vivo studies have been done with the 16-$^{123}$I-hexadecenoic acid. One disadvantage in using terminally radioiodinated fatty acid analogs is that the metabolism is extremely rapid, with the result of Iodide-123 being released to the blood. This iodide increases the blood concentration and therefore decreases the target/non-target ratio for myocardial imaging.

Structural modifications of fatty acid molecules may provide an answer to this problem. The modification must be such that the myocardial extraction of the fatty acid would not be affected while the metabolism will be decreased so that radioactivity remains in the heart. The rate of metabolism of fatty acids could be affected by: 1) synthesis
of branch chain fatty acids which resist beta-oxidation

2) attachment of radioiodine to an omega-phenyl substituted fatty acid (Thrall et al., 1978; Poe et al., 1977).

Substitution of a fluorine atom for a hydrogen atom along the carbon chain may permit retention of the biological behavior and yet detain metabolism once inside the myocardial cell (Poe et al., 1977).

The effect of chain length on myocardial extraction was demonstrated using Erucic acid, an unsaturated 22-carbon fatty acid (Christopherson and Bremer, 1972). Eruric acid concentrated within the myocardium but was metabolized very slowly.

Bilheimer et al. (1978) showed that there is increased fatty acid accumulation around the borders of acute myocardial infarcts as early as 6 hours post-occlusion. By using a nonmetabolizable fatty-acid, the effect of pharmacologic intervention on the extent of recovery of myocardial cells in the areas around the infarct could be investigated.

It appears that labeled fatty acids could provide detailed information regarding the heart shortly after myocardial occlusion. Structural modifications should be investigated in an effort to obtain fatty acids which will provide optimal biological behavior for myocardial scans.
III EXPERIMENTAL METHODS AND MATERIALS
1. **Apparatus**

a) **Reflux Assembly**

A standard reflux assembly was used with a 25 ml round-bottom flask (Ace Glass Incorporated 14/20, 110mm) and a 11cm vertical condenser. (Ace Glass Incorporated). A constant water and heat supply were maintained throughout the refluxing period.

b) **Automated Gamma Counter-1185**

The automated gamma counter was manufactured by Nuclear-Chicago Corporation (Des Plaines, Ill. Lot 026A) and was a 'well' type counter with a 3 inch sodium iodide (Tl activated) crystal. Radioactive samples were placed in counting tubes (15.6 x 125 mm, Amersham Searle #00328) and counted in 4 pi detection geometry.

**Calibration of the Gamma Counter**

The gamma counter was calibrated using a Cs-137 source. (Amersham-Searle: .1 μCi ± 10%, Model 184642) Using the photopeak of 662 KeV, the fine and the high voltages were manipulated until the photopeak fell in the 662nd division. Then, with the attenuator set at 8, the high voltage at 800, the fine voltage at 10, the scale represented 1-1000KeV.

A Sodium Iodide-131 spectrum was plotted to ensure that the photopeak was in the 364th division. For Iodide-131, the base was set at 300, the window at 120, and counted on wide differential. Once calibrated, the gamma counter was stable and seldom drifted, although recalibrations were done every 2 months.
c) **Tubor #8725 (Nuclear-Chicago)**

   The Tubor was used for the whole-body excretion studies. The machine contained two-3 inch NaI crystals and was also calibrated using a Cs-137 source. After adjustment of the high and fine voltages, the Cs-137 peak was in channel 662. This was achieved with the coarse high voltage at 1000, the fine high voltage at 41, and the attenuator at 4. An Iodide-131 source was used to ensure that the photopeak was in channel 364, which represented 364 KeV.

   **Geometry Study of the Tubor**

   The geometrical differences of shelf levels and spatial positioning (horizontal 'A' or vertical 'B') were investigated to observe any changes in counts per minute. A 7-ml vacutainer (Beckton-Dickinson Ltd.) filled with 60 μCi of NaI-131 with water was used to simulate the body of a mouse. Counts were recorded for various shelf levels as well as for each axial direction. Concentric circles marked on the shelf ensured that the vacutainer was in exactly the same position for each shelf level and axial direction.

d) **Uni-melt Capillary Melting Point Apparatus**

   Samples were placed in closed-end melting point capillary tubes (Fisher-Scientific Company) and set in the apparatus. The heat was regulated so that the temperature increase was such as to allow visual inspection of the melting point.

e) **Chromatography Tank**

   The chromatography tank was a standard glass tank with
dimensions of 22 x 21 x 6 cm.

f) **Gamma Camera**

The gamma camera used for the myocardial scans was made by Searle Instrument Ltd. and was of the LFOV type. (Large Field of View). A high energy collimator was used for the Iodide-131 and the radioiodinated fatty acid scans while a low-energy all purpose collimator was used for the Thallium-201 scan.

2) **Chemicals and Reagents**

a) **Fatty Acids**

(i) Lauric Acid-Eastman Kodak, Lot 933, C.P. grade
(ii) Capric Acid-Eastman Kodak, Lot 31, Technical grade
(iii) 2-Bromotetradecanoic Acid-Fluka Chemische, B853822 AG-Purum
(iv) 9-cis Tetradecenoic Acid-Sigma Co., St. Louis, Missouri. (99% pure)
(v) 10-Bromocapric Acid-Sapon Laboratories, Bloombury, N.J. (95-99% pure)
(vi) 12-Bromolauric Acid-Sapon Laboratories, Bloombury, N.J. (95-99% pure)

b) **Solvents**

(i) Acetone N.F.
(ii) Ether Solvent U.S.P.
(iii) Hexane-analytical grade-Mallinckrodt, Lot ASN
(iv) Acetic Acid-made from Glacial acetic acid, reagent ACS
Baker and Adamson, Allied Chemicals, Canada

(v) Alcohol 95%- commercial grade
(vi) Benzene-reagent grade
(vii) Petroleum Ether-30/60
(viii) Cyclohexane-practical grade
(ix) NaOH-1 N Certified Fisher Scientific Co., Lot 770410
     (1.0010±.0005 N)
(x) Propylene Glycol-U.S.P., B.D.H. Lot 35056

c) I-131 (NaI)

NaI-131 was supplied by Atomic Energy of Canada Ltd., Commercial
Products, Ottawa. Its radiochemical purity was stated to be not less than
95%. The Iodide-131 was carrier free and no reducing agents were added.
NaI was provided in Na2SO4 liquid.

d) Normal Serum Albumin (human) (HSA)

HSA was supplied by Connaught Lab. Ltd., Willowdale, Ontario,
and was produced by cold ethanol plasma fractionation. It contained
25gm of normal human serum albumin per 100 ml of diluent. The
diluent contained .02M Na Acetyl tryptophanate and .02 M Na caprylate.

e) Gelman ITLC-SG Sheets

Gelman ITLC-SG sheets were supplied by the Gelman Instrument
Company-5x20 cm., Ann Arbor, Michigan, Lot 20238.

f) Gelman ITLC-SAF Sheets

The polysilicic acid gel impregnated with glass fibre sheets
with fluorescent indicator were supplied from the Gelman Instrument
Company, 5x20cm, Ann Arbor, Michigan, Lot 51436.

g) **Biogel P-10**

Biogel P-10 was supplied by the Biorad Laboratories, Richmond, California. (Exclusion limit, 20,000 daltons, 100-200 mesh) Lot 168702.

h) **Biorad AG 1-X8**

The anion exchange resin was supplied by the Biorad Laboratories, Richmond, California. (100-200 mesh—Chloride form)

i) **Normal Saline**


j) **Bacteriostatic H2O for Injection**

Water for injection was supplied by Abbott Laboratories Ltd., Montreal, Canada. Lot 3977.

k) **Phenothalein—B.P., U.S.P. from BDH (Canada Ltd, Lot 547321)**

Phenothalein T.S. - Prepared according to U.S.P. XVII

l) **Bromo Cresol Green - 3,3’ 5,5’ - Tetrabromo-m-cresol sulfonphthalein**

Bromocresol green was supplied by Pharmaceutic Laboratories, Allied Chemical and Dye Corp. N.Y. Lot 15661.

m) **Rhodamine B**

Rhodamine B was obtained from BDH Chemicals Ltd., Poole, England, Lot 1107320.

n) **Nembutal 60mg/ml (for Veterinary Use Only)**

Nembutal injection was supplied by Abbott Lab. Ltd. (Chemical and Agriculture Products division) Lot 87760 NA.
3. **Fatty Acid Analysis**

a) **Purity of the Brominated Fatty-Acid Analogs**

The fatty acid analogs were stated to be 95-99% pure by GC analysis. However, routine tests were performed to check for degradation and ensure chemical purity.

(i) **Melting Points**

Five melting point determinations were done for each fatty acid analog and the results averaged and compared to those reported in the literature.

(ii) **Thin Layer Chromatography (TLC)**

TLC was performed for each brominated fatty acid analog and its corresponding physiologic fatty acid. The solutions contained 10mg/ml fatty acid in acetone. Twenty microlitres of each solution was chromatographed on ITLC-SA, and ITLC-SAF strips. The chromatograms were air dried and developed to a height of 10-12 cm in four different developing solvent systems.

1. Hexane:diethyl ether:acetic acid (80:20:1)
2. Petroleum ether:diethyl ether: acetic acid (70:30:1)
3. Benzene:diethyl ether: acetic acid (75:25:1)
4. Cyclohexane

Once developed, the solvent fronts were marked and the strips allowed to air dry. Several detecting reagents were used to indicate the migration of the fatty acid as well as the fatty acid analogs. $R_f$ values were calculated for each spot.
The detecting reagents used included iodine vapor, bromocresol green .5%, and rhodamine-B. Iodine vapor was used as a general reagent to visualize organic compounds. Bromocresol green was prepared in an ethanolic solution and adjusted to pH 6. Fatty acids appeared as yellow spots against a background of green-blue. With chromatograms sprayed with rhodamine-B and subjected to either long or short ultraviolet rays, fatty acids appeared as fluorescent orange spots against a background of brownish-orange.

b) Preparation of Radioiodinated Fatty Acids

(i) 9-Iodotetradecanoic Acid

9-cis Tetradecenoic acid was radioiodinated using the iodine monochloride microscale method developed by Robinson and Lee (1975). In this reaction, the iodine adds across the double bond on the ninth carbon, while the chloride adds on to the tenth carbon; thus yielding a saturated radioiodinated fatty acid:

(ii) 2-Iodotetradecanoic Acid, 10-Iodocapric Acid, and 12-Iodolauric Acid

These radioiodinated fatty acids were prepared by refluxing NaI-131 with the bromocarboxylic acids in order for the inter-halogenation replacement to occur. This method was also developed by Robinson and Lee (1975).

The procedure included dissolving 10mg of the bromocarboxylic acid in 10 ml of acetone in the reflux apparatus. Boiling chips and a calculated amount of NaI-131 was added and the mixture refluxed. In
order to determine a minimal refluxing time coupled with a high radio-
chemical yield, different refluxing time periods were considered. In
addition, the quantity of the bromocarboxylic acid as well as the volume
of NaI-131 was varied.

c) Analysis of Radiochemical Yield

Radiochemical yield was determined by chromatography on
Gelman ITLC-SG strips. Approximately 20 µl of the solution was spotted,
air dried, and developed in a solvent system of hexane: diethyl ether;
acetic acid (80:20:1). After 10 minutes, the chromatograms were removed,
the solvent fronts marked, and allowed to air dry.

Using this developing system, free iodide was separated
distinctly from the radioiodinated fatty acids. The location of the
free iodide-131 was confirmed by using a solution of NaI-131 in acetone
and the same chromatography system. The locations of the radioiodinated
fatty acids were ascertained by iodine vapor.

The chromatogram was then cut into 1 cm. segments from the
origin to 1 cm. past the solvent front. The sum of all the counts after
background correction represented 100% activity. The free iodide-131
was retained at the origin while the radioiodinated fatty acids travelled
with the solvent front.

d) Preparation of the Radioiodinated Fatty Acid-Albumin Solution

After determination of the radiochemical yield, the radioiodinated
fatty acid acetone solution was evaporated to dryness under a stream of
dry Nitrogen gas. Three ml. of 25% Human Serum Albumin was added and the
resultant mixture was stirred with a magnetic stirrer until complete dissolution.

e) Removal of Free Iodide-131

The radioiodinated fatty acid albumin mixture was layered on an anion exchange column which consisted of a 3ml syringe filled with Biorad A-X8. This column had been pre-saturated with a 6% solution of HSA. As the mixture flowed through the column, free iodide and bromide were retained. The column was then eluted with 3 ml. of normal saline and the effluent adjusted to a 6% HSA solution by addition of normal saline.

Sterility was obtained by passing the final preparation through a .22 micron millipore filter. Verification of the absence of free iodide-131 was done by gel permeation chromatography using a 10 cm x 1 cm column of Bio-gel P-10. The column was also eluted with physiologic saline.

f) Analysis of the Stability of the Radioiodinated Fatty Acid in 6% Human Serum Albumin

The stability of the radioiodinated fatty acids in 6% HSA was determined for 7 days. The preparation was kept refrigerated for the time period. To check for hydrolysis of iodide-131, aliquots of the preparation were analyzed in an anion-exchange column and by a gel permeation column daily.

4. Animals

a) Mice

Male CD 1 Swiss mice weighing 18-30 gm and being 4-6 weeks
old were used throughout the distribution, excretion, and toxicity studies. On arrival from the UBC animal unit, 25 animals were acclimatized for 24 hours in metal cages with dimensions of 30 x 50 cm. Lobound bedding (Paxton Processing Ltd., Paxton, Ill.) was used and Purina Mouse Chow and tap water were supplied ad libitum.

Prior to injection, 5-6 mice were randomly selected for each experimental group and placed in plastic cages with the same provisions as mentioned above.

b) Rabbits

For the myocardial scans, New Zealand male white rabbits weighing 2-2.5 Kg were used. The rabbits were fed Purina Rabbit Chow and given tap water ad libitum. They were housed in metal cages.

5. Tissue Distribution Studies

In order to evaluate the localization of the radioiodinated fatty acids, .1 ml of each preparation was injected by tail vein into groups of mice. At the time of the injections, three .1 ml standards of each radioiodinated fatty acid were prepared. The mean of the counts obtained from the standards approximated the amount of radioactivity injected. By counting these standards simultaneously with the mouse samples, it was unnecessary to account for decay.

Five mice were used to study distribution at each time period and the mean as well as the standard error was calculated for each group of mice for each organ. The time periods considered included;
1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours.

Following a pre-determined time period post-injection, the mice were anesthetized with ether, weighed, and beheaded. The blood was collected, the volume measured, after which it was placed in a counting tube. The tails were removed and placed in counting tubes as well. The tail counts were subtracted from the standards in order to deduce the true amount of activity which entered the body of the mouse. The percent injected dose remaining in the tail was usually less than 2%. If the counts from the tail were greater than 2%, the results from that particular mouse was rejected and an additional mouse used.

The mice were dissected using midsternal and abdominal incisions. The organs excised included the heart, lung, liver, kidneys, spleen, adrenals, gall bladder, stomach, large and small intestines, bone, and muscle. The contents of the stomach and intestines were emptied and the organs rinsed several times with normal saline and blotted dry. The bone sample consisted of the femur, scraped clean of muscle and fat while the muscle sample was a portion of the quadriceps extensor.

All the tissue samples were blotted free of blood and kept moist until they were weighed. The entire surgical procedure as well as the weighing required less than 15 minutes, after which the samples were counted in the gamma counter. The activity localized in each organ was expressed in 3 ways:

(1) Percent Dose/Organ
(2) Percent Dose/Gm Organ
6. **Toxicity Studies**

The toxicity studies were done with the bromo-fatty acids since any toxic effects would probably not be attributed by the small number of radioiodinated fatty acid molecules. The LD$_{50}$ (lethal dose-50) was calculated by the method of Litchfield and Wilcoxin (1949) after a single intravenous injection.

The low solubility of the brominated fatty acids in 6% HSA was a limiting factor in that the highest concentration obtained was 2mg/ml. At this dose, injections by tail vein of up to 1 ml did not elicit any toxic symptoms or deaths. Therefore different methods were employed to achieve a higher concentration of fatty acid. It must be kept in mind, however, that these vehicles were not identical to the 6% HSA used in the distribution and excretion studies.

a) **Preparation of Sodium Bromolaurate**

The sodium salt of bromolauric acid was prepared according to the method of Van Harken et al. (1969). A 20 ml cold solution of 25% HSA, cooled to 5°C in an ice bath, was added to a hot solution of bromolaurate in 15 ml normal saline. The final concentration obtained was 10mg/ml bromolaurate in 10% HSA. This concentration was chosen so that at the lower doses, no pharmacologic effects were elicited, while at high doses, the final toxic effect was death. After injection, the animals were observed for 7 days.
b) **Preparation of Bromocapric Acid Solution**

It was not possible to make stable complexes of bromocapric acid in albumin at high concentrations of the bromocapric salt. The bromocaprate precipitated out of solution. Therefore, an injectable mixture based on the following formula was used:

**Bromocapric Mixture**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromocapric Acid</td>
<td>500 mg</td>
</tr>
<tr>
<td>Ethanol 95%</td>
<td>5 ml</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>11 ml</td>
</tr>
<tr>
<td>H$_2$O for injection q.s.</td>
<td>30 ml</td>
</tr>
</tbody>
</table>

The final bromocapric acid concentration was 16.67 mg/ml. Again, as for the bromolaurate, increment doses were injected to groups of six mice by tail vein and the animals were observed for 7 days.

7. **Whole Body Excretion**

Whole body counting was performed on mice injected by tail vein to determine the decrease in total body activity with time. The whole body excretion curve provided an indication of the excretion rate of the radioiodinated fatty acids by the mice. The injection volumes were kept constant at 0.1 ml. Immediately after injection, each mouse was placed in a plastic container with dimensions of 7 x 5 x 3.6 cm which was then positioned in a larger container lined with tripads. The smaller container was used to restrict the movements of the mouse while the larger container was used to prevent contamination of the Tubor. Each mouse was counted immediately after injection and at the following time intervals: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120,
Each mouse was counted for 1 minute and corrected for background. On removal from the counting containers, the mice were prompted to urinate. They were then placed in cages with metal grids and given food and water ad libitum. The metal grids provided a platform which prevented the contamination of the mice by excreta.

8. Compartmental Analysis

The corrected counts from the whole body excretion study were plotted on semi-log paper versus time. The activity was expressed as a percentage of the total activity, or the activity in the mouse immediately after injection.

Computer analysis was done using the non-lin program. Weighting, a method of added emphasis to certain data points, was based on the number of animals for the trial divided by the standard deviation squared. The curves were fitted using a one, two, three, and four compartment model. The curve of best fit was analyzed by the computer, a graph drawn, and the slopes calculated for each compartment.

9. Excretion Analysis

In order to analyze the urinary and fecal excretion of the radiiodinated fatty acids, mice were injected with .1 ml of each preparation. After injection, the mice were counted in the Tubor. Subsequently, each mouse was placed in an individual 1000 ml beaker with a platform consisting of a wire grid (8 mm square) under which there
was a fine wire mesh. The purpose of this assembly was to effectively separate the feces from the urine. The feces were retained by the mesh while the urine fell through the mesh onto the beaker.

At selected time intervals of 6, 9, and 12 hours post-injection, whole body counting was repeated. Three mice were used for each time interval. To account for the total activity, the mouse, the urine, as well as the feces were counted. Again, standards were counted simultaneously with the samples so that decay correction would not be necessary. Due to the extremely concentrated urine of the mice, it was necessary to make a dilution, measure the total volume, and count an aliquot.

Approximately 20 μl of each urine sample was chromatographed on Gelman ITLC-SG in a developing solvent of hexane:diethyl ether: acetic acid (80:20:1). The chromatography strips were developed and treated as previously described. To ascertain the form in which the radiiodinated fatty acids were excreted, standards were made using mice urine which were spiked with Iodide-131 in 6% HSA, or the radiiodinated fatty acid in 6% HSA. In addition, a group of mice were injected with the Iodide-131 in 6% HSA solution, and the urine also chromatographed.

Analysis of the feces were not attempted as it was apparent that most of the radioactivity was recovered in the urine. Analysis of feces would entail a very lengthy procedure of homogenization and a series of extractions. Due to the nature of the weak iodide-carbon bond, the analytical procedure itself could conceivably split the iodide-carbon bond, if it was still intact in the feces.
10. The Radionuclide Scan

The scans were done using a Searle (LFOV) gamma camera made by the Searle Instrument Ltd. located in the Nuclear Medicine Department of Vancouver General Hospital. Output consisted of conventional X-ray film which accumulated counts until a certain predetermined count was reached.

The rabbits were anesthetized with 25mg/kg of Nembutal injection prior to scanning. They were placed in the prone position on the face of the gamma camera, so that the scans obtained were anterior views. Once positioned on the camera, the rabbits were injected with the radiolabeled solutions by ear vein.

| TABLE I |
|-----------------|------------------|
| **Radioactivity Injected for Myocardial Scans** |     |
| Scan             | mCi injected     |
| I-131 (HSA)      | 1.70             |
| I-131 lauric acid| 1.97             |
| I-131 capric acid| 1.75             |
| Tl-201           | 1.00             |
IV RESULTS AND DISCUSSIONS
1. Analysis of the Brominated Fatty Acid Analogs
   
a) Melting Points of the Brominated Fatty Acid Analogs

   A series of melting points were determined for each bromo-carboxylic acid. Melting points were not taken for the 9-cis-tetradecenoic acid.

   **TABLE II**
   
   Melting Points of the Brominated Fatty Acid Analogs

<table>
<thead>
<tr>
<th>Acid</th>
<th>Reported Melting Point (° C)</th>
<th>Actually Melting Point (° C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Bromotetradecanoic</td>
<td>41.5</td>
<td>44 (sharp)</td>
</tr>
<tr>
<td>10-Bromocapric</td>
<td>42.0</td>
<td>39-40.5</td>
</tr>
<tr>
<td>12-Bromolauric</td>
<td>53.0</td>
<td>51.5 (sharp)</td>
</tr>
</tbody>
</table>

   Although the melting points observed varied from the reported literature values, these results could be influenced by the melting point apparatus used. More indicative of purity of the compounds is how sharp the melting points are. From this data, it appeared that the 2-bromotetradecanoic acid and the 12-bromolauric acid were very pure due to their very sharp melting points. The 10-bromocapric acid, however, had a small range in the melting points, thus indicating the presence of some impurity. This impurity was not quantitated.
b) Chromatography of the Brominated Fatty Acid Analogs and the Physiologic Fatty Acids

TABLE III

Rf Values of Fatty Acids and Their Analogs Using Various Detecting Reagents

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Iodine Vapor</th>
<th>Rhodamine B</th>
<th>Bromocresol Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-Bromolauric Acid</td>
<td>.96</td>
<td>.95</td>
<td>.96</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>.97</td>
<td>.96</td>
<td>.96</td>
</tr>
<tr>
<td>10-Bromocapric Acid</td>
<td>.97</td>
<td>.93</td>
<td>.95</td>
</tr>
<tr>
<td>Capric Acid</td>
<td>.98</td>
<td>.95</td>
<td>.96</td>
</tr>
</tbody>
</table>

a) Hexane:diethyl ether:acetic acid (80:20:1)

Using this developing system of hexane:diethyl ether:acetic acid, the fatty acids travelled with the solvent fronts, whereas the free iodide remained at the origin. With all three detecting reagents used, there was only one distinct spot at the solvent front indicative of the fatty acid with no evidence of tailing. The Rf values for the brominated fatty acids were similar to the physiologic fatty acids.

Since all three detecting reagents provided consistent visual identification of the fatty acids and their analogs, with fairly consistent Rf values, it was decided to use only one detecting reagent for the other developing solvents. Bromocresol Green was chosen. Iodine vapor was somewhat limited in that the color faded very quickly on exposure to air while with Rhodamine B, it was necessary to use a UV source.
TABLE IV

Rf Values of Fatty Acids and Their Analogs
Using Various Developing Solvents

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Rf Values Using Bromocresol Green</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Developing Systems</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>12-Bromolaurylic Acid</td>
<td>.97</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>.97</td>
</tr>
<tr>
<td>10-Bromocapric Acid</td>
<td>.96</td>
</tr>
<tr>
<td>Capric Acid</td>
<td>.96</td>
</tr>
</tbody>
</table>

(1) Petroleum ether: diethyl ether: acetic acid (70:30:1)
(2) Benzene: diethyl ether: acetic acid (75:25:1)
(3) Cyclohexane

With system (3), cyclohexane, there was severe tailing of all the samples. The physiologic fatty acids migrated approximately double the distance of the brominated fatty acid analogs. This was not a good developing system for fatty acids. It appeared that the addition of small amounts of acetic acid prevented the tailing of the fatty acids.

The other three developing systems showed similar Rf values for the fatty acids when detected by bromocresol green. The fatty acids as well as their analogs appeared to be relatively pure as only one distinct spot could be detected for each fatty acid in the different developing solvent systems.
2. The Radioiodination Procedures

a) 9-Iodotetradecanoic Acid

The iodine monochloride microscale method of Robinson and Lee (1975) provided very poor results. Although this method was attempted in excess of 100 trials, results were inconsistent with very low radiochemical yields. The amounts of the individual reagents were varied in an effort to increase the final yield. However, the problem could not be solved and the reaction was still unpredictable.

Since the iodine monochloride addition reaction begins with an unsaturated fatty acid and results in a saturated fatty acid, it was decided to search for a 14-carbon unsaturated fatty acid which already had a bromine substituted for a hydrogen atom along the hydrocarbon chain. In this manner, radioiodination can be achieved by an interhalogen replacement. This fatty acid analog was found in 2-bromotetradecanoic acid, which was available commercially.

b) 2-Iodotetradecanoic Acid, 10-Iodocapric Acid, and 12-Iodo-Lauric Acid

The radioiodination of the brominated fatty acid analogs was easily achieved by refluxing with a radioactive iodine source. Radioiodination was fairly rapid, while the results were much higher and the reaction much more consistent than the attempts with the iodine monochloride microscale method.
c) **Optimization of the Radiiodination Procedure**

(i) **Radiochemical yields of Radiiodinated Fatty Acids in the Absence of Heat**

It was of interest to determine the radiochemical yield of radiiodinated fatty acids in the absence of heat. This was done by experiments carried out under the identical conditions with the exception of refluxing.

**TABLE V**

The Effect of Time On the Radiochemical Yields of Radiiodinated Fatty Acids (No Réfluxing)

<table>
<thead>
<tr>
<th>Acid</th>
<th>1 hour</th>
<th>2 hours</th>
<th>3 hours</th>
<th>4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodolauric Acid</td>
<td>1.10</td>
<td>1.68</td>
<td>2.74</td>
<td>6.21</td>
</tr>
<tr>
<td>Iodocapric Acid</td>
<td>0.87</td>
<td>2.14</td>
<td>3.21</td>
<td>5.07</td>
</tr>
</tbody>
</table>

It was apparent that heat was mandatory for the interhalogen exchange. The radiochemical yields obtained without refluxing were exceptionally low, with a maximum of approximately 6% in 4 hours by TLC analysis.

(ii) **Radiochemical Yields of Radiiodinated Fatty Acids in the Presence of Heat**

In the experiments where heat was supplied by refluxing, the following reaction occurred:

\[ \text{Br(CH}_2\text{)}_n\text{COOH} + ^{131}\text{I}^- \xrightarrow{\text{heat}} ^{131}\text{I(CH}_2\text{)}_n\text{COOH} + \text{Br}^- \]

In analysis, the radiochemical yield appeared to increase with the
Figure 2  Radiochemical Yields of Radioiodinated Fatty Acids by Bromine Replacement
refluxing time until about four hours, at which time the curve plateaued. (Figure 2) Maximal radiochemical yields were 93% and 97% for 12-iodolauryl and 10-iodocapric acids respectively with 4 hours of refluxing. Radiochemical yields at refluxing times of up to 12 hours were essentially the same as those observed after 4 hours of refluxing.

The radiochemical yields varied marginally for the two radioiodinated fatty acids. The radiochemical yield of 10-iodocapric acid appeared slightly greater than that of the 12-iodolauryl acid, thus being in agreement with the observation of Robinson and Lee (1975) that there was a decrease of labeling rates and radiochemical yield with increasing molecular weight. The observed difference may be small as the two fatty acids under consideration only varied by two carbon units.


The labeling reactions utilized 10mg of the brominated fatty acids initially and, as a result, a considerable amount of unlabeled fatty acid would also be administered intravenously to the experimental animals. In order to reduce the amount of unlabeled fatty acid administered, it was decided to decrease the initial amount of brominated fatty acid used for the refluxing. However, in experiments when 5mg of the brominated fatty acids were used, the radiochemical yields were considerably lower with 4 hours of refluxing. The maximum radiochemical yields were only about 55% for both radioiodinated fatty acids. Consequently, it was decided to proceed with 10mg of the
brominated fatty acids initially for the refluxing.

The use of different volumes of NaI-131 did not appear to affect the radiochemical yield significantly as long as the volume used was less than .1 ml. (Table 6)

<table>
<thead>
<tr>
<th>Acid</th>
<th>Radiochemical yield (%) with 4 hours refluxing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.05 ml</td>
</tr>
<tr>
<td>Iodolauric Acid</td>
<td>90</td>
</tr>
<tr>
<td>Iodocapric Acid</td>
<td>92</td>
</tr>
</tbody>
</table>

From Table 6, it appeared that in volumes of greater than .1 ml of NaI-131, there may be some hydrolysis of the halogen from the fatty acid, thus leading to a lowered radiochemical yield.

d) Analysis of the Stability of the Radioiodinated Fatty Acids in 6% Human Serum Albumin

The stability of the terminal radioiodinated fatty acids with respect to hydrolysis in 6% HSA under conditions of refrigeration was determined. Liberation of free Iodide-131 was analyzed by both anion exchange and gel permeation chromatography and the results are presented in Figure 3.

The relative stability of the radioiodinated fatty acids was studied for 7 days. Both radioiodinated fatty acids appeared to be
Figure 3 Stability of Radioiodinated Fatty Acids in 6% Human Serum Albumin
stable for the first 2 days, after which the percentage of radioiodinated fatty acid dropped below 90%. The 10-iodocapric acid seemed more susceptible to hydrolysis as the intact radioiodinated acid decreased at a faster rate, as noted by the steeper slope. (Figure 3) This may be a reflection of the strength of the carbon-iodide bond. Although radioiodination appeared marginally higher for the 10-iodocapric acid, perhaps the bond formed was not as strong as the carbon-iodide bond of 12-iodolauric acid.

Using both the anion exchange column and the gel permeation column, the results were similar for each radioiodinated fatty acid. The day of refluxing was noted as day 0, in which the radioactivity present was taken to be 100%. The preparations were used for the following one or two days to ensure the absence of free iodide-131, which could alter the biological distribution studies as well as the scan.

3. Preliminary Distribution Studies of 10-Iodocapric, 12-Iodo-
Lauric, and 2-Iodotetradecanoic Acids

Preliminary distribution studies were performed using the above mentioned radioiodinated fatty acids in mice. From Figure 4, which depicted the radiopharmaceutical concentration within the heart, it was apparent that 12-iodolauric acid was concentrated to the greatest extent. This was followed by 10-iodocapric acid and then by 2-iodotetradecanoic acid. The myocardial concentration of 2-iodotetradecanoic acid was about half of that of the other two radioiodinated fatty acids. This apparent lack of uptake of
Radioactivity levels have been determined even after metabolism of the fatty acid.
2-iodotetradecanoic acid can be attributed to stearic hindrance, as noted by Robinson and Lee (1975). The large bulky iodine molecule has the radius of a methyl group and projects out of the plane of the fatty acid molecule, thus decreasing the myocardial uptake. (Robinson and Lee, 1975).

The myocardial concentration of all three radioiodinated fatty acid analogs decreased rapidly with time. By 30 minutes post-injection, the percent injected dose remaining in the myocardium was less than .5%. The same trend was observed in the % dose/gm organ (Figure 5) and the % dose/gm organ/%dose/ml blood. (Figure 6) Although the highest total myocardial uptake was by 12-iodolauric acid, when calculated on the %dose/gm basis, 10-iodocapric acid was concentrated to a greater extent. 2-iodotetradecanoic acid was clearly inferior to the other two iodinated fatty acids in all aspects investigated and thus was excluded from further studies.

a) **Preliminary Radionuclide Scan**

Scans were attempted using the Picker Nuclear Dynacamera to assess the potential usefulness of the two terminal radioiodinated fatty acids as myocardial scanning agents. Scans were taken immediately after an intravenous injection of 12-iodolauric acid and accumulated to 1000 counts. The scans showed some background activity. The liver and heart were outlined although they were indistinguishable from one another. In addition, there was probably some contributing activity from the lungs. The gastrointestinal tract was faintly visualized at the beginning of scanning, but became more evident as the scan progressed.
Figure 5  Relative Myocardial Uptake of 2-Iodotetradecanoic Acid, 10-Iododecanoic Acid, and 12-Iodolauric Acid in Mice\textsuperscript{a}

\textsuperscript{a} Radioactivity levels have been determined even after metabolism of the fatty acid.
Figure 6 Myocardial/Blood Distribution of 2-Iodotetradecanoic Acid, 10-Iododecanoic Acid, and 12-Iodolauryl Acid in Mice

Radioactivity levels have been determined even after metabolism of the fatty acid.
From the initial scans, there appeared to be evidence of myocardial uptake, although the uptake was not definitive. It was decided that a gamma camera with a high energy collimator would provide better resolution and more definitive scans.

4. Tissue Distribution

The tissue distribution of $^{131}$I-capric acid, $^{131}$I-lauric acid, as well as NaI-$^{131}$ in 6% Human Serum Albumin was studied as a function of time in groups of mice. Approximately .5 μCi of radioactivity was administered by tail vein to each mouse and the tissue distribution was done using the procedure previously described.

In the distribution studies, the primary organ of interest was the heart. However, due to the anatomical location of the heart, it was imperative to consider other organs which were in the immediate vicinity as well. These organs included the liver, the lungs, and the stomach. Since these organs are highly vascular and are comparatively large in size, the radiopharmaceutical could localize to a considerable extent, thus interfering with the imaging of the myocardium. For tabulations of the radioactivity distributed throughout the entire mouse body, assumptions were made that 45% of the body weight represented muscle, 6% corresponded to bone, and 77.8 ml. per Kg represented the total blood volume (Wish et al., 1950; Stand et al., 1962).

Other organs considered included the kidneys, the spleen, the stomach, the large and small intestines, the gall bladder, as well as the adrenals. The maximum concentration of radiopharmaceutical by
the gall bladder and the adrenals was less than .1% of the injected dose, thus the results were excluded.

a) $^{131}$I-Capric Acid

The results of the organ distribution studies for $^{131}$I-capric acid, expressed as "percent injected dose per total organ" of the spleen, stomach, intestines, muscle, and bone are listed in Table 7, while the graphical presentation of the "percent injected dose per total organ" of the blood, heart, lungs, liver and kidneys are shown in Figure 7. From these results, it appeared that the concentration of the radiopharmaceutical in the organs examined were the highest immediately following injection, after which there was a steady decline with time.

The highest myocardial uptake of $^{131}$I-capric acid occurred within the first few minutes after injection. The disappearance of radioactivity from the heart occurred in an exponential manner. There appeared to be three components. The first component appeared extremely rapid, within the first few minutes following injection, after which there was a somewhat slower second component, followed by what appeared to be a steady-state, where the concentration in the myocardium remained almost constant.

The first as well as the second component may be indicative of myocardial oxidation of the fatty acid. It is known that once the fatty acids are within the myocardial cells, they are degraded by Beta oxidation to provide energy (Oliver, 1976; Robinson and Lee, 1975; Spilter and Spitzer, 1971). The degradation
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen</th>
<th>Stomach</th>
<th>Intestines</th>
<th>Muscle</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>.017</td>
<td>.24±.02</td>
<td>.76±.11</td>
<td>2.95±.09</td>
<td>16.26±1.8</td>
<td>3.02±.32</td>
</tr>
<tr>
<td>.033</td>
<td>.30±.03</td>
<td>.93±.15</td>
<td>2.99±.10</td>
<td>16.82± .92</td>
<td>3.23±.13</td>
</tr>
<tr>
<td>.050</td>
<td>.32±.02</td>
<td>1.21±.11</td>
<td>2.70±.16</td>
<td>16.12± .58</td>
<td>3.55±.12</td>
</tr>
<tr>
<td>.067</td>
<td>.35±.01</td>
<td>1.34±.20</td>
<td>2.86±.10</td>
<td>17.87± .93</td>
<td>3.49±.12</td>
</tr>
<tr>
<td>.083</td>
<td>.37±.04</td>
<td>1.44±.20</td>
<td>2.72±.18</td>
<td>19.02± .62</td>
<td>3.90±.16</td>
</tr>
<tr>
<td>.100</td>
<td>.28±.02</td>
<td>1.62±.17</td>
<td>2.89±.11</td>
<td>16.68± .82</td>
<td>3.74±.20</td>
</tr>
<tr>
<td>.133</td>
<td>.38±.01</td>
<td>2.73±.38</td>
<td>2.87±.11</td>
<td>14.55± .85</td>
<td>3.46±.12</td>
</tr>
<tr>
<td>.167</td>
<td>.29±.02</td>
<td>2.34±.47</td>
<td>2.55±.17</td>
<td>12.01± .42</td>
<td>3.10±.23</td>
</tr>
<tr>
<td>.250</td>
<td>.29±.02</td>
<td>2.63±.32</td>
<td>2.62±.29</td>
<td>12.44± .56</td>
<td>2.74±.13</td>
</tr>
<tr>
<td>.333</td>
<td>.27±.02</td>
<td>2.21±.48</td>
<td>2.42±.17</td>
<td>11.89± .72</td>
<td>2.69±.09</td>
</tr>
<tr>
<td>.417</td>
<td>.29±.04</td>
<td>3.16±.34</td>
<td>2.11±.07</td>
<td>12.04± .62</td>
<td>2.55±.20</td>
</tr>
<tr>
<td>.500</td>
<td>.18±.01</td>
<td>3.46±.22</td>
<td>1.97±.08</td>
<td>10.83± .78</td>
<td>2.18±.13</td>
</tr>
<tr>
<td>.667</td>
<td>.19±.02</td>
<td>2.86±.40</td>
<td>1.86±.22</td>
<td>9.73± .75</td>
<td>2.39±.17</td>
</tr>
<tr>
<td>.833</td>
<td>.16±.02</td>
<td>2.95±.29</td>
<td>1.69±.17</td>
<td>7.65± .71</td>
<td>1.81±.14</td>
</tr>
<tr>
<td>1.000</td>
<td>.18±.06</td>
<td>3.02±.91</td>
<td>1.50±.19</td>
<td>7.60± .93</td>
<td>1.68±.26</td>
</tr>
<tr>
<td>2.000</td>
<td>.10±.01</td>
<td>2.01±.28</td>
<td>.97±.13</td>
<td>6.89± .41</td>
<td>1.28±.10</td>
</tr>
<tr>
<td>4.000</td>
<td>.05±.004</td>
<td>1.06±.27</td>
<td>.55±.05</td>
<td>2.86± .41</td>
<td>.61±.07</td>
</tr>
<tr>
<td>6.000</td>
<td>.06±.01</td>
<td>1.26±.30</td>
<td>.74±.13</td>
<td>3.20± .41</td>
<td>.69±.13</td>
</tr>
<tr>
<td>12.000</td>
<td>.006±.001</td>
<td>.06±.02</td>
<td>.06±.01</td>
<td>1.35± .13</td>
<td>.12±.01</td>
</tr>
<tr>
<td>24.000</td>
<td>.005±.001</td>
<td>.04±.01</td>
<td>.06±.01</td>
<td>1.48± .10</td>
<td>.10±.01</td>
</tr>
</tbody>
</table>

a Expressed as per cent injected 131I-capric acid per total organ
b Mean values from five mice ± standard error of the mean
c Total muscle was calculated on 45 percent of body weight (Stand et al., 1962)
d Total bone was calculated as 6 percent of body weight (Stand et al., 1962)
Figure 7 Total Organ Uptake of 10-Iodocapric Acid by Liver, Heart, Lung, Kidneys and Blood in Mice

Radioactivity levels have been determined even after metabolism of the fatty acid.
process occurs rapidly, hence the estimated myocardial half-time of $^{123}$I-hexadecenoic acid is less than 25 minutes (Poe et al., 1976a). Iodoacetate, the metabolic end product, is subjected to hydrolysis, thus releasing the iodine-label which diffused from the myocardium to the blood stream (Robinson and Lee, 1975).

Poe et al. (1975a) obtained similar results of myocardial clearance after intracoronary injections of $^{131}$I-hexadecenoic acid and $^{131}$I-oleic acid in dogs. A biphasic myocardial clearance curve was obtained for each radioiodinated fatty acid within the first two minutes after injection. Assumptions were made that the first phase of the curve represented a wash out of non-extracted fatty acid, whereas the second phase represented the fatty acid retained in the heart and metabolized. Subsequently, Poe et al. (1976a) found that the disappearance of $^{123}$I-hexadecenoic acid from the myocardium approximated a monoexponential function, although there was a slightly slower clearance rate after 10 minutes. These results, however, were obtained using a different animal model, a different method of injection, as well as different radioiodinated fatty acids.

The third component probably represented the radioiodinated fatty acid which was not oxidized by Beta-oxidation, but was presumably incorporated into intracellular triglycerides or other complex lipids within the myocardium, thus accounting for the apparent steady state (Evans et al., 1963a). In earlier studies, it was demonstrated that over 2/3 of the radioactivity present in the heart 4 hours after injection of $^{131}$I-oleic acid was in the triglyceride form (Evans et al., 1965).
A similar pattern was observed for the other organs with the exception of the stomach and the kidneys (Table 7, Figure 7). The highest $^{131}$I-capric acid accumulation was in the muscle, followed by the blood, bone, intestines, liver, kidney, heart, lung, stomach and spleen. When the results are expressed in this manner, no organ appears to have any special affinity to incorporate $^{131}$I-capric acid. This was evident by the rapid decrease of radioactivity within each organ during the first hour. The high accumulation of radioactivity in the muscle and the blood was due to the large total mass or volume in consideration.

The blood level reflected a rapid clearance of the $^{131}$I-capric acid with little residual activity remaining at 24 hours. However, the results tended to fluctuate somewhat although a steady decrease of radioactivity was seen. This fluctuation could conceivably be due to the iodide-$^{131}$ ion diffusing into and out of various tissues.

Compartmental analysis of the blood concentration with time was not attempted although it has been reported in the literature (Evans et al., 1965; Poe et al., 1976a). A biphasic decrease in blood radioactivity was found by Evans et al. (1965) after intravenous administration of $^{131}$I-oleic acid. The initial phase was rapid, within the first 10 minutes, followed by a second, slower phase. Partition of the blood into lipid bound and water soluble radioactivity indicated that the initial rapid phase was due to the disappearance of the $^{131}$I-oleic acid with a half-life of about one and one-half minutes. The remaining radioactivity in the blood was derived from "free" radiiodine present in the original injection and from radiiodine released by the
metabolism of fatty acid in tissues. The clearance of the radioiodinated fatty acid from blood was virtually complete in 20 minutes.

Additional blood clearance results were obtained by Poe et al. (1976a) using serial venous sampling of mongrel dogs. Again, a biphasic clearance pattern was seen. Half-time values for the first phase were extremely rapid, 1.7 minutes for $^{123}$I-hexadecenoic acid and 2.2 minutes for $^{11}$C-oleic acid. Analysis of the blood at 10 minutes after injection showed that over 75% of the activity was attributed to free iodide, which presumably resulted from complete metabolism of the labeled fatty acid, with hydrolysis of the terminal iodine atom (Poe et al., 1976a). The bound component was a mixture of residual labeled hexadecenoic acid, iodinated breakdown products, and released iodine bound to proteins.

Recent studies by Machulla et al. (1978) determined the free blood Iodine-123 after administration of labeled fatty acid into mice using high pressure liquid chromatography. They found that at 1 minute after injection, 100% of the total blood activity existed as the free halide. The mice were sacrificed between 15 seconds to 10 minutes after injection. It appears questionable as to how precisely timed these experiments were as it would be very difficult to inject and sacrifice a mouse within 15 seconds of injection.

The renal concentration of the $^{131}$I-capric acid also fluctuated. This was expected since the kidneys are the major route of elimination of the Iodide-131 and as the iodide was filtered, a portion of it would be reabsorbed, and the remainder would enter the
bladder and be voided (Myant et al., 1950).

The initial concentration of radioactivity in the stomach was low, but increased with time as metabolism of the $^{131}$I-capric acid and subsequent hydrolysis released free iodide-131 into the blood stream (Poe et al., 1976a; Myant et al., 1950; Nelson et al., 1947). The gastric concentration of the $^{131}$I-capric acid increased gradually to a maximum between .5 hour and 1 hour, after which the concentration decreased. The concentration of radioactivity in the stomach was shown by Evans et al. (1965) to be due to the rapid excretion of radioiodide from the blood by the gastric mucosa.

The large and the small intestine activity decreased from a maximum of 2.95% at .017 hour to .06% at 12 hours. This indicated that only small quantities of the $^{131}$I-capric acid was eliminated via the feces in the time periods under observation. This is in agreement with our findings since it appears that degradation and subsequent hydrolysis released free Iodide-131 into the blood stream, and it was the free Iodide-131 which was excreted via the urine. It has been shown that the iodide ion is so efficiently absorbed by the intestines that only minute traces of iodide escaped from the body in the feces (Nelson et al., 1947).

The $^{131}$I-capric acid was rapidly cleared by the organs under investigation. By 24 hours after injection, only the muscle had greater than one percent of the injected dose remaining.

In view of the different shapes and sizes of the organs under consideration, relative accumulation of the $^{131}$I-capric acid was
expressed as "percent injected dose per gram of organ". This is shown in Table 8 and Figure 8. The heart showed the highest concentration of $^{131}$I-capric acid initially for the first .05 hours after injection. The concentration then decreased rapidly until about .167 hours, at which time the concentration in the blood, the kidneys, and the lungs were higher than that of the heart. After .167 hours, the decrease in radioactivity appeared slower. The maximum concentration of $^{131}$I-capric acid by the heart was observed at 1 minute after injection, at which time the concentration was 9.25% of the injected dose per gram of myocardial tissue. Machulla et al. (1978) measured the radioactivity in mouse hearts after intravenous injections of various labeled fatty acids. They found the highest myocardial accumulation of $^{123}$I-heptadecanoic acid to be 36% of the injected dose per gram of myocardial tissue at 30 seconds after injection. This value appeared to be somewhat high compared to our results as well as those reported by other investigators.

In our results, at 5 minutes after injection, 4.73% of the injected dose was concentrated in each gram of myocardium. In similar experiments carried out in rats by Beierwaltes et al. (1975), the concentration of $^{131}$I-oleic acid in the heart at 5 minutes was found to be 1.78% of the injected dose per gram of tissue. This lower myocardial concentration could be attributed to stearic hindrance due to the bulky iodine, as previously discussed. In the same experiment, the concentration of $^{14}$C-oleic acid was found to be 3.42% of the injected dose per gram of myocardium, which was comparable to the results we obtained. However, the decrease in the myocardial concentration of $^{131}$I-capric
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen</th>
<th>Stomach</th>
<th>Intestines</th>
<th>Muscle</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>.017</td>
<td>1.56± .14</td>
<td>2.80± .43</td>
<td>1.73± .06</td>
<td>1.22±.16</td>
<td>1.67±.14</td>
</tr>
<tr>
<td>.033</td>
<td>2.00± .11</td>
<td>3.62± .54</td>
<td>1.68± .04</td>
<td>1.24±.06</td>
<td>1.78±.08</td>
</tr>
<tr>
<td>.050</td>
<td>2.12± .17</td>
<td>5.23± .61</td>
<td>1.69± .04</td>
<td>1.21±.04</td>
<td>2.00±.05</td>
</tr>
<tr>
<td>.067</td>
<td>2.04± .06</td>
<td>4.41± .35</td>
<td>1.57± .04</td>
<td>1.27±.08</td>
<td>1.85±.05</td>
</tr>
<tr>
<td>.083</td>
<td>2.25± .10</td>
<td>5.23± .66</td>
<td>1.69± .09</td>
<td>1.44±.06</td>
<td>2.22±.11</td>
</tr>
<tr>
<td>.100</td>
<td>1.99± .04</td>
<td>6.57± .64</td>
<td>1.57± .05</td>
<td>1.24±.04</td>
<td>2.10±.09</td>
</tr>
<tr>
<td>.133</td>
<td>2.10± .08</td>
<td>9.23± .99</td>
<td>1.49± .03</td>
<td>1.07±.04</td>
<td>1.92±.08</td>
</tr>
<tr>
<td>.167</td>
<td>1.90± .10</td>
<td>10.45±1.82</td>
<td>1.42± .09</td>
<td>.90±.05</td>
<td>1.76±.19</td>
</tr>
<tr>
<td>.250</td>
<td>1.83± .14</td>
<td>10.05±1.20</td>
<td>1.51± .12</td>
<td>.93±.05</td>
<td>1.55±.06</td>
</tr>
<tr>
<td>.333</td>
<td>1.59± .11</td>
<td>8.12± .68</td>
<td>1.29± .03</td>
<td>.90±.05</td>
<td>1.53±.06</td>
</tr>
<tr>
<td>.417</td>
<td>1.64± .08</td>
<td>11.82± .82</td>
<td>1.29± .06</td>
<td>.92±.04</td>
<td>1.45±.12</td>
</tr>
<tr>
<td>.500</td>
<td>1.43± .09</td>
<td>13.56± .79</td>
<td>1.10± .05</td>
<td>.80±.05</td>
<td>1.21±.07</td>
</tr>
<tr>
<td>.667</td>
<td>1.39± .07</td>
<td>13.27±2.13</td>
<td>1.06± .11</td>
<td>.68±.05</td>
<td>1.24±.07</td>
</tr>
<tr>
<td>.833</td>
<td>1.17± .14</td>
<td>11.93± .86</td>
<td>.90± .08</td>
<td>.58±.05</td>
<td>1.81±.08</td>
</tr>
<tr>
<td>1.00</td>
<td>1.41± .59</td>
<td>12.56±4.2</td>
<td>.80± .11</td>
<td>.41±.08</td>
<td>.89±.15</td>
</tr>
<tr>
<td>2.00</td>
<td>.69± .10</td>
<td>7.31±1.4</td>
<td>.53± .07</td>
<td>.43±.03</td>
<td>.60±.05</td>
</tr>
<tr>
<td>4.00</td>
<td>.36± .05</td>
<td>4.26± .96</td>
<td>.33± .05</td>
<td>.22±.04</td>
<td>.34±.05</td>
</tr>
<tr>
<td>6.00</td>
<td>.06±0.08</td>
<td>4.7± 1.0</td>
<td>.38± .07</td>
<td>.23±.03</td>
<td>.37±.07</td>
</tr>
<tr>
<td>12.00</td>
<td>.05±0.00</td>
<td>.23± .06</td>
<td>.04± .01</td>
<td>.10±.01</td>
<td>.06±.00</td>
</tr>
<tr>
<td>24.00</td>
<td>.036±.01</td>
<td>.15± .03</td>
<td>.029±.00</td>
<td>.10±.01</td>
<td>.053±.00</td>
</tr>
</tbody>
</table>

a Expressed as percent of injected $^{131}$I-capric acid per gm organ weight

b Mean value from five mice ± standard error of the mean
Figure 8  Relative Uptake of 10-Iodocapric Acid by Liver, Heart, Lung, Kidneys, and Blood in Mice

Radioactivity levels have been determined even after metabolism of the fatty acid.
acid was comparatively faster than that observed by Beierwaltes et al. (1975) for $^{131}$I-oleic acid. This could be due to the fact that $^{131}$I-capric acid is a medium chain length fatty acid, and therefore an activation step is not necessary in order for it to cross the inner mitochondrial membrane in order to be oxidized, whereas $^{131}$I-oleic acid, a long chain fatty acid, would require activation prior to metabolism. In addition, in $^{131}$I-oleic acid, the iodine group may conceivably slow down the Beta oxidation cycle, thus accounting for the slower clearance with time.

The same trend in the decrease of radioactivity from the liver, the lungs, and the kidneys was observed from our experimental results as those reported by Beierwaltes et al. (1975). The spleen, the intestines, the muscle, and the bone all demonstrated similar concentrations of radioactivity accompanied by similar decreases with time.

To further investigate an optimum scan time, the "percent injected dose per gram organ/percent injected dose per ml blood" was also calculated. This data is presented in Table 9 and Figure 9. The highest organ/blood ratio for the heart was found at 2 minutes after injection, at which time the concentration in the liver and lungs was considerably lower. The optimal scan time for $^{131}$I-capric acid therefore appeared to be immediately following injection. The subject should be positioned under the camera so that the scan could be started immediately after the injection of the radiopharmaceutical.

The maximal organ/blood ratio obtained for the heart was 1.40:1. This was considerably lower than the 9.40:1 ratio reported
### TABLE IX

**Organ/blood Distribution of ¹³¹I-Capric Acid in Mice a,b**

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen Blood</th>
<th>Stomach Blood</th>
<th>Intestines Blood</th>
<th>Muscle Blood</th>
<th>Bone Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>.016</td>
<td>.23±.02</td>
<td>.41±.07</td>
<td>.25±.01</td>
<td>.18±.03</td>
<td>.25±.02</td>
</tr>
<tr>
<td>.033</td>
<td>.38±.02</td>
<td>.63±.09</td>
<td>.29±.01</td>
<td>.22±.01</td>
<td>.31±.01</td>
</tr>
<tr>
<td>.050</td>
<td>.35±.02</td>
<td>.85±.10</td>
<td>.28±.00</td>
<td>.20±.01</td>
<td>.33±.01</td>
</tr>
<tr>
<td>.067</td>
<td>.39±.01</td>
<td>.84±.06</td>
<td>.30±.01</td>
<td>.24±.01</td>
<td>.36±.01</td>
</tr>
<tr>
<td>.083</td>
<td>.38±.02</td>
<td>.89±.14</td>
<td>.28±.01</td>
<td>.24±.01</td>
<td>.37±.01</td>
</tr>
<tr>
<td>.100</td>
<td>.39±.01</td>
<td>1.28±.13</td>
<td>.31±.01</td>
<td>.24±.01</td>
<td>.41±.01</td>
</tr>
<tr>
<td>.133</td>
<td>.44±.01</td>
<td>1.97±.25</td>
<td>.32±.01</td>
<td>.23±.02</td>
<td>.40±.01</td>
</tr>
<tr>
<td>.167</td>
<td>.47±.04</td>
<td>2.55±.39</td>
<td>.35±.01</td>
<td>.22±.00</td>
<td>.43±.03</td>
</tr>
<tr>
<td>.250</td>
<td>.48±.01</td>
<td>2.70±.36</td>
<td>.40±.02</td>
<td>.25±.02</td>
<td>.42±.02</td>
</tr>
<tr>
<td>.330</td>
<td>.43±.02</td>
<td>2.33±.27</td>
<td>.37±.01</td>
<td>.26±.02</td>
<td>.44±.02</td>
</tr>
<tr>
<td>.417</td>
<td>.46±.02</td>
<td>3.32±.24</td>
<td>.36±.01</td>
<td>.26±.02</td>
<td>.40±.01</td>
</tr>
<tr>
<td>.500</td>
<td>.50±.03</td>
<td>4.72±.29</td>
<td>.38±.01</td>
<td>.28±.01</td>
<td>.42±.01</td>
</tr>
<tr>
<td>.567</td>
<td>.50±.03</td>
<td>4.59±.48</td>
<td>.37±.02</td>
<td>.24±.01</td>
<td>.44±.02</td>
</tr>
<tr>
<td>.833</td>
<td>.50±.03</td>
<td>5.20±.38</td>
<td>.39±.01</td>
<td>.25±.01</td>
<td>.44±.01</td>
</tr>
<tr>
<td>1.000</td>
<td>.65±.18</td>
<td>5.81±1.23</td>
<td>.41±.02</td>
<td>.27±.00</td>
<td>.45±.01</td>
</tr>
<tr>
<td>2.000</td>
<td>.52±.02</td>
<td>5.36±.48</td>
<td>.39±.01</td>
<td>.34±.03</td>
<td>.46±.02</td>
</tr>
<tr>
<td>4.000</td>
<td>.52±.04</td>
<td>6.07±.99</td>
<td>.47±.04</td>
<td>.31±.03</td>
<td>.50±.04</td>
</tr>
<tr>
<td>6.000</td>
<td>.50±.02</td>
<td>5.65±.56</td>
<td>.45±.02</td>
<td>.30±.05</td>
<td>.44±.01</td>
</tr>
<tr>
<td>12.000</td>
<td>.80±.15</td>
<td>3.59±.30</td>
<td>.61±.07</td>
<td>1.91±.58</td>
<td>1.19±.31</td>
</tr>
<tr>
<td>24.000</td>
<td>.70±.07</td>
<td>2.75±.41</td>
<td>.57±.04</td>
<td>2.05±.10</td>
<td>1.07±.12</td>
</tr>
</tbody>
</table>

---

a Expressed as ratio of percent injected dose per gm. of organ to percent injected dose per ml. of blood.

b Mean values from five mice ± standard error of the mean.
Radioactivity levels have been determined even after metabolism of the fatty acid.
for $^{131}$I-oleic acid by Evans et al. (1965, 1962). However, the results of Evans et al. (1965) were obtained from dogs and the administration of the $^{131}$I-oleic acid was accompanied by intravenous infusions of glucose and insulin. It has been observed that insulin and glucose both promoted myocardial storage of fatty acids derived from the circulation and decreased oxidation of the fatty acids in all body tissues (Evans et al., 1965).

In the studies of Schelbert et al. (1974) myocardium/blood ratios were calculated for rats injected with radionuclides such as $^{131}$I-oleic acid. The highest myocardial/blood level was found at 10 minutes after injection, and was found to be 2.40:1. However, these results were obtained using different methodology for the labeling process, different animals, and a different vein for injection. These factors may have affected the results.

There appears to be an inverse relationship between the dose of iodofatty acid administered and the myocardium/blood ratio (Schelbert et al., 1974). Ratios of less than one were observed with doses as high as 8mg/Kg body weight while a ratio of 6.7:1 was observed using .76 mg/Kg. High specific-activity fatty acids appeared to provide higher myocardium/blood ratios. The dose of the total fatty acid used in our experiments was approximately 2.67 mg/Kg. However, this included the unlabeled bromocapric acid as well. Although the unlabeled bromocapric acid was not detected during gamma counting of the samples, it may have influenced the myocardium/blood ratio.
The results of the organ distribution studies for \( ^{131} \text{I}-\text{lauric acid} \), expressed as "percent injected dose per total organ" are presented in Table 10 and Figure 10. Again, a general trend was observed that the concentration of the radiopharmaceutical was the highest immediately following injection, after which there was a decline of radioactivity with time.

The highest myocardial uptake of \( ^{131} \text{I}-\text{lauric acid} \) was within one minute after injection. The decrease in radioactivity with time within the myocardium was again observed to occur in an exponential manner, being similar to that of \( ^{131} \text{I}-\text{capric acid} \). It can be assumed that both \( ^{131} \text{I}-\text{lauric acid} \) and \( ^{131} \text{I}-\text{capric acid} \) are cleared in the same manner from the heart, since the fatty acids differed only by 2 carbon units in the hydrocarbon chain. Initial myocardial extraction presumably accounted for the peak concentration immediately following injection. Subsequently, a portion of the \( ^{131} \text{I}-\text{lauric acid} \) was probably metabolized by Beta oxidation (Oliver, 1976; Spitzer and Spitzer, 1971). The fatty acid not metabolized was presumably incorporated into triglycerides or other phospholipids (Evans et al., 1965).

The \( ^{131} \text{I}-\text{lauric acid} \) uptake by the myocardium and the lungs were almost identical, and the decline of the radioactivity with time was almost indistinguishable. This suggested that there may have been no significant preferential uptake of the \( ^{131} \text{I}-\text{lauric acid} \) by the myocardium, or that the \( ^{131} \text{I}-\text{lauric acid} \) was being retained by the lungs.
## TABLE X

Total Uptake of $^{131}$I-Lauric Acid by the Spleen, Stomach, Intestines, Muscle, and Bone of Mice $^a,b$

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen</th>
<th>Stomach</th>
<th>Intestines</th>
<th>Muscle $^c$</th>
<th>Bone $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>.017</td>
<td>.41±.03</td>
<td>.81±.10</td>
<td>4.74±.11</td>
<td>29.28±.84</td>
<td>4.34±.32</td>
</tr>
<tr>
<td>.033</td>
<td>.37±.07</td>
<td>1.50±.14</td>
<td>4.46±.23</td>
<td>24.43±1.17</td>
<td>4.27±.28</td>
</tr>
<tr>
<td>.050</td>
<td>.31±.02</td>
<td>1.25±.12</td>
<td>3.10±.10</td>
<td>17.54±1.23</td>
<td>3.52±.21</td>
</tr>
<tr>
<td>.067</td>
<td>.37±.02</td>
<td>1.54±.18</td>
<td>3.64±.15</td>
<td>23.73±1.15</td>
<td>4.59±.25</td>
</tr>
<tr>
<td>.083</td>
<td>.29±.03</td>
<td>1.60±.18</td>
<td>2.77±.23</td>
<td>16.56±.72</td>
<td>3.39±.13</td>
</tr>
<tr>
<td>.100</td>
<td>.36±.05</td>
<td>1.94±.15</td>
<td>3.91±.17</td>
<td>21.83±1.09</td>
<td>4.45±.30</td>
</tr>
<tr>
<td>.133</td>
<td>.47±.06</td>
<td>2.50±.36</td>
<td>4.00±.26</td>
<td>21.64±1.47</td>
<td>4.39±.28</td>
</tr>
<tr>
<td>.167</td>
<td>.28±.02</td>
<td>2.64±.43</td>
<td>3.80±.28</td>
<td>17.61±.86</td>
<td>3.58±.17</td>
</tr>
<tr>
<td>.250</td>
<td>.34±.03</td>
<td>3.82±.49</td>
<td>3.02±.19</td>
<td>15.38±.63</td>
<td>3.24±.14</td>
</tr>
<tr>
<td>.333</td>
<td>.27±.04</td>
<td>2.21±.26</td>
<td>2.30±.13</td>
<td>12.79±.65</td>
<td>2.60±.09</td>
</tr>
<tr>
<td>.417</td>
<td>.23±.03</td>
<td>2.08±.33</td>
<td>2.08±.14</td>
<td>11.57±.88</td>
<td>2.39±.17</td>
</tr>
<tr>
<td>.500</td>
<td>.24±.02</td>
<td>4.05±.95</td>
<td>2.31±.24</td>
<td>11.76±1.17</td>
<td>2.43±.17</td>
</tr>
<tr>
<td>.667</td>
<td>.19±.02</td>
<td>3.48±.14</td>
<td>2.28±.12</td>
<td>11.37±1.03</td>
<td>2.20±.11</td>
</tr>
<tr>
<td>.833</td>
<td>.21±.04</td>
<td>3.97±.82</td>
<td>2.60±.39</td>
<td>11.86±1.54</td>
<td>2.57±.29</td>
</tr>
<tr>
<td>1.000</td>
<td>.16±.02</td>
<td>2.08±.44</td>
<td>1.46±.17</td>
<td>7.29±.78</td>
<td>1.44±.12</td>
</tr>
<tr>
<td>2.000</td>
<td>.20±.02</td>
<td>5.84±.71</td>
<td>1.63±.13</td>
<td>8.24±.32</td>
<td>1.87±.09</td>
</tr>
<tr>
<td>3.000</td>
<td>.19±.03</td>
<td>4.77±1.01</td>
<td>1.63±.18</td>
<td>8.07±.97</td>
<td>1.86±.21</td>
</tr>
<tr>
<td>4.000</td>
<td>.05±.01</td>
<td>1.07±.30</td>
<td>.75±.14</td>
<td>5.02±1.02</td>
<td>.66±.06</td>
</tr>
<tr>
<td>6.000</td>
<td>.07±.01</td>
<td>1.16±.17</td>
<td>.72±.06</td>
<td>3.23±.39</td>
<td>.71±.11</td>
</tr>
<tr>
<td>12.000</td>
<td>.01±.001</td>
<td>.06±.004</td>
<td>.18±.01</td>
<td>2.56±.45</td>
<td>.35±.04</td>
</tr>
<tr>
<td>24.000</td>
<td>.02±.001</td>
<td>.08±.02</td>
<td>.11±.02</td>
<td>1.31±.64</td>
<td>.35±.06</td>
</tr>
</tbody>
</table>

$^a$Expressed as percent of $^{131}$I-lauric acid per total organ.

$^b$Mean values for five mice ± standard error of the mean.

$^c$Total muscle was calculated on 45 percent of body weight (Stand et al., 1962).

$^d$Total bone was calculated as 6 percent of body weight (Stand et al., 1962).
Figure 10  Total Organ Uptake of 12-Iodolauric Acid by Liver, Heart, Lung, Kidneys, and Blood in Mice

Radioactivity levels have been determined even after metabolism of the fatty acid.
The decline of the total blood concentration of $^{131}$I-lauric acid appeared to be very sporadic, with peaks occurring at 0.1 hours, 0.5 hours, and at 2-3 hours. Again, a rapid blood clearance of the $^{131}$I-lauric acid was noted, as with $^{131}$I-capric acid. However, the total blood concentration seemed to fluctuate much more than the $^{131}$I-capric acid. The hepatic concentration of 12-iodolauric acid fluctuated in a manner parallel to that of the blood, thus implying that the concentration in the liver was due to diffusion of the $^{131}$I-lauric acid rather than by selective retention (Perlman et al., 1941). The kidney concentration was again variable with peak levels noted at 2-3 hours. The $^{131}$I-lauric acid was rapidly cleared by the blood as well as the spleen, the intestines, the muscle, and the bone. The concentration of radioactivity within these organs were less than 1% by 24 hours after injection. The concentration of $^{131}$I-lauric acid by the intestines was somewhat higher than the value observed for $^{131}$I-capric acid, 4.74% compared to 2.95% at 0.017 hour respectively. This difference could possibly be attributed to the preparations themselves, since it would appear that the concentration of the $^{131}$I-lauric acid was higher in the muscle, the bone, as well as the spleen for the time intervals observed than the $^{131}$I-capric acid.

The uptake by the stomach was highest between 2-3 hours after injection, although a peak level was also noted at 0.25 hours and at 0.5 hours. This is in agreement with the hypothesis that it is the free Iodide-$^{131}$ which is excreted by the gastric mucosa (Nelson et al., 1947). The free Iodide-$^{131}$ is probably the result of hydrolysis of iodoacetate, the end product of Beta oxidation of the iodofatty acid (Robinson and Lee, 1975).
In Table 11 and Figure 11, the results of the "percent injected dose per gram of organ" are shown. The initial uptake at 1 minute by the heart was 13.2%, compared to 11% for the liver. The observed decline of $^{131}$I-laury acid per gram of tissue for the heart, the liver, the lungs, and the kidneys was very similar. The values were clustered, following a general trend with the decline of radioactivity in the blood with time. In view of the close anatomical proximity of these organs, it would be very difficult to clearly identify the myocardium on a scan.

The "percent injected dose per gram organ/percent injected dose per ml blood" was also calculated. (Table 12 and Figure 12). The heart had the highest uptake of $^{131}$I-laury acid at the commencement of the studies, followed by the lung, the liver, and the kidneys. However, the results were very similar and appeared to be clustered. There was a general decline of the $^{131}$I-laury acid with time, until 3 hours post-injection. After 3 hours, the organ/blood ratios increased as the radioactivity was cleared from the blood. An exception to this was the kidneys, which showed a very high concentration of radioactivity at 2-3 hours after injection. The reason for this is not understood.

The organ/blood ratios for the spleen, the intestines, the muscle, and the bone were relatively low and showed an almost identical increase with time. The highest ratios for the spleen and stomach, however, occurred at 2 hours after injection. The other organs or tissues showed a continued increase in organ/blood ratios as the blood was cleared of the radioactivity.
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen</th>
<th>Stomach</th>
<th>Intestines</th>
<th>Muscle</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>.017</td>
<td>2.41±.23</td>
<td>3.25±.35</td>
<td>2.67±.11</td>
<td>2.16±.13</td>
<td>2.51±.17</td>
</tr>
<tr>
<td>.033</td>
<td>3.01±.15</td>
<td>6.07±.41</td>
<td>2.46±.21</td>
<td>2.07±12</td>
<td>2.72±.22</td>
</tr>
<tr>
<td>.050</td>
<td>2.23±.08</td>
<td>5.02±.65</td>
<td>1.80±.06</td>
<td>1.37±.10</td>
<td>2.17±.11</td>
</tr>
<tr>
<td>.067</td>
<td>2.91±.11</td>
<td>5.85±.73</td>
<td>2.18±.08</td>
<td>1.84±.17</td>
<td>2.65±.20</td>
</tr>
<tr>
<td>.083</td>
<td>2.17±.10</td>
<td>5.02±.82</td>
<td>1.67±.05</td>
<td>1.39±.06</td>
<td>2.13±.07</td>
</tr>
<tr>
<td>.100</td>
<td>2.70±.10</td>
<td>6.97±.52</td>
<td>1.95±.11</td>
<td>1.60±.10</td>
<td>2.45±.22</td>
</tr>
<tr>
<td>.133</td>
<td>2.62±.10</td>
<td>9.13±1.10</td>
<td>1.89±.07</td>
<td>1.60±.09</td>
<td>2.45±.14</td>
</tr>
<tr>
<td>.167</td>
<td>2.31±.17</td>
<td>9.32±1.94</td>
<td>1.72±.14</td>
<td>1.38±.10</td>
<td>2.10±.16</td>
</tr>
<tr>
<td>.250</td>
<td>2.19±.19</td>
<td>12.99±.84</td>
<td>1.48±.08</td>
<td>1.16±.05</td>
<td>1.83±.09</td>
</tr>
<tr>
<td>.333</td>
<td>1.72±.07</td>
<td>10.48±1.33</td>
<td>1.38±.03</td>
<td>1.06±.04</td>
<td>1.62±.07</td>
</tr>
<tr>
<td>.417</td>
<td>1.45±.10</td>
<td>10.42±.94</td>
<td>1.14±.06</td>
<td>1.14±.07</td>
<td>1.47±.12</td>
</tr>
<tr>
<td>.500</td>
<td>1.58±.14</td>
<td>14.29±2.05</td>
<td>1.28±.12</td>
<td>.91±.10</td>
<td>1.41±.14</td>
</tr>
<tr>
<td>.667</td>
<td>1.47±.21</td>
<td>12.71±.35</td>
<td>1.12±.07</td>
<td>.89±.09</td>
<td>1.29±.05</td>
</tr>
<tr>
<td>.833</td>
<td>1.58±.19</td>
<td>14.40±.25</td>
<td>1.18±.12</td>
<td>.89±.11</td>
<td>1.46±.18</td>
</tr>
<tr>
<td>1.000</td>
<td>1.11±.10</td>
<td>8.09±1.36</td>
<td>.94±.06</td>
<td>.74±.05</td>
<td>.99±.09</td>
</tr>
<tr>
<td>2.000</td>
<td>1.21±.13</td>
<td>20.92±3.21</td>
<td>.92±.10</td>
<td>.62±.03</td>
<td>1.06±.05</td>
</tr>
<tr>
<td>3.000</td>
<td>1.15±.14</td>
<td>16.28±2.27</td>
<td>.85±.12</td>
<td>.58±.08</td>
<td>1.01±.12</td>
</tr>
<tr>
<td>4.000</td>
<td>.34±.06</td>
<td>4.31±1.47</td>
<td>.48±.12</td>
<td>.42±.08</td>
<td>.58±.16</td>
</tr>
<tr>
<td>6.000</td>
<td>.40±.06</td>
<td>4.11±.56</td>
<td>.38±.04</td>
<td>.24±.02</td>
<td>.40±.06</td>
</tr>
<tr>
<td>12.000</td>
<td>.079±.01</td>
<td>.24±.02</td>
<td>.08±.01</td>
<td>.19±.03</td>
<td>.20±.02</td>
</tr>
<tr>
<td>24.000</td>
<td>.12±.01</td>
<td>.30±.05</td>
<td>.06±.01</td>
<td>.18±.04</td>
<td>.19±.03</td>
</tr>
</tbody>
</table>

a Expressed as percent of injected $^{131}$I-Lauric acid per gram organ weight.

b Mean value from five mice ± standard error of the mean.
Radioactivity levels have been determined even after metabolism of the fatty acid.
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen Blood</th>
<th>Stomach Blood</th>
<th>Intestines Blood</th>
<th>Muscle Blood</th>
<th>Bone Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>.016</td>
<td>.23±.03</td>
<td>.31 ±.03</td>
<td>.26±.01</td>
<td>.21±.01</td>
<td>.24±.01</td>
</tr>
<tr>
<td>.033</td>
<td>.45±.05</td>
<td>.93 ±.14</td>
<td>.36±.04</td>
<td>.32±.04</td>
<td>.42±.07</td>
</tr>
<tr>
<td>.050</td>
<td>.36±.01</td>
<td>.80 ±.08</td>
<td>.29±.01</td>
<td>.22±.02</td>
<td>.35±.01</td>
</tr>
<tr>
<td>.067</td>
<td>.44±.02</td>
<td>.88 ±.11</td>
<td>.33±.01</td>
<td>.28±.01</td>
<td>.40±.02</td>
</tr>
<tr>
<td>.083</td>
<td>.42±.00</td>
<td>.95 ±.11</td>
<td>.32±.01</td>
<td>.27±.02</td>
<td>.41±.01</td>
</tr>
<tr>
<td>.100</td>
<td>.49±.04</td>
<td>1.26 ±.14</td>
<td>.35±.01</td>
<td>.29±.02</td>
<td>.44±.75</td>
</tr>
<tr>
<td>.133</td>
<td>.50±.02</td>
<td>1.73 ±.17</td>
<td>.36±.02</td>
<td>.31±.02</td>
<td>.48±.03</td>
</tr>
<tr>
<td>.167</td>
<td>.43±.03</td>
<td>1.70 ±.28</td>
<td>.32±.01</td>
<td>.26±.01</td>
<td>.40±.02</td>
</tr>
<tr>
<td>.250</td>
<td>.49±.03</td>
<td>2.90 ±.16</td>
<td>.33±.01</td>
<td>.26±.02</td>
<td>.41±.02</td>
</tr>
<tr>
<td>.330</td>
<td>.48±.01</td>
<td>2.61 ±.08</td>
<td>.39±.01</td>
<td>.31±.02</td>
<td>.46±.02</td>
</tr>
<tr>
<td>.417</td>
<td>.54±.02</td>
<td>4.00 ±.49</td>
<td>.43±.02</td>
<td>.36±.03</td>
<td>.55±.04</td>
</tr>
<tr>
<td>.500</td>
<td>.49±.02</td>
<td>4.36 ±.46</td>
<td>.40±.01</td>
<td>.28±.02</td>
<td>.51±.06</td>
</tr>
<tr>
<td>.667</td>
<td>.49±.04</td>
<td>4.39 ±.36</td>
<td>.38±.01</td>
<td>.30±.02</td>
<td>.44±.03</td>
</tr>
<tr>
<td>.833</td>
<td>.58±.02</td>
<td>5.26 ±.78</td>
<td>.44±.02</td>
<td>.33±.01</td>
<td>.54±.03</td>
</tr>
<tr>
<td>1.000</td>
<td>.55±.03</td>
<td>4.14 ±.80</td>
<td>.48±.04</td>
<td>.34±.04</td>
<td>.50±.04</td>
</tr>
<tr>
<td>2.000</td>
<td>.60±.05</td>
<td>10.31 ±.95</td>
<td>.46±.03</td>
<td>.32±.03</td>
<td>.54±.04</td>
</tr>
<tr>
<td>3.000</td>
<td>.58±.03</td>
<td>8.57 ±1.38</td>
<td>.43±.02</td>
<td>.30±.02</td>
<td>.53±.01</td>
</tr>
<tr>
<td>4.000</td>
<td>.46±.12</td>
<td>5.12 ±1.17</td>
<td>.61±.08</td>
<td>.54±.07</td>
<td>.73±.13</td>
</tr>
<tr>
<td>6.000</td>
<td>.55±.06</td>
<td>5.72 ± .59</td>
<td>.53±.03</td>
<td>.34±.02</td>
<td>.56±.08</td>
</tr>
<tr>
<td>12.000</td>
<td>.55±.04</td>
<td>1.72 ± .05</td>
<td>.58±.03</td>
<td>1.37±.19</td>
<td>1.46±.23</td>
</tr>
<tr>
<td>24.000</td>
<td>1.64±.18</td>
<td>3.93 ± .69</td>
<td>.86±.18</td>
<td>2.54±.74</td>
<td>2.66±.61</td>
</tr>
</tbody>
</table>

a Expressed as a ratio of percent injected dose per gm of organ to percent injected dose per ml of blood.

b Mean values from five mice ± standard error of the mean.
Figure 12  Organ/Blood Distribution of 12-Iodolauryc Acid by Liver, Heart, Lung and Kidneys in Mice

Radioactivity levels have been determined even after metabolism of the fatty acid.
In reviewing these results, it does not appear that 12-iodolauric acid would be an ideal myocardial scanning agent, although the initial uptake by the myocardium may allow visualization. It would appear questionable if the heart could be differentiated from the lungs and the liver due to the similar concentrations of radiopharmaceutical within each organ.

c) NaI-131 (6% HSA)

NaI-131 in a 6% solution of human serum albumin was investigated to observe its biological behavior in comparison to the radioiodinated fatty acids. The NaI-131 was dissolved in the 6% HSA to provide the same vehicle for injection as the radioiodinated fatty acids. Since it was believed that Iodide-131 was released by hydrolysis of the metabolic product of Beta oxidation, the distribution studies should be similar in the latter stages of the study.

The results of the "percent injected dose per total organ" are shown in Table 13 and Figure 13. The blood level of NaI-131 was extremely high and showed a steady decrease with time. This blood level was higher than that observed for the two radioiodinated fatty acids. The myocardial concentration was lower than the concentration in the liver, the kidney, as well as the lungs. In the 6 hour study, the myocardial level of NaI-131 appeared to be constant. This was also true for the radioactivity in the lungs, which was almost indistinguishable from that of the heart. The kidney concentration of the NaI-131 did not fluctuate noticeably but decreased gradually with time. The hepatic concentration peaked at .033 hours, after which there was a gradual
TABLE XIII

Total Uptake of NaI-131(6% HSA) by the Spleen, Stomach, Intestines, Muscle, and Bone of Mice \(^a\),\(^b\)

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen</th>
<th>Stomach</th>
<th>Intestines</th>
<th>Muscle(^c)</th>
<th>Bone(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.016</td>
<td>.37±.04</td>
<td>.82±.07</td>
<td>2.79±.21</td>
<td>17.92±.82</td>
<td>4.28±.16</td>
</tr>
<tr>
<td>.033</td>
<td>.33±.03</td>
<td>.97±.08</td>
<td>3.52±.77</td>
<td>15.84±.97</td>
<td>3.69±.09</td>
</tr>
<tr>
<td>.050</td>
<td>.32±.03</td>
<td>1.16±.08</td>
<td>2.26±.16</td>
<td>15.55±.41</td>
<td>4.06±.22</td>
</tr>
<tr>
<td>.083</td>
<td>.32±.02</td>
<td>1.51±.17</td>
<td>1.82±.07</td>
<td>14.05±.50</td>
<td>3.55±.10</td>
</tr>
<tr>
<td>.167</td>
<td>.22±.01</td>
<td>2.12±.43</td>
<td>1.73±.11</td>
<td>12.72±.38</td>
<td>3.43±.32</td>
</tr>
<tr>
<td>.500</td>
<td>.21±.01</td>
<td>1.81±.27</td>
<td>1.44±.11</td>
<td>7.95±.37</td>
<td>1.88±.03</td>
</tr>
<tr>
<td>1.000</td>
<td>.18±.03</td>
<td>1.77±.19</td>
<td>1.19±.07</td>
<td>6.51±.31</td>
<td>1.58±.05</td>
</tr>
<tr>
<td>4.000</td>
<td>.09±.01</td>
<td>1.34±.25</td>
<td>.68±.04</td>
<td>3.40±.56</td>
<td>.81±.09</td>
</tr>
<tr>
<td>6.000</td>
<td>.06±.01</td>
<td>1.12±.31</td>
<td>.59±.11</td>
<td>2.82±.37</td>
<td>.72±.13</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as percent of \(^{131}\)I-HSA per total organ

\(^b\) Mean values from five mice ± standard deviation of the mean

\(^c\) Total muscle was calculated on 45 percent of body weight (Stand et al., 1962)

\(^d\) Total bone was calculated as 6 percent of body weight (Stand et al., 1962)
Figure 13  Total Organ Uptake of NaI-131(HSA) by Liver, Heart, Lung, Kidneys, and Blood in Mice

Radioactivity levels have been determined even after metabolism of the fatty acid.
decrease in concentration of NaI-131.

The stomach concentration of NaI-131 peaked at .167 hours, and then decreased slowly. This peak probably represented excretion of Iodide-131 into the gastic juices (Myant et al., 1950). The muscle, the bone, the intestines, and the spleen showed no affinity for the radioiodide, and the decrease in the organs was rapid, showing less than 1% of the injected dose at 6 hours, with the exception of the muscle.

Table 14 and Figure 14 represented the "percent injected dose per gram organ" or the "percent injected dose per ml blood". The blood concentration was higher than that of the other organs. The kidney accumulated a peak concentration of 6.5% per gram organ at .033 and .050 hours. This was probably due to the blood flow to the kidneys. In the kidney, the iodide is filtered by the glomeruli and about 73% is reabsorbed by the tubules (Myant et al., 1950; Perlman et al., 1941). The kidney concentration was followed by the concentration of NaI-131 by the lungs, the heart, and then the liver. The relative organ uptake was similar for the bone and the spleen, in which radioactivity also decreased in a similar manner. For the spleen, the intestines, as well as the bone, the calculated percent injected dose per gram was less than .61% by 6 hours. The exception was the concentration of the NaI-131 by the stomach. Although the peak level was observed at .5 hours, the concentration was still 5.79% at 6 hours after injection.

The distribution studies showing "percent injected dose per gram organ/percent injected dose per ml blood" are depicted in Table 15 and Figure 15. The kidneys showed the highest organ/blood
TABLE XIV

Relative Organ Uptake of NaI-131 (5% HSA) in Mice\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen</th>
<th>Stomach</th>
<th>Intestines</th>
<th>Muscle</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>.016</td>
<td>2.47±.14</td>
<td>3.87±.40</td>
<td>1.85±.07</td>
<td>1.34±.09</td>
<td>2.74±.12</td>
</tr>
<tr>
<td>.033</td>
<td>2.09±.06</td>
<td>4.40±.47</td>
<td>3.07±.70</td>
<td>1.38±.06</td>
<td>2.43±.10</td>
</tr>
<tr>
<td>.050</td>
<td>2.43±.15</td>
<td>5.73±.20</td>
<td>1.78±.05</td>
<td>1.35±.02</td>
<td>2.61±.14</td>
</tr>
<tr>
<td>.083</td>
<td>2.17±.08</td>
<td>6.37±.46</td>
<td>1.48±.04</td>
<td>1.18±.04</td>
<td>2.24±.03</td>
</tr>
<tr>
<td>.167</td>
<td>2.07±.11</td>
<td>9.94±1.39</td>
<td>1.61±.04</td>
<td>1.14±.02</td>
<td>2.33±.26</td>
</tr>
<tr>
<td>.500</td>
<td>1.56±.11</td>
<td>8.72±1.01</td>
<td>1.11±.07</td>
<td>.69±.04</td>
<td>1.21±.04</td>
</tr>
<tr>
<td>1.000</td>
<td>1.24±.03</td>
<td>8.87±1.19</td>
<td>.99±.05</td>
<td>.57±.03</td>
<td>1.04±.04</td>
</tr>
<tr>
<td>4.000</td>
<td>.60±.07</td>
<td>5.69±.92</td>
<td>.49±.03</td>
<td>.29±.05</td>
<td>.53±.06</td>
</tr>
<tr>
<td>6.000</td>
<td>.61±.09</td>
<td>5.79±1.38</td>
<td>.58±.07</td>
<td>.27±.07</td>
<td>.53±.08</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Expressed as percent of injected 131\textsuperscript{I}-HSA per gm of organ weight.

\textsuperscript{b}Mean value from five mice ± standard error of the mean.
Radioactivity levels have been determined even after metabolism of the fatty acid.
TABLE XV

Organ/Blood Distribution of NaI-131 (6% HSA) in Mice \(^a,b\)

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spleen Blood</th>
<th>Stomach Blood</th>
<th>Intestines Blood</th>
<th>Muscle Blood</th>
<th>Bone Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>.016</td>
<td>.23±.02</td>
<td>.36±.03</td>
<td>.18±.00</td>
<td>.13±.01</td>
<td>.26±.01</td>
</tr>
<tr>
<td>.033</td>
<td>.25±.01</td>
<td>.53±.05</td>
<td>.37±.09</td>
<td>.17±.01</td>
<td>.30±.01</td>
</tr>
<tr>
<td>.050</td>
<td>.29±.02</td>
<td>.68±.03</td>
<td>.21±.01</td>
<td>.16±.01</td>
<td>.31±.02</td>
</tr>
<tr>
<td>.083</td>
<td>.33±.02</td>
<td>.97±.08</td>
<td>.22±.00</td>
<td>.18±.01</td>
<td>.34±.00</td>
</tr>
<tr>
<td>.167</td>
<td>.36±.01</td>
<td>1.73±.24</td>
<td>.28±.01</td>
<td>.20±.00</td>
<td>.40±.04</td>
</tr>
<tr>
<td>.500</td>
<td>.42±.02</td>
<td>2.35±.27</td>
<td>.29±.01</td>
<td>.19±.01</td>
<td>.33±.00</td>
</tr>
<tr>
<td>1.000</td>
<td>.41±.01</td>
<td>2.92±.37</td>
<td>.33±.01</td>
<td>.19±.01</td>
<td>.34±.01</td>
</tr>
<tr>
<td>4.000</td>
<td>.39±.02</td>
<td>3.64±.37</td>
<td>.33±.03</td>
<td>.19±.02</td>
<td>.34±.01</td>
</tr>
<tr>
<td>6.000</td>
<td>.38±.03</td>
<td>3.44±.53</td>
<td>.35±.02</td>
<td>.17±.01</td>
<td>.32±.03</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as a ratio of percent injected dose per gm of organ to percent injected dose per ml of blood.

\(^b\) Mean values from five mice ± standard error of the mean.
Radioactivity levels have been determined even after metabolism of the fatty acid.
ratio, which was followed by the lungs, the heart, and then the liver. This could be expected as the kidneys are highly vascular and would filter the Iodide-131 presented by the blood. The organ/blood ratios for the spleen, the intestines, the muscle, and the bone were relatively constant in the 6 hour study. The stomach/blood ratio peaked at 4 hours, and then decreased.

d) **Comparative Myocardial Uptake of $^{131}$I-Capric Acid, $^{131}$I-Lauric Acid, and NaI-131 (6% HSA)**

It is apparent from Figure 16 that the radioiodinated fatty acids were concentrated by the heart in significantly higher quantities than the NaI-131 (6% HSA). The higher uptake of the radioiodinated fatty acids by the heart was evident up to .1 hours post-injection, after which the rate of decline of all three compounds closely resembled one another. This was to be expected since one of the metabolic end products of the Beta oxidation of iodofatty acids was iodoacetate, which was liberated into the blood stream (Robinson and Lee, 1975). However, the iodoacetate is reactive and unstable due mainly to the weakness of the carbon-iodine bond (Webb, 1966). Decomposition occurs releasing iodide ions and iodine. This released Iodide-131 behaved in an identical manner to the NaI-131 (6% HSA), thus confirming that it was the existence of the iodide ion which accounted for the biological distribution of the iodofatty acids after the first .1 hour.

In Figure 17, a comparable decline in radiopharmaceutical
Radioactivity levels have been determined even after metabolism of the fatty acid.
Figure 17  Relative Myocardial Uptake of 10-Iododecanoic Acid, 12-Iodolauric Acid, and NaI-131(HSA) in Mice\textsuperscript{a}

\textsuperscript{a}Radioactivity levels have been determined even after metabolism of the fatty acid.
concentration per gram of myocardium could be seen. The higher myocardial uptake of radioiodinated fatty acids was again evident in comparison to NaI-131, thus indicating the preferential uptake of fatty acids by the heart. It appeared that 12-iodolauric acid had a higher initial myocardial concentration than the 10-iodocapric acid although this difference was no longer evident at .05 hours.

Figure 18 graphically indicated the differences of the myocardial/blood ratios for the $^{131}$I-capric acid, the $^{131}$I-lauric acid, and the NaI-131 (6% HSA). The figure clearly depicted the superior myocardial/blood ratios of the radioiodinated fatty acids. The ratios for the $^{131}$I-capric acid and $^{131}$I-lauric acid were similar, showing decreasing ratios until 1 hour after injection. From 1 hour, the myocardium/blood ratios increased as the blood was cleared of radioactivity.

For a myocardial scanning agent, the localization of the radionuclide as well as the target/non-target ratios are equally important. From the distribution studies, it was evident that both radioiodinated fatty acids appeared to be extracted by the heart to a greater extent than the NaI-131(6% HSA). While the myocardial concentration of 12-iodolauric acid appeared higher than the 10-iodocapric acid, the myocardium/blood ratios were similar. In addition, the similar concentration of 12-iodolauric acid by the liver and the lung compared to the heart could present potential problems in myocardial scanning.

The differences noted in the distribution studies did not
Radioactivity levels have been determined even after metabolism of the fatty acid.
appear conclusive as to which iodofatty acid would be superior for myocardial scanning. Therefore it was decided to proceed with further studies using both radioiodinated fatty acids. In these distribution studies the molar ratios of fatty acid/albumin were approximately 3. This was calculated on the basis of the total bromofatty acid used in the final volume of 6% human serum albumin. The molar ratios used were within physiologic limits and should not have interfered with myocardial extraction.

5. **Toxicity Studies**

a) 12-Bromolauroic Acid

The sodium bromolauroate solution used had a final concentration of 10mg/ml and a molar ratio of 24.35:1 of fatty acid to albumin. At least 7 groups of mice were used to study the toxicity of 12-bromolauroic acid. Each group consisted of a minimum of 6 mice. The control groups also consisted of 6 mice. The mice were observed immediately after injection and for a period of 7 succeeding days to observe any toxic effects which may not manifest itself immediately. However, it appeared that if the mouse survived for the first 5 minutes after the injection, it would not die and would appear to be normal.

For sodium bromolauroate, the injection volumes varied from .25 ml to .50 ml. Since the injection volumes were inconsistent, samples of 10% human serum albumin equivalent to the largest volume administered was injected into a control group. This was to ascertain that any toxic effects observed were not due to the volume injected.
The volume acceptable for rapid intravenous injection varies from .10 ml - .50 ml for rodents (Balaz, 1970). Therefore, the maximum dose injected was within the acceptable volume range. The control group of mice demonstrated no adverse effects when injected with the maximum volume of .50 ml. The injection was made over a period of 10 seconds. The results of the toxicity study for sodium bromolaurate are given in Table 16, according to the method of Litchfield and Wilcoxin (1949).

The value of \( \chi^2 \) for 4 degrees of freedom was 9.49. Since 2.72 is less than 9.49, the data was not significantly heterogeneous.

The \( L_{D16} \) was 183 mg/Kg; the \( L_{D50} \) was 210 mg/Kg; while the \( L_{D84} \) was 236 mg/Kg. The slope function \( s \) was computed as:

\[
S = \frac{L_{D84}/L_{D50} + L_{D50}/L_{D16}}{2} = \frac{236/210 + 210/183}{2} = 1.14
\]

The total number of animals tested between the expected effects of 16% - 84% was designated \( N' \) and consisted of 20 mice.

The \( f(L_{D50}) \) was calculated by the formula:

\[
S^2 \sqrt{N'} = s^{\text{exponent}} = 1.14^{(0.6194)} = 1.0846
\]

The upper limits and lower limits for the 95% confidence level were calculated by \( (L_{D50}) \times f(L_{D50}) \) and \( (L_{D50})/f(L_{D50}) \) respectively. The upper limit was found to be 228 mg/Kg while the lower limit was found to be 194 mg/Kg. Therefore, the intravenous \( L_{D50} \) of sodium bromolaurate was 210 mg/Kg (194mg/Kg - 228 mg/Kg).
TABLE XVI

Determination of the Intravenous LD\textsubscript{50} for Na-Bromolaurate in 10\% Human Serum Albumin Solution in Mice

<table>
<thead>
<tr>
<th>Dose mg/Kg</th>
<th>No. dead</th>
<th>Observed % Dead</th>
<th>Expected % Dead</th>
<th>No. Observed</th>
<th>No. Expected</th>
<th>Contribution to (Chi){\textsuperscript{2}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>139</td>
<td>0/6</td>
<td>0 (1.15)</td>
<td>.05</td>
<td>.10</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>167</td>
<td>0/6</td>
<td>0 (1.1)</td>
<td>3.3</td>
<td>2.2</td>
<td>.015</td>
<td></td>
</tr>
<tr>
<td>175</td>
<td>1/6</td>
<td>16.7</td>
<td>7.5</td>
<td>9.2</td>
<td>.120</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>5/10</td>
<td>50</td>
<td>37.0</td>
<td>13.0</td>
<td>.105</td>
<td></td>
</tr>
<tr>
<td>225</td>
<td>6/10</td>
<td>60</td>
<td>72.0</td>
<td>12.0</td>
<td>.095</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>6/6'</td>
<td>100 (97.7)</td>
<td>93.0</td>
<td>4.7</td>
<td>.034</td>
<td></td>
</tr>
</tbody>
</table>

Total No. of Animals = 44

Degrees of freedom N=K-2=4

No. of doses K=6

Animals/dose = 7.33

\((\text{Chi})^2 = 7.33 \times .371 = 2.72\)
The sodium salt of bromolauric acid can be considered a relatively toxic substance since the LD$_{50}$ was found to be 210 mg/Kg as compared to 750 mg/Kg for non-toxic compounds (Christianson, 1973). The published LD$_{50}$ for intravenously administered lauric acid is 131 mg/Kg (Oro and Wretlind, 1961). There is no LD$_{50}$ value listed for bromolauric acid. It must be remembered that the results obtained by Oro and Wretlind (1961) were for a 2% emulsion of lauric acid in cottonseed oil. This lower toxicity observed in our experimental results could probably be attributed in part to the vehicle used. While albumin solution can be considered physiological, cottonseed oil cannot be considered physiological, although cottonseed oil has shown little or no toxic effects during intravenous injections into mice (Oro and Wretlind, 1961).

For sodium bromolaurate, the first indications of toxicity included slackening of the limbs and loss of the righting reflex, accompanied by profuse defecation. At the higher injection doses, the mice suffered from palpitations, sedation, twitching, tremors, followed by tonic convulsions which resulted in death.

The toxic effects observed for 2% lauric acid were comparable to those observed for sodium bromolaurate. In both cases, death appeared to be caused by respiratory failure (Balaz, 1970; Oro and Wretlind, 1961). The dyspnea and convulsions were probably secondary to anoxia (Oro and Wretlind, 1961). Massive pulmonary edema has been suggested as the cause of death after administration of oleic acid to dogs (Jefferson and Necheles, 1948). Attempts were made in our experiments to examine the lungs immediately following death of the
mice. Although the lungs were sometimes speckled with blood, the thoracic cavity was not edematous. However, only gross macroscopic appearance was noted and no further microscopic examinations were performed, thus the precise cause of death was difficult to interpret.

Bromoacetate, a metabolite of terminal labeled bromofatty acid metabolism, may also contribute to the toxic effects observed after the administration of the bromofatty acids. The administration of the radioiodinated fatty acid is always accompanied by the simultaneous administration of the parent compound, the bromofatty acid. Iodoacetate, the metabolite of iodofatty acid metabolism, is also a toxic compound. However, only trace quantities are administered so it need not be of great concern. Almost 10 mg of the bromofatty acid could be administered along with the radioiodinated fatty acid. From 10 mg of bromolauric or bromocapric acid, 4.3 mg of bromoacetate may be produced by complete metabolism by Beta oxidation (Robinson, 1977). The bromoacetate is subject to hydrolysis, as is apparent with the iodoacetate, with the major part of the compound being hydrolysed to the halide ion within 10 minutes in the blood (Poe et al., 1976b).

The LD$_{50}$ of bromoacetate has been reported to be 100mg/Kg in white mice forced fed an aqueous solution (Morrison, 1946). It has been found to cause progressive respiratory and cardiac depression (Webb, 1966). Tremors, tonic convulsions, and changes in the muscular system cause weakness and paralysis, followed by death of the animal. Intravenous injections of 50 mg/Kg of bromoacetate has been observed to cause general muscle rigor, followed by death (Webb, 1966).
It is apparent that care must be taken to ensure that the minimal amount of unlabeled bromofatty acid is administered. However, even with the administration of 10 mg of terminal brominated fatty acid, the amount of bromoacetate produced is well below the toxic dose. Robinson (1977) has since devised a method whereby smaller initial quantities of omega bromocarboxylic acids could be used, thus eliminating the administration of unnecessary unlabeled brominated fatty acids.

b) **10-Bromocapric Acid**

The vehicle for the bromocapric acid was distinctly different from 10% HSA, as previously described. The results obtained from these toxicity studies would not necessarily reflect the toxic effects of the bromocapric acid alone. The toxicity of the other components of the vehicle must also be taken into consideration.

A total of 7 groups of mice were used for the increment doses for the toxicity study in addition to the control groups. Each group consisted of a minimum of six mice. The volumes injected varied from .12 ml to .18 ml. Control groups were injected with varying quantities of vehicle without the bromocapric acid up to a volume of .20 ml. The only effect observed was that the mice appeared inactive and sluggish. However, these effects appeared to be transient, with the mice recovering to full activity within a few minutes.

The value of \((\chi^2)\) for 5 degrees of freedom was calculated to be 11.1 (Litchfield and Wilcoxon, 1949). The experimental \((\chi^2)\)
### TABLE XVII

**Determination of the Intravenous LD\(_{50}\) for 10-Bromocapric Acid Solution in Mice**

<table>
<thead>
<tr>
<th>Dose mg/Kg</th>
<th>No. dead</th>
<th>Observed % Dead</th>
<th>Expected % Dead</th>
<th>No. - No. Observed</th>
<th>Contribution to (Chi)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>71</td>
<td>0/6</td>
<td>0 (.03)</td>
<td>.1</td>
<td>.07</td>
<td>.001</td>
</tr>
<tr>
<td>77</td>
<td>1/10</td>
<td>10</td>
<td>5</td>
<td>5.0</td>
<td>.050</td>
</tr>
<tr>
<td>84</td>
<td>2/6</td>
<td>33.3</td>
<td>33</td>
<td>.30</td>
<td>---</td>
</tr>
<tr>
<td>86</td>
<td>4/10</td>
<td>40.0</td>
<td>40</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>87</td>
<td>8/10</td>
<td>80.0</td>
<td>56</td>
<td>24</td>
<td>.235</td>
</tr>
<tr>
<td>98</td>
<td>9/10</td>
<td>90.0</td>
<td>97.5</td>
<td>7.5</td>
<td>.230</td>
</tr>
<tr>
<td>99</td>
<td>6/6</td>
<td>100.0 (99.7)</td>
<td>99.0</td>
<td>.70</td>
<td>.005</td>
</tr>
</tbody>
</table>

\[
\Sigma = .521
\]

Total No. of Animals = 58  
No. of doses \(K = 7\)  
Animal/dose = 8.29

\[
N = K - 2 = 5  
(\text{Chi})^2 = 8.29 \times .521 = 4.32
\]
was found to be 4.32, which is less than 11.1. Therefore there was not significant heterogeneity in the data. The LD$_{16}$ was 80.9 mg/Kg; the LD$_{50}$ was 86.1 mg/Kg; and the LD$_{84}$ was 92.3 mg/Kg. The slope function (S) was found to be 1.04. The upper and lower limits for the 95% confidence level was calculated to be 89.3 mg/Kg; and 83.0 mg/Kg. Thus, it can be observed that for our particular vehicle, the intravenous lethal dose for 50% of the mice was 86.1 mg/Kg (83.0 mg/Kg-- 89.3 mg/Kg).

The 10-bromocapric acid in this particular vehicle was very toxic as the LD$_{50}$ was observed to be 86.1 mg/Kg. There may be an additive or synergistic effect of the propylene glycol along with the bromocapric acid. However, when considered individually, the highest dose of propylene glycol administered was well below that of the minimum lethal dose (Latven and Molitier, 1939). However, this formula was maintained as an alteration of the components led to separation of the phases and a milky solution.

The toxic effects were much more prominent with 10-bromocapric acid than with the 12-bromolauric acid. Twitching and tremors were noted to accompany the lowest dose causing any adverse effects. The muscular system appeared to be affected and the animal often collapsed, although recovery was noted within 3 minutes. As the dosage injected was increased, collapse was prominent, along with muscle rigidity, palpitations, and loss of the righting reflex. Hypernea appeared to be accompanied by choking, and finally tonic convulsions which were followed by death.
The LD$_{50}$ reported for capric acid was 129 mg./Kg (Oro and Wretlind, 1961). This LD$_{50}$ was slightly higher than our experimental results. The discrepancy could not be attributed to species differences, as mice were used in both studies. However, the acids differed in a bromo group, which could attribute to differences in the toxicity. The most probable reason for the different LD$_{50}$ obtained appeared to be the inherent property of the vehicle. Oro and Wretlind (1939) used a 2% emulsion of capric acid in cottonseed oil while our vehicle was a distinctly different aqueous solution. The bromoacetate may also have contributed to the toxic effects of the bromocapric acid, as previously discussed.

6. Excretion Studies

The excretion studies were performed to determine the rate and the route by which the radioiodinated fatty acids were eliminated from the body. Mice were injected with .5-1.0 μCi of the labeled material by tail vein and the activity remaining in the body was determined by whole body counting using the Tubor. The urine and the feces were collected for the first 12 hours after injection to determine the amount excreted by each route.

The geometry study done using the Tubor revealed that the shelf level as well as the axial position influenced the count rates. The horizontal axis was designated A and the vertical axis B. The results are as follows when a 60 μCi sample was counted for 1.0 minute and corrected for background activity.
It was apparent that as the shelf level was lowered, the counts per minute decreased accordingly. Counts obtained from shelf level 5.25 were the highest of the shelf levels, as were the counts obtained in the vertical as opposed to the horizontal position. It was decided to maintain the shelf at the 5.25 level and use the vertical axis for positioning the containers for the whole body counting.

Whole body counting was carried out for $^{131}$I-lauric acid, $^{131}$I-capric acid, and NaI-$^{131}$ (6% HSA). A minimum of 6 and a maximum of 18 mice were used for each data point. The counts obtained were corrected for background activity. During the analysis, any counts

<table>
<thead>
<tr>
<th>Axial Direction</th>
<th>Shelf Level</th>
<th>CPM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.25</td>
<td>98.4</td>
</tr>
<tr>
<td>B</td>
<td>5.25</td>
<td>100.0</td>
</tr>
<tr>
<td>A</td>
<td>5.50</td>
<td>84.1</td>
</tr>
<tr>
<td>B</td>
<td>5.50</td>
<td>85.9</td>
</tr>
<tr>
<td>A</td>
<td>5.75</td>
<td>76.7</td>
</tr>
<tr>
<td>B</td>
<td>5.75</td>
<td>77.0</td>
</tr>
<tr>
<td>A</td>
<td>6.00</td>
<td>65.5</td>
</tr>
<tr>
<td>B</td>
<td>6.00</td>
<td>65.8</td>
</tr>
<tr>
<td>A</td>
<td>6.25</td>
<td>59.7</td>
</tr>
<tr>
<td>B</td>
<td>6.25</td>
<td>60.0</td>
</tr>
<tr>
<td>A</td>
<td>6.50</td>
<td>56.3</td>
</tr>
<tr>
<td>B</td>
<td>6.50</td>
<td>57.0</td>
</tr>
<tr>
<td>A</td>
<td>6.75</td>
<td>53.6</td>
</tr>
<tr>
<td>B</td>
<td>6.75</td>
<td>54.1</td>
</tr>
<tr>
<td>A</td>
<td>7.00</td>
<td>53.2</td>
</tr>
<tr>
<td>B</td>
<td>7.00</td>
<td>52.0</td>
</tr>
<tr>
<td>A</td>
<td>7.25</td>
<td>52.9</td>
</tr>
<tr>
<td>B</td>
<td>7.25</td>
<td>52.2</td>
</tr>
<tr>
<td>A</td>
<td>7.50</td>
<td>53.9</td>
</tr>
<tr>
<td>B</td>
<td>7.50</td>
<td>54.9</td>
</tr>
</tbody>
</table>
which deviated in excess of 3 standard deviations were rejected. The mean for each group of mice at each time interval was calculated along with the standard deviation and the standard error of the mean. However, these counts were not corrected for physical decay.

The excretion data was entered into the U.B.C. Amdahl #470 computer and analyzed using the nonlin program (Metzler et al., 1964). The nonlin program estimates the parameters in the nonlinear system of equations using weighted least squares. The data was weighted by the number of animals per time period/standard deviation squared.

a) $^{131}$I-Capric Acid

The $^{131}$I-capric acid seemed to be eliminated rapidly from the mouse body. At 3, 12, and 24 hours respectively after intravenous injections, 43.7%, 78.4%, and 87.7% had been eliminated from the mouse body.

(i) Compartmental Analysis

In computer analysis, the excretion data was adequately described by three exponentials of time. (Figure 19) The tri-exponential equation can be described as follows:

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Fe^{-\gamma t}$$

$C =$ concentration at time $t$
$A, B, F =$ Y-intercepts of the elimination curve
$\alpha, \beta, \gamma =$ rate constants of the individual exponential lines

The values of $A, B, F, \alpha, \beta, \gamma$ were provided by the computer. Hence, the effective $t_{1/2}$ (half-life) for each component can be
calculated by the term: \( t_{1/2} = \frac{.693}{\text{rate constant}} \)

From the results, there appeared to be three components, a short lived component of 0.90 hours, a medium component of 3.91 hours, and a long component of 74.9 hours. From the y-intercepts of the individual components from the nonlin analysis, it appeared that approximately 36.7% of the injected dose was cleared at the fastest rate, while 61.6% was cleared at the medium rate, and about 14.5% was accounted for by the terminal elimination phase.

Since this is a triexponential curve representing whole body counting, it is very difficult to interpret which processes of elimination each component represents. Whole body counting accounts for the total activity remaining in the mouse body, regardless of the distribution. It appears that as a mixed function, there is some contribution from the first component to the second and the third component. There is also some contribution from the second component to the third component. The first and the second components may represent elimination by urinary excretion, since both these components have relatively short half-lives. This appears to be in agreement with the urinary excretion data, where 57.6% of the injected activity was recovered in the urine at 6 hours after injection. The terminal elimination phase may represent radioactivity which is bound tightly to some tissue and is released very slowly. This tightly bound radioactivity may be thyroidal Iodide-131 or protein bound iodide. This third component probably represents elimination by fecal excretion and only accounts for about 14% of the injected dose.
Radioactivity levels have been determined even after metabolism of the fatty acid.
As defined in biopharmaceutics, the biological half-life is the time required for the plasma, serum, or whole blood to drop to one-half of its value following the establishment of equilibrium (Ritschel, 1972). The loss of drug from plasma, serum, or blood is due to metabolism or excretion into urine or feces. However, in nuclear medicine, the term biological half-life is used to denote the time required by the body to eliminate one-half of the dose of any substance by the regular processes of elimination (Early et al., 1969). From the triexponential whole body excretion curve of $^{131}$I-capric acid, three effective half-lives were calculated. Since a biological half-life is dependent on the physical half-life and the effective half-life, the biological half-life for the terminal elimination phase can be calculated as follows:

$$t_{\text{1/2 (biol)}} = \frac{t_{\text{1/2 (phys)}} \times t_{\text{1/2 (eff)}}}{t_{\text{1/2 (phys)}} - t_{\text{1/2 (eff)}}}$$

For $^{131}$I, $t_{\text{1/2 (phys)}} = 8.05$ days

$$t_{\text{1/2 (eff)}} = 3.12$$

Therefore, the $t_{\text{1/2 (biol)}}$ can be calculated to be 5.11 days.

(ii) Urinary Analysis

From the whole body counting data it was evident that the injected radioactivity was eliminated rapidly from the body. At 24 and 48 hours respectively after intravenous administration, only 12.3% and 9.72% of the injected dose remained in the body. In order to determine the route of elimination, the urine and feces were collected and counted simultaneously with the mice in order to account for total
injected activity. Prepared standards were also counted at the same time so that decay was not necessary. Approximately .02 µCi/gm of $^{131}$I-capric acid was injected into each mouse. The results of these urine and fecal collections are shown in Table 19.

Table XIX

Excretion of $^{131}$I-Capric Acid in Urine and Feces of Mice$^a$

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Urine</th>
<th>Feces</th>
<th>Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>57.6</td>
<td>2.80</td>
<td>39.1</td>
</tr>
<tr>
<td>10</td>
<td>73.0</td>
<td>3.85</td>
<td>29.9</td>
</tr>
<tr>
<td>24</td>
<td>82.4</td>
<td>8.88</td>
<td>11.8</td>
</tr>
</tbody>
</table>

$^a$ The mean of 3 mice calculated as a percent of injected dose.

It can be observed after 24 hours that approximately 91.3% of the injected dose had been eliminated. Of this, 82.4% was excreted in the urine and 8.88% was excreted in the feces. The percent excreted in the feces appears to be high. This could be due to contamination of the feces with urine.

In order to ascertain the chemical species of the radioactive compound excreted, urinary analysis was done. Approximately 10 µl of each individual urine sample was chromatographed on Gelman ITLC-SG sheets and developed in the same solvent system used for radiochemical purity of the radiiodinated fatty acids. For comparison, urine was collected from control mice and dilutions of the urine were made. Individual samples were spiked with NaI-131 (6% HSA), NaI-131, and
\(^{131}\)I-capric acid. In addition, mice were injected with NaI-\(^{131}\)I (6% HSA) and the urine collected. These samples were also spotted and chromatographed. The \(^{131}\)I-capric acid spiked urine sample showed \(R_f\) values of .93 while the NaI-\(^{131}\)I, the NaI-\(^{131}\)I (6%HSA), as well as the urine collected from the mice injected with NaI (6% HSA) showed \(R_f\) values of 0. The free Iodide-\(^{131}\)I ion remained at the origin. The chromatogram of the mice injected with \(^{131}\)I-capric acid showed a spot with a \(R_f\) value of 0. This suggested that the excreted radioactivity was in the form of iodide and that presumably the metabolism of the radioiodinated fatty acid resulted in the urinary excretion of the free Iodide-\(^{131}\)I as the ion. This data appears to be in agreement with the distribution studies, in which it appeared that the end product of Beta oxidation, iodoacetate, diffused from the myocardium and was subsequently hydrolyzed to free iodide in the blood (Poe et al., 1976; Robinson and Lee, 1975).

b) \(^{131}\)I-Lauric Acid

The \(^{131}\)I-lauric acid was eliminated in a similar manner from the mouse body as the \(^{131}\)I-capric acid. At 3, 12, and 24 hours respectively after injection, 42.2%, 68.2%, and 83.4% had been eliminated from the mouse body.

(i) Compartmental Analysis

In the same computer analysis as previously described, the excretion curve fitted into a triexponential equation. The effective half-lives of the three components were found to be 1.67 hours, 7.68 hours, and 71.6 hours. It appeared that 46.6% of the injected dose
Figure 20  Whole-Body Excretion of 12-Iodolauric acid after Intravenous Injection in Mice$^a$

$^a$Radioactivity levels have been determined even after metabolism of the fatty acid.
was cleared by the fastest rate, 38.4% cleared by the medium rate, while 17.8% was cleared by the slowest rate.

It was assumed that the elimination of the \(^{131}\)I-lauric acid was approximately the same as that of the \(^{131}\)I-capric acid from the similar triexponential curves. The first as well as the second component presumably represented a decrease in the whole body radioactivity due to urinary excretion. This appears to agree with the urinary excretion data, in which 53.8% of the injected dose was recovered in the urine at 6 hours after injection. The third component probably represents elimination by fecal excretion. The biological half-life for each component can be calculated from the effective half-life. The effective half-life for \(^{131}\)I-lauric acid for the terminal elimination phase was 71.6 hours. Thus the biological half-life was calculated to be 4.74 days.

(ii) Urinary Analysis

Quantitative urinary and fecal collections were made for the first 24 hours following intravenous injections of the \(^{131}\)I-lauric acid. Approximately .02 \(\mu\)Ci/gm of \(^{131}\)I-lauric acid was injected into each mouse and the results are shown in Table 20.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Urine</th>
<th>Feces</th>
<th>Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>53.8</td>
<td>5.48</td>
<td>50.6</td>
</tr>
<tr>
<td>9</td>
<td>65.5</td>
<td>5.77</td>
<td>37.7</td>
</tr>
<tr>
<td>12</td>
<td>70.4</td>
<td>7.33</td>
<td>32.6</td>
</tr>
<tr>
<td>24</td>
<td>78.9</td>
<td>9.4</td>
<td>14.5</td>
</tr>
</tbody>
</table>

\(^{a}\) The mean of 3 mice calculated as a percent of injected dose
At 24 hours after injection, 88.3% of the injected dose had had been eliminated from the mouse body. Of this, 78.9% was excreted via the urine and 9.4% was excreted via the feces.

Urine samples of mice injected with $^{131}$I-lauric acid, NaI-131 (6% HSA), as well as urine from a control group of mice spiked with $^{131}$I-lauric acid as well as NaI-131 were chromatographed on Gelman ITLC-SG and compared. The $R_f$ value of $^{131}$I-lauric acid spiked urine was .92, while the NaI-131 samples had $R_f$ values of zero. The excreted radioactivity appeared to be in the form of iodide-131 as the ion.

a) NaI-131 (6% HSA)

A similar whole body elimination curve was constructed from the data obtained after intravenous injections of NaI-131 in 6% human serum albumin. The human serum albumin was used only to provide an identical vehicle for intravenous injection purposes. The Iodide-131 appeared to be cleared more rapidly than the radioiodinated fatty acids. At 3 and 24 hours respectively post-injection, the percentages eliminated were 59.1% and 90.9%.

(i) Compartmental Analysis

Computer analysis revealed a triexponential decline in radioactivity within the mouse body with time. The effective half-lives were estimated to be 1.25 hours (69.6%), 6.38 hours (33.4%), and 71.1 hours (9.31%). Again, the terminal elimination phase was assumed to represent the elimination by fecal excretion. The biological
half-life was calculated to be 4.69 days for the terminal elimination phase.

**TABLE XXI**

Comparison of Half-lives for $^{131}$I-Capric Acid $^{131}$I-Lauric Acid, and NaI-131 (6% HSA)$^a$

<table>
<thead>
<tr>
<th>Sample</th>
<th>$t_{1/2}$ (eff) (hours)</th>
<th>$t_{1/2}$ (biol) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{131}$I-Capric Acid</td>
<td>74.98</td>
<td>122.5</td>
</tr>
<tr>
<td>$^{131}$I-Lauric Acid</td>
<td>71.56</td>
<td>113.6</td>
</tr>
<tr>
<td>NaI-131 (6% HSA)</td>
<td>71.09</td>
<td>112.5</td>
</tr>
</tbody>
</table>

$^a$Half-lives were calculated using the terminal phase of the whole body excretion curve.

It was evident that the $^{131}$I-lauric acid, $^{131}$I-capric acid, as well as the NaI-131 (6% HSA) exhibited similar properties in compartmental analysis. (Table 22),

**TABLE XXII**

Comparison of the Effective Half-lives and the Percentages Eliminated by the Three Components of the Elimination Curves

<table>
<thead>
<tr>
<th>Sample</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaI-131</td>
<td>1.25 hours (69.6%)</td>
<td>6.38 hours (33.4%)</td>
<td>71.7 hours (31.0%)</td>
</tr>
<tr>
<td>$^{131}$I-Lauric acid</td>
<td>1.67 hours (46.6%)</td>
<td>7.68 hours (38.4%)</td>
<td>71.6 hours (17.8%)</td>
</tr>
<tr>
<td>$^{131}$I-Capric acid</td>
<td>0.90 hours (36.6%)</td>
<td>3.91 hours (61.0%)</td>
<td>74.9 hours (14.5%)</td>
</tr>
</tbody>
</table>
Figure 21 Whole-Body Excretion of NaI-131(HSA) after Intravenous Injection in Mice

Radioactivity levels have been determined even after metabolism of the fatty acid.
Although the half-lives were similar for the 3 components of the whole body excretion curve, the percentages accounted for by each component varied. This was particularly true of the amount attributed to the first component. The NaI-131 was eliminated much more rapidly than the radioiodinated fatty acids, as shown by the whole body excretion curve as well as the compartmental analysis. A larger portion of NaI-131 was accounted for by the first component than for $^{131}$I-lauric acid and $^{131}$I-capric acid. This could possibly be due to the fact that the radioactivity was already in the iodide ion form in the blood and thus was readily available for renal excretion while the radioiodinated fatty acids had to undergo metabolism prior to renal excretion.

Although the effective half-lives for the first two components varied, it was expected as the mice excreted very concentrated urine and it was difficult to induce the mice to urinate. On voiding, the whole body counts could decrease from 20-30%.

A larger portion of the radioactivity was attributed to the third component for NaI-131 than for the radioiodinated fatty acids. This component was presumably due to tightly bound iodide. This compartment may conceivably include the uptake by the thyroid or binding to plasma proteins. Again, the reason for the larger amount being accounted for by the third component could be due to the availability of the free iodide ion for uptake by the thyroid and binding to the proteins.
d) Calculation of the Renal Half-life

From the radioactivity excreted in the collected urine samples, a urinary excretion curve was constructed. From this data, the effective half-life in the kidney was found to be 4.8 hours for the pooled results from the urine samples. This value was in agreement with the renal half-life of 4.43 hours (Keating et al., 1947) and 4.46 hours (Berson et al., 1952) calculated from human studies after orally administered NaI-131. McConahey et al. (1951) found the renal rate factor to be $2.02 \times 10^{-3}$ min$^{-1}$ from which the effective half-life was calculated to be 5.71 days. The renal half-life can be calculated to be 7.65 days from the urinary excretion rate constants found by Oddie et al. (1955).

It can be seen that the renal half-lives were similar for the radioiodinated fatty acids and the orally administered NaI-131. This can probably be attributed to the rapid metabolism of the radioiodinated fatty acids, liberating iodoacetate into the blood stream (Robinson and Lee, 1975). Once in the blood, the iodoacetate is hydrolyzed thus releasing the free iodide ion (Poe et al., 1976b). Thus, the biological behavior of the radioiodinated fatty acids appear to be similar to that of the Iodide-131 ion after an initial period of metabolism.
Radionuclide Scans were performed using the Searle Gamma Camera at selected time intervals after intravenous injection of the radiolabeled compounds. Serial scans were done using $^{131}$I-capric acid, $^{131}$I-lauric acid, NaI-$^{131}$ (6% HSA), and Thallium = 201.

a) $^{131}$I-Capric Acid

The first series of scans are shown in Figure 22 and gives a visual display of the distribution of $^{131}$I-capric acid within the rabbit body with time. The images depicted in Figure 22 were recorded immediately after injection, 4 minutes after injection, and 8 minutes after injection. The immediate scan taken just after the administration of $^{131}$I-capric acid showed activity in the liver, heart and the lungs although these organs were difficult to distinguish from one another. The 4 minute scan displayed a prominent image of the liver. The heart area was less pronounced. Images of the kidneys and the bladder were faint but recognizable. In the 8 minute scan, the background activity appeared to have increased as the outline of the rabbit body became apparent. The liver image was still discernible but the heart showed a marked decrease in activity. The right and the left kidney images were more apparent and the bladder image was observed.

These scans support the distribution studies done in mice which indicated that maximum activity in the heart occurs immediately after injection of the $^{131}$I-capric acid.
Figure 22  Serial Scans of $^{131}$I-Capric Acid (Anterior Projection in Rabbit)

a = Immediate  b = 4 minutes  c = 8 minutes
b) $^{131}$I-Lauric Acid

Scans were performed for $^{131}$I-lauric acid immediately following injection and at 5, 10 and 15 minutes after injection. (Figure 23) The results were similar to those observed for $^{131}$I-capric acid. The immediate scan showed definite activity in the area of the heart and the liver although the inferior border of the heart could not be differentiated from the liver. In addition, there was probably some contribution from the radioactivity in the lungs. The left kidney image was also faintly visible. In the 5 minute scan the heart image was vague. The kidneys were more pronounced and the background activity appeared to have increased with time. In the 10 minute scan the liver image was still noticeable. The heart appeared to have lost most of the activity and was presented as a faint image. The bladder activity was evident in the 15 minute scan. The kidney images were very prominent and the liver appeared to contain a substantial amount of activity. The heart activity diminished with time and was indistinguishable by 15 minutes.

c) Na I - 131 (6% HSA)

The scans for injected NaI-131 in 6% HSA are depicted in Figure 24. The scans were taken immediately after injection, and at intervals of 5, 10 and 15 minutes after injection. In the immediate scan, both kidney images were already visible as contrasted to the scans of the radioiodinated fatty acids, in which the kidneys became visible with the passage of time after the injection. There was apparent uptake by the liver, the heart and probably the lungs. The
Figure 23 Serial Scans of $^{131}$I-Lauric Acid (Anterior Projection in Rabbit)

a = Immediate  b = 5 minutes  
c = 10 minutes  d = 15 minutes
Figure 24  Serial Scans of NaI-131 (6% HSA) (Anterior Projection in Rabbit)

a = Immediate  b = 5 minutes

 c = 10 minutes  d = 15 minutes
background activity appeared slightly higher than that observed for the radioiodinated fatty acids. In the 5 minute scan, the activity in the heart had diminished considerably. The liver image and the kidney images were still visible. The bladder image was increasing in intensity. There also appeared to be some activity observed in the head region. In the 10 and 15 minute scan, the background activity increased. There appeared to be some uptake of activity by the muscles. The kidney images were pronounced and the heart image was indistinguishable from the background.

d) Thallium - 201

The Thallium - 201 scan was done at 15 minutes after intravenous injection. There appeared to be significantly less activity in the liver, thus the heart image was more definitive on the scan. The activity in the kidney was prominent and there was a faint outline of the rabbit body.

e) Comparison of the Radionuclide Scans

The $^{131}$I-capric acid and the $^{131}$I-lauric acid did not appear to be good myocardial scanning agents although there appeared to be definite uptake by the heart. Both of the radioiodinated fatty acids appeared to be inferior to Thallium-201, the present myocardial agent of choice for cold-spot imaging.

It must be noted that the experimental scans obtained were done with Iodine-131 as the radioactive label. If Iodine - 123 had been available, undoubtedly the quality of the scans would have been superior. The rabbits were not pretreated prior to administration
Figure 25  Thallium - 201 Scan in Rabbit
(Anterior projection at 15 minutes)
of the radioiodinated fatty acids. The myocardial uptake of the radioiodinated fatty acids could have been enhanced by the simultaneous administration of glucose and insulin to promote storage of the radioiodinated fatty acids and to prevent Beta Oxidation (Gunton et al., 1965).

In our experimental scans, no background subtraction was done and no attempt was made to enhance the scan by magnifying the counts obtained in the heart. Poe et al. (1976b) obtained scans using $^{123}$I-hexadecenoic acid injected intravenously into mongrel dogs previously subjected to coronary ligation. The scans were performed 3 minutes after administration of the $^{123}$I-hexadecenoic acid using a Pho/Gamma HP Scintillation camera with a pinhole collimator. The collimator was placed 6-10 cm from the chest, a distance estimated to provide a magnification factor of 2. The data was further analyzed for subsequent background correction and an additional twofold magnification. The background value was uniformly subtracted from the final image.

Subsequently, Poe et al. (1977b) performed clinical studies in patients undergoing cardiac catherization using the same specifications as mentioned above. Good quality scans were obtained in 3 minutes in human subjects. The quality of the scans obtained by Poe et al., (1976b, 1977b) were superior to our scans obtained with $^{131}$I-capric acid and $^{131}$I-lauric acid. However, it would be unjust to compare the scans as a superior radioisotope, different animal species, and a different imaging system was used by Poe et al. (1976b, 1977b). In addition, the quality of our experimental scans would have improved
with magnification and background correction.

8. Dosimetry

The ultimate aim of this study was to perform myocardial scans using $^{123}$I-labeled fatty acids. However, due to some production problems, the Iodine-123 was not available but it was decided to calculate the radiation dosimetry for $^{123}$I-labeled fatty acids. The radiation doses were calculated for the whole body, liver, kidneys, muscle and heart. The following assumptions were made for the dose calculations:

(1) The distribution and excretion data determined in mice also applied to humans.

(2) The whole body excretion complied with the whole body excretion curve.

(3) The urinary excretion collections were valid, and the renal biological half-life was 4.92 hours. The effective half-life can be calculated to be 3.57 hours for $^{123}$I-labeled fatty acids.

(4) The liver and the muscle followed the first two components of the whole body excretion curve.

(5) The bladder and the heart contributions to the absorbed dose were neglected because appropriate values have not been published.

(6) The distribution and biological half-life of $^{123}$I-labeled fatty acids will be the same as the $^{131}$I-labeled fatty acids.
The absorbed doses were calculated using the equation proposed by Loevinger and Berman (1968) and modified by Snyder et al. (1975)

\[
D = \sum \frac{A_2}{m_i} \Delta_i \phi_i (r_1 + r_2) \text{ rads}
\]

Where

- \( A_2 \) = The cumulated activity in source organ \( r_2 \) (\( \muCi = h \))
- \( D (r_1 + r_2) \) = The mean absorbed dose to a target organ \( r_1 \) from a radionuclide distributed uniformly in a source organ \( r_2 \). (rads)

\( \Delta_i \) = the equilibrium dose constant for radiation of type \( i=1,2,3,4 \) with a fractional frequency \( n_i \) per disintegration, and a mean energy \( E_i \) in MeV.

\[
= 2.13 n_i E_i \ (g\text{-rads})
\]

\( m_i \) = the mass of the target organ \( r_1 \) (g)

\( \phi_i (r_1 + r_2) \) = The absorbed dose fraction of energy for target organ \( r_1 \) for particles \( i \) emitted in source organ \( r_2 \).

\( \phi_i (r_1 + r_2) \) = The specific absorbed fraction of energy for target organ \( r_1 \) for particles \( i \) emitted in source organ \( r_2 \).
Most of the biological data needed for the estimation of dose are embodied in the cumulative activity $A_2$, while the remaining portion involves physical and anatomic data. (Snyder et al., 1975).

Thus, $S(r_1 + r_2) = \sum_{i} \Delta_i \phi_i(r_1 + r_2)$

The biological data used in estimating dose relate only to the anthropomorphic model used (size, shape, density, and composition) and to the distribution and retention of the radionuclide in source organ $r_2$. Values of $S$ used were obtained from Snyder et al., (1975).

The average dose can be calculated as:

$$\bar{D}(r_1 + r_2) = A_2 S(r_1 + r_2)$$

Since there are generally a number of source organs, the total average dose to target organ $r_2$ is

$$\bar{D}(r_2) = \sum_{2}^{\infty} \bar{D}(r_1 + r_2)$$

$$= \sum_{2}^{\infty} A_2 S(r_1 + r_2)$$

$$A_2 = \int_{t_1}^{t_2} A_0 e^{-\lambda t} dt$$

Where $\lambda = \lambda_{\text{eff}}$.

a) Theoretical Absorbed Doses from $^{123}$I-capric or $^{123}$I-Lauric Acids

(i) Whole Body Dosimetry

The values for 'S' for Iodine-123, assuming uniform distribution in the whole body, are from Snyder et al. (1975). The results are based upon a 69,880 g standard body weight. The administered
The diagram illustrates the decay scheme of $^{123}$Iodine. The table below provides the energy and other nuclear parameters for various transitions:

<table>
<thead>
<tr>
<th>Radiation Type</th>
<th>Energy (MeV)</th>
<th>Other Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron capture-1</td>
<td>2.0</td>
<td>Allowed</td>
</tr>
<tr>
<td>Electron capture-2</td>
<td>0.5</td>
<td>Allowed</td>
</tr>
<tr>
<td>Electron capture-3</td>
<td>0.3</td>
<td>Allowed</td>
</tr>
<tr>
<td>Electron capture-4</td>
<td>0.5</td>
<td>Allowed</td>
</tr>
<tr>
<td>Gamma-1</td>
<td>99.7</td>
<td>$M_1, Q = 0.161(T)$, $K/(L+M) = 6.6$</td>
</tr>
<tr>
<td>Gamma-2</td>
<td>0.14</td>
<td>0.274 Assumed all unconverted</td>
</tr>
<tr>
<td>Gamma-3</td>
<td>0.16</td>
<td>0.342 Assumed all unconverted</td>
</tr>
<tr>
<td>Gamma-4</td>
<td>0.44</td>
<td>0.436 Assumed all unconverted</td>
</tr>
<tr>
<td>Gamma-5</td>
<td>0.28</td>
<td>0.504 Assumed all unconverted</td>
</tr>
<tr>
<td>Gamma-6</td>
<td>2.0</td>
<td>0.530 Assumed all unconverted</td>
</tr>
<tr>
<td>All other gammas</td>
<td>&lt;0.1</td>
<td>--</td>
</tr>
</tbody>
</table>

Ref. Nuclear Data Sheets, 60-6-63, 60-6-71
(T) = Theoretical value.

Figure 26 Decay Scheme of $^{123}$Iodine$^a$

$^a$(from Dillman)
dose was 2 mCi of radioiodinated fatty acid.

The \( \lambda \) effective for \(^{131}\text{I}\)-capric and \(^{131}\text{I}\)-lauric acid were supplied by computer compartmental analysis. From this, the \( \lambda \) biological can be calculated.

\[
\lambda_{\text{eff}} = \lambda_p - \lambda_b
\]

where:

- \( \lambda_{\text{eff}} \) = effective decay constant
- \( \lambda_p \) = physical decay constant
- \( \lambda_b \) = biological decay constant

(1) \(^{131}\text{I}\)-Capric Acid

\[
\lambda_p \text{ for } ^{131}\text{I} = .003587 \text{ h}^{-1}
\]

\( \lambda_{\text{eff}} \) for \(^{131}\text{I}\)-capric acid:

\[
\begin{align*}
\lambda_1 \text{ eff} &= .7709 \text{ h}^{-1} \\
\lambda_2 \text{ eff} &= .1774 \text{ h}^{-1} \\
\lambda_3 \text{ eff} &= .00919 \text{ h}^{-1}
\end{align*}
\]

The biological decay constant for \(^{123}\text{I}\)-capric acid would be the same. Therefore the \( t_{\frac{1}{2}} \) biological (\( t_{\frac{1}{2}} b \)) can be calculated to be:

\[
t_{\frac{1}{2}}b = \frac{.693}{\lambda_b}
\]

\( t_{\frac{1}{2}}b(1) \) = .9032 h

\( t_{\frac{1}{2}}b(2) \) = 3.987 h

\( t_{\frac{1}{2}}b(3) \) = 123.7 h
For $^{123}\text{I}$-Capric Acid

$$t_{1/2}^{\text{eff}} = \frac{t_{1/2}^{\text{b}} \times t_{1/2}^{\text{p}}}{t_{1/2}^{\text{b}} + t_{1/2}^{\text{p}}}$$

$$t_{1/2}^{\text{b}} = 13.0 \text{ h}$$

$$t_{1/2}^{\text{p}}$$

- $t_{1/2}^{\text{eff}}(1) = 0.8445 \text{ h}$
- $t_{1/2}^{\text{eff}}(2) = 3.051 \text{ h}$
- $t_{1/2}^{\text{eff}}(3) = 11.76 \text{ h}$

$$A = A_0 \sum_{i} 1.44 \times t_{1/2}^{\text{eff}} \alpha_i$$

Where $\alpha_i$ = the initial value of $i^{th}$ exponential component of the iodine that appears in the source.

The cumulated activity in the whole body is $11215 \mu\text{Ci} - \text{h}$.

$$D = \sum_{2} \sim A_i S(r_1 - r_2)$$

where $S = 3.1 \times 10^{-6} \text{ rad} \mu\text{Ci-h}$.

$$D = 0.03476 \text{ rad} / 2 \mu\text{Ci}$$

$$= 34.76 \text{ mrad} / 2 \mu\text{Ci}$$

(2) $^{131}\text{I}$-Lauric Acid

<table>
<thead>
<tr>
<th>$\lambda$ biological</th>
<th>$t_{1/2}^{\text{biol}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_1^b$ = 0.4116 h$^{-1}$</td>
<td>$t_{1/2}^{\text{biol}}(1)^b$ = 1.684 h</td>
</tr>
<tr>
<td>$\lambda_2^b$ = 0.08661 h$^{-1}$</td>
<td>$t_{1/2}^{\text{biol}}(2)^b$ = 8.001 h</td>
</tr>
<tr>
<td>$\lambda_3^b$ = 0.006098 h$^{-1}$</td>
<td>$t_{1/2}^{\text{biol}}(3)^b$ = 113.6 h</td>
</tr>
</tbody>
</table>
For $^{123}$I-Lauric Acid

\[ t_{1/2}^{\text{eff}} = \frac{t_{1/2}^{\text{b}} \times t_{1/2}^{\text{p}}}{t_{1/2}^{\text{b}} + t_{1/2}^{\text{p}}} \quad \text{tp} = 13.0 \text{ h} \]

\[ t_{1/2}^{\text{eff}} \]
\[ t_{1/2}^{\text{(1) eff}} = 1.491 \text{ h} \]
\[ t_{1/2}^{\text{(2) eff}} = 4.953 \text{ h} \]
\[ t_{1/2}^{\text{(3) eff}} = 11.67 \text{ h} \]

The cumulated activity in the whole body is:

\[ A_{\text{WB}} = A_0 \sum \alpha \cdot t_{1/2} \times h_j \]
\[ = 13461 \mu\text{Ci-h} \]

\[ D = \sum \frac{A_2}{2} \times S(r_1 + r_2) \]

where \( S = 3.1 \times 10^{-6} \text{ rad/} \mu\text{Ci-h} \)

\[ D = 0.04173 \text{ rad/}2\text{mCi} \]
\[ = 41.73 \text{ mrad/}2\text{mCi} \]
### TABLE XXIII

<table>
<thead>
<tr>
<th>Target Organ</th>
<th>Whole body</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.4x10^-6</td>
<td>7.9x10^-5</td>
<td>5.1x10^-6</td>
<td>1.5x10^-6</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.3x10^-6</td>
<td>5.1x10^-6</td>
<td>3.4x10^-4</td>
<td>2.0x10^-6</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.9x10^-6</td>
<td>1.5x10^-6</td>
<td>2.0x10^-6</td>
<td>4.4x10^-6</td>
</tr>
</tbody>
</table>

\[a\text{From Snyder et al., 1975}\]

#### (ii) The Cumulated Activity

\[
D = \sum_{k=1}^{\infty} A_k \cdot S(r_1 + r_2)
\]

\[A = A_0 \cdot 1.44 \cdot t_{\text{eff}} \cdot a_h j\]

where \(h_j\) = the initial value of the \(i^{th}\) exponential component of the iodine that appears in the source

#### (1) 123I-Capric Acid

\[\hat{A}_{\text{WB}} = \text{Cumulated activity in the whole body per 2mCi administered.}\]

\[= 11215 \mu\text{Ci-h}\]

\[\hat{A}_k = \text{Cumulated activity in the kidneys per 2mCi administered.}\]

A maximum of 2.697% of the injected dose was concentrated in the kidney. The effective half-life of 123I-Capric acid in the kidneys can be calculated to be 3.57 hours.

\[= 277.3 \mu\text{Ci-h}\]
\[ A_L = \text{Cumulated activity in the liver per 2mCi administered.} \]

The disappearance of radioactivity from the liver presumably complies with the first and the second component of the whole body excretion curve for \(^{131}\text{I}-\text{capric acid}.\) A maximum of 9.129% of the injected dose concentrated in the liver.

\[ = 575.5 \ \mu\text{Ci-h} \]

\[ A_M = \text{Cumulated activity in the muscle per 2mCi administered.} \]

The disappearance of radioactivity from the muscle presumably complies with the first and the second component of the whole body excretion curve. A maximum of 16.26% of the injected dose concentrated in the muscle.

\[ = 1025.2 \ \mu\text{Ci-h} \]

\[ (2)^{123}\text{I}-\text{Lauric Acid} \]

\[ A_{\text{WB}} = \text{Cumulated activity in the whole body per 2mCi administered.} \]

\[ = 13461 \ \mu\text{Ci-h} \]

\[ A_k = \text{Cumulated activity in the kidneys per 2mCi administered.} \]

A maximum of 4.069% of the injected dose concentrated in the kidneys.

\[ = 418.4 \ \mu\text{Ci-h} \]

\[ A_L = \text{Cumulated activity in the liver per 2mCi administered.} \]

The disappearance of radioactivity from the liver presumably complies with the first and the second component of the whole body excretion curve for \(^{131}\text{I}-\text{lauric acid}.\) A maximum of 22.80% of the injected dose was concentrated in the liver.

\[ = 1705 \ \mu\text{Ci-h} \]

\[ A_M = \text{Cumulated activity in the muscle per 2mCi administered.} \]

The disappearance of radioactivity from the muscle...
presumably complies with the first and second component of the whole body excretion curve. A maximum of 29.28% of the injected dose was concentrated in the liver.

= 2190 μCi-h

(iii) The Mean Dose

(1) $^{123}$I-Capric Acid

Calculation of Mean Dose to the Kidneys for $^{123}$I-capric acid

\[
D_k = \sum A_{\text{WB}} S(\text{Kidneys + Whole Body}) + A_k S(\text{Kidney + Kidney}) + A_L S(\text{Kidney + Liver}) + A_M S(\text{Kidney + Muscle})
\]

= 0.1363 rad / 2mCi

= 136.3 mrad / 2mCi

Calculation of Mean Dose to the Liver

\[
D_L = \sum A_{\text{WB}} S(\text{Liver + Whole Body}) + A_L S(\text{Liver + Liver}) + A_k S(\text{Liver + Kidney}) + A_M S(\text{Liver + Muscle})
\]

= 0.0866 rads / 2mCi

= 86.6 mrads / 2mCi
Calculation of Mean Dose to the Muscle

\[ D = \sum A_2 S(r_1 - r_2) \]

\[ D_M = \sum \tilde{A}_{WB} S(\text{Muscle + Whole Body}) + \tilde{A}_M S(\text{Muscle + Muscle}) + \tilde{A}_L S(\text{Muscle + Liver}) + \tilde{A}_K S(\text{Muscle + Kidney}) \]

\[ = .0385 \text{ rads / 2mCi} \]
\[ = 38.5 \text{ mrads / 2mCi} \]

(2) \text{ I23-I-Lauric Acid}

Calculation of the Mean Dose to the Kidneys

\[ D = \sum A_2 S(r_1 - r_2) \]

\[ D_K = \tilde{A}_{WB} S(\text{Kidneys + Whole Body}) + \tilde{A}_K S(\text{Kidney + Kidney}) + \tilde{A}_L S(\text{Kidney + Liver}) + \tilde{A}_M S(\text{Kidney + Muscle}) \]

\[ = .1998 \text{ rads / 2mCi} \]
\[ = 199.8 \text{ mrads / 2mCi} \]

Calculation of the Mean Dose to the Liver

\[ D_L = \tilde{A}_{WB} S(\text{Liver + Whole Body}) + \tilde{A}_L S(\text{Liver + Liver}) + \tilde{A}_K S(\text{Liver + Kidney}) + \tilde{A}_M S(\text{Liver + Muscle}) \]

\[ = .1859 \text{ rads / 2mCi} \]
\[ = 185.9 \text{ mrads / 2mCi} \]
Calculation of the Mean Dose to the Muscle

\[ D_M = A_{WB} S(\text{Muscle + Whole Body}) + A_M S(\text{Muscle + Muscle}) \]
\[ + A_K S(\text{Muscle + Kidney}) + A_L S(\text{Muscle + Liver}) \]
\[ = 0.05207 \text{ rads/2mCi} \]
\[ = 52.07 \text{ mrads/2mCi} \]

TABLE XXIV

The Mean Absorbed Dose to the Whole Body, Kidneys, Liver, and Muscle Using $^{123}$I-Capric and $^{123}$I-Lauric Acids

<table>
<thead>
<tr>
<th>Mean Absorbed Dose (mrads)(^a)</th>
<th>Whole Body</th>
<th>Kidneys</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{123}$I-Capric Acid</td>
<td>34.76</td>
<td>136.3</td>
<td>86.6</td>
<td>38.5</td>
</tr>
<tr>
<td>$^{123}$I-Lauric Acid</td>
<td>41.73</td>
<td>199.8</td>
<td>185.9</td>
<td>52.07</td>
</tr>
</tbody>
</table>

\(^a\)2mCi of radioiodinated fatty acid administered.

The mean absorbed dose for the $^{123}$I-lauric acid appeared to be somewhat higher than $^{123}$I-capric acid from the data recorded from the whole body distribution studies. The mean absorbed dose for the whole body was 34.76 mrad/2mCi or 17.38 mrad/mCi for $^{123}$I-capric acid and 41.73 mrad/2mCi or 20.87 mrad/mCi for $^{123}$I-capric acid. This was comparable to the absorbed radiation dose of 30 mrads/mCi to the total body calculated for $^{123}$I-hexadecenoic acid. (Poe et al., 1976b)
Calculation of the Mean Dose to the Heart

Radiation dosimetry for the heart was difficult to calculate as 'S' values were not readily available. The absorbed dose fractions $\phi$ from the other organs to the heart were available although the contribution from the heart to the heart was not available. Using the formula:

$$D = \frac{A}{M} \Sigma \Delta_i \phi_i$$

the mean absorbed dose to the heart can be approximated. From the dosimetry calculations of Poe et al. (1976b) the $\Sigma \Delta_i \phi_i$ value for the heart to the heart is approximately .1734. This was based on the assumption of a 300 g heart (for non-penetrating radiation only) and a myocardial biological half-life of 30 min. Other assumptions were made that:

- Kidney mass = 310 g
- Liver mass = 1800 g
- Muscle mass = 48,480 g
- Body mass = 69,880 g

Therefore, the dose to the heart becomes

$$D_{\text{heart}} = \frac{A_{\text{heart}}}{M_{\text{heart}}} \Sigma \phi_i \phi_i (\text{Heart} \rightarrow \text{Heart}) + \frac{A_{\text{Liver}}}{M_{\text{Liver}}} \Sigma \Delta_i \phi_i (\text{Heart} \rightarrow \text{Liver})$$

$$+ \frac{A_{\text{Kidneys}}}{M_{\text{Kidneys}}} \Sigma \Delta_i \phi_i (\text{Heart} \rightarrow \text{Kidneys}) + \frac{A_{\text{Muscle}}}{M_{\text{Muscle}}} \Sigma \Delta_i \phi_i (\text{Heart} \rightarrow \text{Muscle})$$

$$+ \frac{A_{\text{Whole body}}}{M_{\text{Whole body}}} \Sigma \Delta_i \phi_i (\text{Heart} \rightarrow \text{Whole Body})$$
### TABLE XXV

Absorbed Dose Fractions for the Heart

<table>
<thead>
<tr>
<th>$E_i$ (MeV)</th>
<th>$n_i$</th>
<th>$\Delta_i$ (g-rad)</th>
<th>$\phi_1$</th>
<th>$\phi_2$</th>
<th>$\phi_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1590</td>
<td>.835</td>
<td>.2828</td>
<td>.00420</td>
<td>.00674</td>
<td>.00185</td>
</tr>
<tr>
<td>.1272</td>
<td>.134</td>
<td>.0363</td>
<td>.00420</td>
<td>.00674</td>
<td>.00185</td>
</tr>
<tr>
<td>.1544</td>
<td>.0153</td>
<td>.0050</td>
<td>.00420</td>
<td>.00674</td>
<td>.00185</td>
</tr>
<tr>
<td>.1582</td>
<td>.0051</td>
<td>.0017</td>
<td>.00420</td>
<td>.00674</td>
<td>.00185</td>
</tr>
<tr>
<td>.2740</td>
<td>.0014</td>
<td>.0008</td>
<td>.00337</td>
<td>.00570</td>
<td>.00170</td>
</tr>
<tr>
<td>.3420</td>
<td>.0016</td>
<td>.0012</td>
<td>.00337</td>
<td>.00570</td>
<td>.00170</td>
</tr>
<tr>
<td>.4360</td>
<td>.0044</td>
<td>.0041</td>
<td>.00372</td>
<td>.00573</td>
<td>.00208</td>
</tr>
<tr>
<td>.5040</td>
<td>.0028</td>
<td>.0030</td>
<td>.00372</td>
<td>.00573</td>
<td>.00208</td>
</tr>
<tr>
<td>.5300</td>
<td>.020</td>
<td>.0226</td>
<td>.00372</td>
<td>.00573</td>
<td>.00208</td>
</tr>
<tr>
<td>.0271</td>
<td>.472</td>
<td>.0276</td>
<td>.00769</td>
<td>.00531</td>
<td>0.000</td>
</tr>
<tr>
<td>.0272</td>
<td>.242</td>
<td>.0140</td>
<td>.00769</td>
<td>.00531</td>
<td>0.000</td>
</tr>
<tr>
<td>.0310</td>
<td>.127</td>
<td>.0084</td>
<td>.00635</td>
<td>.00531</td>
<td>0.000204</td>
</tr>
<tr>
<td>.0318</td>
<td>.0264</td>
<td>.0018</td>
<td>.00635</td>
<td>.00531</td>
<td>0.000204</td>
</tr>
<tr>
<td>.3038</td>
<td>.133</td>
<td>.0011</td>
<td>.00756</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>.0227</td>
<td>.0877</td>
<td>.0042</td>
<td>.00756</td>
<td>.00132</td>
<td>0.000</td>
</tr>
<tr>
<td>.0264</td>
<td>.0370</td>
<td>.0021</td>
<td>.00756</td>
<td>.00132</td>
<td>0.000</td>
</tr>
<tr>
<td>.0302</td>
<td>.006</td>
<td>.0004</td>
<td>.00635</td>
<td>.00531</td>
<td>0.000204</td>
</tr>
<tr>
<td>.0029</td>
<td>.922</td>
<td>.3057</td>
<td>.00756</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>.0008</td>
<td>2.18</td>
<td>.3037</td>
<td>.00756</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\[ \sum \Delta_i n_i = .01151 \quad .01668 \quad .005230 \]

1. Contribution from whole body to heart
2. Contribution from liver to heart
3. Contribution from kidney to heart
The approximated $\Sigma \phi_i$ for the muscle to the heart is .09696, assuming 48,480 g for muscle.

(1) $^{123}$I-Capric Acid

Assuming a maximum uptake of 1.5% by the heart.

$A_{\text{heart}} = 20.74 \mu \text{Ci} \cdot \text{h}$

$D_{\text{heart}} = .02589 \text{ rads/2mCi}$

= $\frac{.02589}{2} = .01295 \text{ rads/mCi}$

(2) $^{123}$I-Lauric Acid

The same assumptions for masses of the organs were made. A maximum of 2.12% of the injected dose was taken up by the heart.

$A_{\text{heart}} = 29.31 \mu \text{Ci-h}$

$D_{\text{heart}} = .04639 \text{ rads/2mCi}$

= $\frac{.04639}{2} = .02319 \text{ rad/mCi}$

The mean absorbed dose to the heart was calculated to be .01295 rad/mCi for $^{123}$I-capric acid and .02319 rad/mCi for $^{123}$I-lauric acid. This is somewhat lower than the dosimetry calculations of Poe et al. (1976b) in which they obtained a value of .04 rad/mCi for the heart. This lower value could be due to the fact that we assumed the distribution of radioiodinated fatty acids in mice also applied to humans. In addition, the maximum myocardial uptake from our results was 2.12% compared to an assumed myocardial uptake of 5% in the studies of Poe et al. (1976b).
V. SUMMARY AND CONCLUSIONS
1. 10-Bromocapric acid, 12-bromolauric acid, and 2 bromotetradecanoic acid were labeled by interhalogen replacement affected by refluxing with NaI-131 in acetone. Radiochemical purity was ascertained by chromatography.

2. Preliminary scans and distribution studies showed that 2-iodotetradecanoic acid was inferior to the two terminal radioiodinated fatty acids in myocardial uptake and thus was excluded from further studies.

3. Tissue distribution studies showed that the highest accumulation of radioactivity was in the muscle and the blood for both $^{131}$I-capric acid and $^{131}$I-lauric acid. Other organs investigated included the heart, liver, lung, kidneys, spleen, stomach, intestines, bone, and adrenals. When the results were expressed as "percent injected dose per total organ", no organ appeared to have special affinity to incorporate the radioiodinated fatty acids. However, when the relative accumulation of the radioiodinated fatty acids were expressed as "percent injected dose per gram organ", the heart showed the highest uptake of the labeled fatty acids for the first .05 hours, after which there was a decline in radioactivity. From the observations of the time course and the localization of the labeled compounds within the mouse body, it appeared that the optimum time for myocardial imaging would be immediately after injection of the radioiodinated fatty acids.

4. The $\text{LD}_{50}$ of sodium bromolaurate was calculated after intravenous administration in mice and found to be 210 mg/kg (194 mg/kg - 228 mg/kg). The bromocapric acid was dissolved in a solvent system similar to that
of Nembutal U.S.P. The LD$_{50}$ values may not necessarily reflect the toxicity of the bromocapric acid solely. The LD$_{50}$ was found to be 86.1 mg/kg (83.0 mg/kg - 89.3 mg/kg).

5. The excretion of the radioactivity after administration of the radioiodinated fatty acids was primarily by the kidneys. Whole body counting revealed a triexponential excretion curve in mice. For $^{131}$I-capric acid, the effective half-lives of the short lived component was .90 hours (36.7%), the medium component was 3.91 hours (61.6%), and the long component was 74.9 hours (14.5%). For $^{131}$I-lauric acid, the effective half-lives of the short lived component was 1.67 hours (46.6%), the medium component 7.68 hours (38.4%), and the long component was 71.6 hours (17.8%). For both the radioiodinated fatty acids, the first as well as the second component presumably represented a decrease in the whole body radioactivity due primarily to urinary excretion. The third component may represent radioactivity tightly bound to some tissue and slowly released. The radioactivity may be thyroidal Iodide-131 or protein bound iodide. This third component presumably represents elimination by fecal excretion.

6. For $^{131}$I-capric acid, urine collections showed that at 10 and 24 hours respectively post-injection, 73.0% and 82.4% of the injected activity had been recovered. Fecal excretion was found to be 3.85% and 8.88% of the injected dose at 10 and 24 hours respectively post-injection. For $^{131}$I-lauric acid, 70.4% and 78.9% of the injected dose was recovered in the urine at 12 and 24 hours respectively. Fecal recovery was 7.33% and 9.4% of the injected
doese at 12 and 24 hours respectively. Chromatography of the urine showed that the radioactivity was excreted in the form of the iodide-131 ion.

7. Urinary results of both radioiodinated fatty acids revealed an effective half-life of 4.8 hours for the kidneys. From this, the biological half-life for the kidney can be calculated to be 4.92 hours.

8. Serial scans were performed for $^{131}$I-capric acid, $^{131}$I-lauric acid, NaI-131 (6% HSA), and Thallium-201 using the Searle gamma camera. Better quality scans would have been possible with the use of Iodine-123 as the radionuclide, background subtraction from the final image, and magnification of the area of interest.

9. Theoretical radiation doses were calculated from the results of both the distribution and the excretion studies in mice. This data was extrapolated to humans. For $^{123}$I-capric acid, the mean absorbed dose was calculated to be 34.76 mrads/2 mCi for the whole body, 136.3 mrads/2 mCi for the kidneys, 86.6 mrads/2 mCi for the liver, 38.5 mrads/2 mCi for the muscle, and 25.89 mrads/2 mCi for the heart. For $^{123}$I-lauric acid, the mean absorbed dose was 41.73 mrads/2 mCi for the whole body, 199.8 mrads/2 mCi for the kidneys, 185.9 mrads/2 mCi for the liver, 52.07 mrads/2 mCi for the muscle, and 46.39 mrads/2 mCi for the heart.

The scope of this investigation was to evaluate the possible use of $^{123}$I-capric acid and $^{123}$I-lauric acid as myocardial scanning agents. However, due to the problems encountered with the production of Iodine-123,
the experiments were carried out using Iodine-131. The preliminary distribution studies revealed that radioiodinated fatty acids are indeed taken up by the heart. The toxicity studies showed that care must be taken to ensure that a minimum amount of unlabeled fatty acid is administered with the radioiodinated fatty acids. The excretion study showed that the radioactive label was removed from the fatty acid and was primarily excreted as the ion in the urine. The radiation doses calculated showed that millicurie quantities of Iodine-123 labeled compounds could be administered and still be within the permissible radiation exposure.

The $^{131}\text{I}$-capric acid and $^{131}\text{I}$-lauric acid scans were somewhat disappointing in that the heart image was difficult to differentiate from the liver image. It was realized that the fatty acid analogs used in this investigation were not the fatty acids of choice. However, it was felt that a lengthy synthesis procedure to obtain a more suitable fatty acid was not within the scope of this study. In performing the investigation, the necessary protocol for the development of a new radiopharmaceutical was followed. If more suitable terminal brominated fatty acids should become commercially available in the near future, the same experimental procedures could be followed to evaluate the possible use of them as myocardial scanning agents.
VI  BIBLIOGRAPHY


Anthony, G.J. and Landau, B.R.: Relative contributions of $\alpha$-, $\beta$-, and $\gamma$-oxidative pathways to in vitro fatty acid oxidation in rat liver. J. Lipid Res. 9: 267, 1968.


