THE TRANSPORT PROPERTIES OF ARTERIAL TISSUE

by

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24
Professor Fredrick J. McGarry  
Secretary of the Faculty  
Massachusetts Institute of Technology  
Cambridge, Massachusetts 02139  

Dear Professor McGarry:

In accordance with the regulations of the Faculty, I herewith submit a thesis, entitled "The Transport Properties of Arterial Tissue", in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemical Engineering at the Massachusetts Institute of Technology.

Respectfully submitted,

Signature redacted

Robert L. Bratzler
I. ABSTRACT

THE TRANSPORT PROPERTIES OF ARTERIAL TISSUE

by Robert L. Bratzler

Submitted to the Department of Chemical Engineering in September, 1974, in partial fulfillment of the requirements for the degree of Doctor of Philosophy

The purpose of this thesis was to develop a more fundamental understanding of the factors affecting the transport of biological macromolecules into and across the walls of large blood vessels. Healthy rabbits were injected with radioiodinated low-density lipoprotein (LDL) or albumin and sacrificed 10 minutes to 67 hours later. The thoracic aorta was removed and quickly frozen to preserve the intramural distribution of the labeled solute. This distribution was subsequently determined by counting successive 20\mu thick radial sections prepared with a refrigerated microtome. The resulting profiles demonstrated that both albumin and LDL label had penetrated the entire 240\mu thick aortic wall in less than 10 minutes. Labeled solute entered both through the arterial intima and from the capillaries in the adventitia. After 10 minutes and 30 minutes, labeled albumin accumulation was greater than that for LDL. At four hours, no significant overall difference was observed. For both labeled solutes, net accumulation increased up to four hours. At 67 hours, LDL accumulation was less than at four hours indicating efflux of label had occurred.

A mathematical model was developed to aid in the interpretation of the data. The experimental data were found to be in accord with the following concepts: (1) Intramural solute transport is the result of both diffusion and pressure-driven convection; (2) LDL reacts reversibly with components within the arterial wall; (3) LDL crosses the arterial endothelium from the blood plasma exclusively by vesicular transport.

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Robert S. Lees  
Professor of Nutrition and Food Science
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Others who contributed to this thesis include Mr. Stanley Mitchell, who prepared many of the figures for the final manuscript, Mrs. Laura Chisolm and Ms. Gwen Terry, who helped with the typing of the final manuscript, Mr. Ernest Yamartino, who ably assisted with experiments and data analysis, and Mr. Vince Vilker, with whom many stimulating discussions, technical and otherwise, were held.

The people to whom I am most indebted include my supervi-
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And finally, and above all, I am eternally grateful to my wife, Lori, whose constant encouragement, overworked understanding, and deep love sustained me throughout the course of this work. This thesis is as much hers as mine.
TO
LORI
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Abstract</td>
<td>3</td>
</tr>
<tr>
<td>II. Summary</td>
<td>22</td>
</tr>
<tr>
<td>A. Background</td>
<td>22</td>
</tr>
<tr>
<td>B. Experimental Methods</td>
<td>29</td>
</tr>
<tr>
<td>C. Experimental Results</td>
<td>33</td>
</tr>
<tr>
<td>D. Theory</td>
<td>39</td>
</tr>
<tr>
<td>III. Introduction</td>
<td>60</td>
</tr>
<tr>
<td>A. Scope and Purpose</td>
<td>60</td>
</tr>
<tr>
<td>B. Relevance</td>
<td>60</td>
</tr>
<tr>
<td>C. Pathology of Atherosclerosis</td>
<td>61</td>
</tr>
<tr>
<td>D. Possible Factors Involved in Blood-Artery Macromolecular Transport</td>
<td>72</td>
</tr>
<tr>
<td>1. Transendothelial Transport Mechanisms</td>
<td>72</td>
</tr>
<tr>
<td>2. Effect of Basement Membrane on Transmural Solute Transport</td>
<td>79</td>
</tr>
<tr>
<td>3. Effects of Elastic Lamina on Transmural Transport</td>
<td>80</td>
</tr>
<tr>
<td>4. Effect of Ground Substance on Transmural Transport</td>
<td>81</td>
</tr>
<tr>
<td>5. Effect of Lymphatic System on Transmural Transport</td>
<td>83</td>
</tr>
<tr>
<td>6. Transmural Transport Driving Forces</td>
<td>85</td>
</tr>
<tr>
<td>7. The Relative Importance of Diffusion and Convection</td>
<td>90</td>
</tr>
<tr>
<td>E. Specific Purposes of This Study</td>
<td>99</td>
</tr>
<tr>
<td>IV. Apparatus and Procedures</td>
<td>100</td>
</tr>
<tr>
<td>A. Solute Distribution Studies</td>
<td>100</td>
</tr>
<tr>
<td>1. Preparation of Proteins</td>
<td>100</td>
</tr>
<tr>
<td>2. Radioisotope Protein Labeling</td>
<td>101</td>
</tr>
<tr>
<td>3. Injection of Labeled Proteins</td>
<td>104</td>
</tr>
<tr>
<td>4. Sacrifice of Animal and Removal of Aorta</td>
<td>106</td>
</tr>
</tbody>
</table>
5. Sectioning and Preparation of the Aorta 107
6. Radioactivity Determinations 113
7. Expressing Results 114
8. Plasma Chemical Analysis 115
9. Special Experiments 115
10. Aortic Tissue Homogenate Analysis 116

B. Hydraulic Permeability Studies 117
1. Apparatus 117
2. Procedures 119

V. Experimental Results 121
A. Solute Distribution Studies 121
1. Testing of Injected Labeled Proteins 121
2. Plasma Isotope Analysis 136
3. Intramural Distribution of LDL and Albumin 149
4. Chemical Identity of Labeled Solute in the Vessel Wall 174

B. Hydraulic Permeability 176

VI. Theory—Convection and Diffusion Across Aortic Tissue 183
A. General Formulation 188
1. Governing Partial Differential Equation 188
2. Endothelial-side Boundary Condition 191
3. Media-Adventitia Interface: Boundary Condition 194

B. Convection and Diffusion—No Reaction 204
1. Solution 204
2. Effect of $T_1$ 214
3. Effect of Peclet Number, $P$ 220
4. Effect of effective Diffusivity, $D_{eff}$ 226
5. Effect of Equilibrium Constant, $\kappa$ 227
6. Effect of $\eta=1$ Boundary Condition 233
7. Effect of Solute Rejection at $\eta=1$ 251

C. Convection and Diffusion with Reaction 256
1. Binding Model 256
2. Solution 260
3. Effect of Binding 263
4. Nonuniform Binding
5. Spatially Nonuniform $D_{\text{eff}}$ and Binding
6. Analysis of the Experimental Albumin Profiles

D. Summary

VII. Discussion of Results
A. Entry of Labeled Albumin and LDL into Arterial Tissue
B. Distribution of Labeled LDL and Labeled Albumin
   1. Behavior of Concentration Profile Near $\eta = 0$
   2. Behavior of Concentration Profile Near $\eta = 1$
   3. Concentration Profiles Between $\eta = 0$ and $\eta = 1$
C. Comparison of LDL and Albumin Total Solute Accumulation
D. Experimental Techniques
E. Hydraulic Permeability of Rabbit Aorta

VIII. Conclusions and Recommendations

IX. Appendix
A. Theories of Atherogenesis Relating to the Transport Properties of Arterial Tissue
   1. Injury Theories
   2. Theories Related to the Metabolism of the Blood Vessel Wall—Hypoxia
   3. Summary of Theories of Atherogenesis
B. Sample Calculations
   1. Solute Distribution Studies
   2. Hydraulic Permeability Calculations
C. Details of Experimental Apparatus and Procedures
   1. Hydraulic Permeability Studies
   2. Double Isotope Radioactivity Analysis
D. Details of Experimental Results—Solute Distribution Studies
E. Solutions to Partial Differential Equations
1. Convection and Diffusion without Internal Reaction 470
2. Convection and Diffusion with Reversible Internal Reaction 479
3. Convection and Diffusion with Nonuniformly Reversible Internal Reaction 487

F. Computer Solutions 496
   1. Analytical Solution 496
   2. Numerical Solution 505

G. Data Tables 518

H. Nomenclature 536

I. Literature Citations 539

J. Biographical Note 559
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Number</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cross-Sectional View of Aortic Wall</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>Morphology of Adjoining Endothelial Cells</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>Possible Transport Modes</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>Aortic Hydraulic Permeability</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>Distribution of $^{125}$I-Low Density Lipoprotein Across Canine Aorta</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>Rabbit Aortic Distributions</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>Distribution of $^{131}$I-Fibrinogen and $^{131}$I-Albumin Across Hog Aortic Wall</td>
<td>95</td>
</tr>
<tr>
<td>8</td>
<td>Distribution of ($^3$H)Cholesterol Across Hog Aortic Wall</td>
<td>96</td>
</tr>
<tr>
<td>9</td>
<td>An Integrated View of Transport Processes Within the Aortic Wall</td>
<td>98</td>
</tr>
<tr>
<td>10</td>
<td>Summary of Experimental Procedures</td>
<td>105</td>
</tr>
<tr>
<td>11</td>
<td>Tissue Slicing Techniques—Area Corrections</td>
<td>110</td>
</tr>
<tr>
<td>12</td>
<td>Hydraulic Permeability Experimental Apparatus</td>
<td>118</td>
</tr>
<tr>
<td>13</td>
<td>Immunoelectrophoresis of Iodinated LDL</td>
<td>122</td>
</tr>
<tr>
<td>14</td>
<td>Immunodiffusion of Iodinated LDL</td>
<td>124</td>
</tr>
<tr>
<td>15</td>
<td>Gel Permeation Chromatography—Human $^{125}$I-LDL Elution Through G-200 Sephadex</td>
<td>126</td>
</tr>
<tr>
<td>16</td>
<td>Gel Permeation Chromatography—Rabbit $^{125}$I-LDL Elution Through G-200 Sephadex</td>
<td>127</td>
</tr>
<tr>
<td>17</td>
<td>Gel Permeation Chromatography—Rabbit $^{125}$I-Albumin Elution Through G-200 Sephadex</td>
<td>128</td>
</tr>
<tr>
<td>18</td>
<td>Paper Electrophoresis of $^{125}$I-LDL Rabbit Plasma</td>
<td>130</td>
</tr>
</tbody>
</table>
Paper Electrophoresis of Rabbit $^{125}$I-LDL Injectates: Radioactivity Distribution

Paper Electrophoresis of Human $^{125}$I-LDL Injectates: Radioactivity Distribution

Paper Electrophoresis of Rabbit $^{125}$I-Albumin Plasma: Radioactivity Distribution

$^{125}$I-LDL Plasma Decay: Range Over All Experiments

$^{125}$I-Albumin Plasma Decay: Range Over All Experiments

Double Exponential Decay of Labeled Albumin and LDL

Double Exponential Decay for all Albumin Experiments 4 Hours and Longer—Least Squares Analysis

Double Exponential Decay for all LDL Experiments 4 Hours and Longer—Least Squares Analysis

Distribution of Label in Terminal Rabbit Plasma Ultracentrifugation Fractions

Paper Electrophoresis—Human $^{125}$I-LDL—Exp 154—Terminal Plasma (30 minutes)

Paper Electrophoresis—Rabbit $^{125}$I-LDL—Exp 155—Terminal Plasma Sample (30 minutes)

Effect of Prescreening on the Clearance of Labeled Proteins from Rabbit Plasma

Grand Summary of LDL Distribution Data—10 minutes through 4 Hours

Grand Summary of LDL Distribution Data—4 Hours Through 67 Hours

Average Net Accumulation of Labeled LDL Expressed Relative to Initial Plasma Isotope Concentration

Average Net Accumulation of Labeled LDL Expressed Relative to the Time-Averaged Plasma Isotope Concentration

Comparison of Raw Profile Data and the Experimental Average Profile (Exp 157)—t=10 minutes
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>Comparison of Individual Profile Data with the Experimental Average-LDL-t=4 Hours</td>
</tr>
<tr>
<td>37</td>
<td>Comparison of Experimental Average Profiles for 4 Hour Labeled LDL Penetration</td>
</tr>
<tr>
<td>38</td>
<td>Comparison of Labeled Rabbit LDL and Human LDL Distributions</td>
</tr>
<tr>
<td>39</td>
<td>Comparison of Penetration of Screened and Unscreened Labeled LDL in the Same Animal</td>
</tr>
<tr>
<td>40</td>
<td>Grand Summary of Albumin Distribution Data</td>
</tr>
<tr>
<td>41</td>
<td>Comparison of Labeled LDL and Labeled Albumin Average Net Accumulation Expressed Relative to the Initial Plasma Isotope Concentration</td>
</tr>
<tr>
<td>42</td>
<td>Comparison of Labeled LDL and Labeled Albumin Average Net Accumulation Expressed Relative to the Time-Averaged Plasma Radioactivity</td>
</tr>
<tr>
<td>43</td>
<td>Distribution of $^{131}$I-Albumin and $^{125}$I-LDL After 30 Minutes</td>
</tr>
<tr>
<td>44</td>
<td>Comparison of Hydraulic Permeability of Normal and Atherosclerotic Aortas</td>
</tr>
<tr>
<td>45</td>
<td>Comparison of Apparent Flows Observed When Both Chambers Are Covered (Rabbit Aorta)</td>
</tr>
<tr>
<td>46</td>
<td>Difference in Apparent Flow Rates (Both Chambers Covered)</td>
</tr>
<tr>
<td>47</td>
<td>Apparent Volumetric Flux Through Normal Rabbit Aorta (Exp 107)</td>
</tr>
<tr>
<td>48</td>
<td>Apparent Volumetric Flux Through Normal Rabbit Aorta (Exp 121)</td>
</tr>
<tr>
<td>49</td>
<td>Apparent Volumetric Flux Across Atherosclerotic Rabbit Artery</td>
</tr>
<tr>
<td>50</td>
<td>Apparent Volumetric Flux Across Atherosclerotic Rabbit Aorta (Exp 120)</td>
</tr>
<tr>
<td>51</td>
<td>Microscopic En Face View of Aortic Endothelium</td>
</tr>
<tr>
<td>52</td>
<td>Lesion Coverage of Atherosclerotic Aortas</td>
</tr>
<tr>
<td>53</td>
<td>Material Balance on Infinitesimal Element of the Aortic Wall</td>
</tr>
</tbody>
</table>
54 Schematic Diagram of Capillaries and Lymphatic Terminals at Media-Adventitia Interface

55 Material Balance Over Extravascular Adventitial Space

56 Effect of $T_1$-Equilibrium Boundary Condition - $t=0.167$ Hours

57 Effect of $T_1$-Equilibrium Boundary Condition - $t=0.5$ Hours

58 Effect of $T_1$-Equilibrium Boundary Condition - $t=4$ Hours

59 Effect of $T_1$-Equilibrium Boundary Condition - $t=24$ Hours

60 Effect of $T_1$-Equilibrium Boundary Condition - $t=67$ Hours

61 Effect of $P$-Equilibrium Boundary Condition - $t=0.167$ Hours

62 Effect of $P$-Equilibrium Boundary Condition - $t=0.5$ Hours

63 Effect of $P$-Equilibrium Boundary Condition - $t=4$ Hours

64 Effect of $P$-Equilibrium Boundary Condition - $t=24$ Hours

65 Effect of $P$-Equilibrium Boundary Condition - $t=67$ Hours

66 Effect of $D_{eff}$-Equilibrium Boundary Condition - $t=0.167$ Hours

67 Effect of $D_{eff}$-Equilibrium Boundary Condition - $t=0.5$ Hours

68 Effect of $D_{eff}$-Equilibrium Boundary Condition - $t=4$ Hours

69 Effect of $D_{eff}$-Equilibrium Boundary Condition - $t=24$ Hours

70 Effect of $D_{eff}$-Equilibrium Boundary Condition - $t=67$ Hours
<p>| Effect of $T_1$ - No Diffusion at $\eta=1-t=0.167$ Hours | 71 |
| Effect of $T_1$ - No Diffusion at $\eta=1-t=0.5$ Hours | 72 |
| Effect of $T_1$ - No Diffusion at $\eta=1-t=4$ Hours | 73 |
| Effect of $T_1$ - No Diffusion at $\eta=1-t=24$ Hours | 74 |
| Effect of $T_1$ - No Diffusion at $\eta=1-t=67$ Hours | 75 |
| Effect of $P$ - No Diffusion at $\eta=1-t=0.167$ Hours | 76 |
| Effect of $P$ - No Diffusion at $\eta=1-t=0.5$ Hours | 77 |
| Effect of $P$ - No Diffusion at $\eta=1-t=4$ Hours | 78 |
| Effect of $P$ - No Diffusion at $\eta=1-t=24$ Hours | 79 |
| Effect of $P$ - No Diffusion at $\eta=1-t=67$ Hours | 80 |
| Effect of $T_1$ - Complex Boundary Condition - $t=0.167$ Hours | 81 |
| Effect of $T_1$ - Complex Boundary Condition - $t=0.5$ Hours | 82 |
| Effect of $T_1$ - Complex Boundary Condition - $t=4$ Hours | 83 |
| Effect of $T_1$ - Complex Boundary Condition - $t=24$ Hours | 84 |
| Effect of $T_1$ - Complex Boundary Condition - $t=67$ Hours | 85 |
| Effect of $T_1/T_2$ - Complex Boundary Condition | 86 |
| Effect of $P$ and $R_2$ - Complex Boundary Condition - $t=0.5$ Hours | 87 |
| Effect of $P$ and $R_2$ - Complex Boundary Condition - $t=4$ Hours | 88 |
| Effect of $P$ and $R_2$ - Complex Boundary Condition - $t=24$ Hours | 89 |
| Effect of Binding - $t=0.167$ Hours | 90 |
| Effect of Binding - $t=0.5$ Hours | 91 |
| Effect of Binding - $t=4$ Hours | 92 |</p>
<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
</tr>
<tr>
<td>94</td>
</tr>
<tr>
<td>95</td>
</tr>
<tr>
<td>96</td>
</tr>
<tr>
<td>97</td>
</tr>
<tr>
<td>98</td>
</tr>
<tr>
<td>99</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>101</td>
</tr>
<tr>
<td>102</td>
</tr>
<tr>
<td>103</td>
</tr>
<tr>
<td>104</td>
</tr>
<tr>
<td>105</td>
</tr>
<tr>
<td>106</td>
</tr>
<tr>
<td>107</td>
</tr>
<tr>
<td>108</td>
</tr>
<tr>
<td>109</td>
</tr>
<tr>
<td>110</td>
</tr>
<tr>
<td>111</td>
</tr>
<tr>
<td>112</td>
</tr>
<tr>
<td>113</td>
</tr>
</tbody>
</table>
114 Effect of P-Nonuniform Diffusivity and Nonuniform Binding-\( t=0.5 \) Hours

115 Effect of P-Nonuniform Diffusivity and Nonuniform Binding-\( t=4 \) Hours

116 Effect of P-Nonuniform Diffusivity and Nonuniform Binding-\( t=24 \) Hours

117 Effect of P-Nonuniform Diffusivity and Nonuniform Binding-\( t=64 \) Hours

118 Effect of \( D_{\text{eff}} \)-Nonuniform Diffusivity and Nonuniform Binding-\( t=0.167 \) Hours

119 Effect of \( D_{\text{eff}} \)-Nonuniform Diffusivity and Nonuniform Binding-\( t=0.5 \) Hours

120 Effect of \( D_{\text{eff}} \)-Nonuniform Diffusivity and Nonuniform Binding-\( t=4 \) Hours

121 Effect of \( D_{\text{eff}} \)-Nonuniform Diffusivity and Nonuniform Binding-\( t=24 \) Hours

122 Effect of \( D_{\text{eff}} \)-Nonuniform Diffusivity and Nonuniform Binding-\( t=64 \) Hours

123 Effect of Binding Parameters-Nonuniform Diffusivity-\( t=0.167 \) Hours

124 Effect of Binding Parameters-Nonuniform Diffusivity-\( t=0.167 \) Hours

125 Effect of Binding Parameters-Nonuniform Diffusivity-\( t=0.5 \) Hours

126 Effect of Binding Parameters-Nonuniform Diffusivity-\( t=4 \) Hours

127 Effect of Binding Parameters-Nonuniform Diffusivity-\( t=24 \) Hours

128 Distribution of Bound and Mobile Solute-\( t=4 \) Hours

129 Distribution of Bound and Mobile Solute-\( t=24 \) Hours

130 Labeled LDL Distribution Near Media-Adventitia Interface

131 Preparation of LDL-Experimental Procedures
C.1 Hydraulic Permeability Filtration Cell
C.2 Rabbit Aorta Stretching Rack
C.3 $^{125}$I-Energy Spectrum, $^{131}$I-Energy Spectrum
D.1 Distribution of H$_2$O Across Rabbit Thoracic Aorta In Vitro
D.2 Average Distribution of $^{3}$H$_2$O Across Rabbit Thoracic Aorta In Vitro
D.3 "Labeled Free Iodide" Distribution Across Rabbit Thoracic Aorta In Vivo
D.4 Comparison of Experimental Average Profiles for 10 Minute Labeled LDL Penetration
D.5 Comparison of Experimental Average Profile for 30 Minute Labeled LDL Penetration
D.6 Comparison of Experimental Average Profile for 24 Hour Labeled LDL Penetration
D.7 Comparison of Experimental Averages for 67 Hour Labeled LDL Penetration
D.8 LDL Plasma Decay—67 Hour Experiments
D.9 Comparison of Experimental Average Profiles for 10 Minute Labeled Albumin Penetration
D.10 Comparison of Experimental Average for 30 Minute Labeled Albumin Penetration
D.11 Comparison of Experimental Average Profiles for 4 Hour Labeled Albumin Penetration
D.12 Comparison of Experimental Average Profiles for 24 Hour Labeled Albumin Penetration
D.13 Comparison of 30 Minute and 4 Hour Labeled LDL Accumulation in Rabbit Thoracic Aorta of the Same Animal
D.14 Comparison of 4 Hour and 24 Hour Labeled LDL Accumulation in Rabbit Thoracic Aorta of the Same Animal
D.15 Effect of Anesthetizing the Rabbit on Labeled LDL Distribution (t=30 minutes)
<table>
<thead>
<tr>
<th>Number</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Comparison of Serum LDL, Perifibrous Lipid in Normal Intima and Early Fibrous Lesions, and the Intracellular Lipid in Fatty Streaks</td>
<td>67</td>
</tr>
<tr>
<td>II.</td>
<td>The Plasma Lipoprotein Spectrum (10)</td>
<td>70</td>
</tr>
<tr>
<td>III.</td>
<td>Distribution of Radioactivity ($^{125}$I-LDL) After Analytical Ultracentrifugation in SW41 Rotor</td>
<td>135</td>
</tr>
<tr>
<td>IV.</td>
<td>Plasma Isotope Half-life</td>
<td>139</td>
</tr>
<tr>
<td>V.</td>
<td>Mean LDL Accumulation</td>
<td>152</td>
</tr>
<tr>
<td>VI.</td>
<td>Mean Albumin Accumulation</td>
<td>167</td>
</tr>
<tr>
<td>VII.</td>
<td>Comparison of Grand Average Albumin and LDL Distribution Data</td>
<td>169</td>
</tr>
<tr>
<td>VIII.</td>
<td>Radioactivity Extracted From Abdominal Aortic Tissue Homogenates</td>
<td>175</td>
</tr>
<tr>
<td>IX.</td>
<td>Condition of Endothelium at the End of Hydraulic Permeability Experiments</td>
<td>186</td>
</tr>
<tr>
<td>X.</td>
<td>Uptake of Labeled LDL by Human Aorta (21)</td>
<td>330</td>
</tr>
<tr>
<td>XI.</td>
<td>Total Volume of Fluid Displaced From Covered Tissue</td>
<td>353</td>
</tr>
<tr>
<td>B.I.</td>
<td>Range of Dimensionless Depths Used For Grouping Relative Tissue Concentration Data</td>
<td>394</td>
</tr>
<tr>
<td>B.II.</td>
<td>Calculated Average Profile-Experiment 165-LDL-24 Hours</td>
<td>397</td>
</tr>
<tr>
<td>B.III.</td>
<td>Grand Average-24 Hour LDL Data</td>
<td>399</td>
</tr>
<tr>
<td>C.I.</td>
<td>Spill Over of $^{125}$I and $^{131}$I into Alternate Counting Channel</td>
<td>411</td>
</tr>
<tr>
<td>D.I.</td>
<td>Reproducibility of Slicing Technique-Successive 20μ Sections of $^3$H₂O at -10.9°C</td>
<td>413</td>
</tr>
</tbody>
</table>
D.II. Volume Fraction of $^3$H$_2$O Found in Aortic Tissue Slices After In Vitro Incubation

D.III. Distribution of $^{125}$I Across Rabbit Aorta In Vivo 30 Minutes After Free $^{125}$I Injection

D.IV. Ratio of $^3$H$_2$O and $^{125}$I Volume Fractions in Aortic Tissue

D.V. Effect of TCA Washings on the Removal of Free Iodine From Tissue Slices

D.VI. Fraction of Radioactivity Removed By Successive Washes with 10% TCA-30 Minute Experiment

D.VII. Fraction of Radioactivity Removed By Successive Washes with 10% TCA-24 Hour Experiments

D.VIII. Fraction of Tissue $^{125}$I(LDL) Radioactivity Attributable to Blood Contamination

D.IX. 10 Minute LDL Data-Media-Adventitia Junctions Matched

D.X. 30 Minute LDL Data-Media-Adventitia Junctions Matched

D.XI. Four Hour LDL Data-Media-Adventitia Junctions Matched

D.XII. 24 Hour LDL Data-Media-Adventitia Junctions Matched

D.XIII. 67 Hour LDL Data-Media-Adventitia Junctions Matched

D.XIV. 10 Minute Albumin Data-Media-Adventitia Junctions Matched

D.XV. 30 Minute Albumin Data-Media-Adventitia Junctions Matched

D.XVI. 4 Hour Albumin Data-Media-Adventitia Junctions Matched

D.XVII. 24 Hour Albumin Data-Media-Adventitia Junctions Matched

D.XVIII. LDL Iodination Efficiencies and Injectate Precipitable Radioactivity
D.IX. Albumin Iodination Efficiencies and Injectate Precipitable Radioactivity 466

D.XX. % Radioactivity in Ethanol-Ether Extracts of Iodinated LDL Preparations 468

D.XXI. Summary of Plasma Cholesterol Analysis 469

G.I. Summary of LDL Plasma Isotope Decay 519

G.II. Summary of Albumin Plasma Isotope Decay 525

G.III. Summary of Tissue-Slice Area Data 529

G.IV. Normal Rabbit Aorta Hydraulic Flux Data 532

G.V. Hydraulic Permeability Control Experiment—Both Chambers Covered 533

G.VI. Atherosclerotic Rabbit Aorta: Hydraulic Permeability 534

G.VII. Rabbit Aorta Hydraulic Flux Variation Over Time 535
II. SUMMARY

The purpose of this thesis is to develop a more fundamental understanding of the factors affecting the transport of macromolecules into and across the walls of large blood vessels. Attention is focused on elucidating the phenomena which influence the distribution of solute across aortic tissue, in particular the relative importance of pressure-driven convection, diffusion and reaction with wall components. Fundamental knowledge of the transport properties of arterial tissue may be relevant to understanding the processes which cause the disease, atherosclerosis. Atherosclerosis, broadly defined, is the disease state characterized by the accumulation of fatty substances within the arterial wall. The largest single constituent of this lipid accumulation is cholesterol, which is carried in the bloodstream by lipoproteins. Although blood vessels do have the capability of synthesizing small quantities of this lipid, most investigators believe that the majority of the accumulated cholesterol originates in the circulating blood plasma. Thus, an integral part of the disease process, if not also the cause, involves the transport of cholesterol into the blood vessel wall and its subsequent accumulation therein.

A. Background

The aorta is the largest blood vessel in mammals, being the main conduit for blood leaving the heart. It is composed of three distinct layers, the intima, the media and the adventitia. The intima, or innermost layer, consists in part of a layer of cells
(endothelium) which separate the flowing blood from the rest of the aortic wall (Fig. 1). The endothelium is thought to regulate the transfer of solutes into the vessel wall, only partially restricting the passage of small solutes (e.g., < 40Å Stokes radius) while almost totally rejecting larger solutes. The media is made up of structural tissue, mainly smooth muscle cells, collagen and elastin. The adventitia, or outer layer, is penetrated with capillaries and lymphatic vessels. In large mammals, capillaries enter the media as well. However, cells in the center of the media are quite distant from the nearest capillary both in small and large mammal aorta. This suggests solute transport by convection or diffusion either from the adventitia or from the intima is required to meet the nutritional demands of these cells.

In the diseased state the aortic wall takes on different characteristics. The intima becomes thickened, and, in the advanced stages of disease, large deposits of extracellular cholesterol and other lipids are found. The chemical composition of the lipids in these deposits is similar to the lipid composition of the blood (8). This is taken as evidence that the accumulated cholesterol originates in the blood plasma. Most of the cholesterol in blood is carried as part of a class of molecules known as low-density lipoproteins (LDL). As the name implies, these are lipid-protein complexes which have a characteristic hydrated density between 1.006 and 1.063 gm/ml. Because of the correlation between plasma cholesterol concentration and the incidence of atherosclerosis, LDL transport into the arterial wall has been implicated, although not proven as a causal
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24
factor in the development of the disease.

The uptake of plasma cholesterol by arterial tissue is thought to occur both by transport from the adventitial capillaries and from the lumen of the large arteries across the intimal endothelium. The question of whether or not the cholesterol crosses the endothelium as part of an intact LDL molecule is unresolved. One school of thought is that the plasma lipid dissociates from its protein carrier at the blood-endothelium interface and, because of its hydrophobic properties, subsequently diffuses within the endothelial cell membrane to the subendothelial space. Another possibility is that intact LDL is carried from the plasma to the subendothelial space by a process known as vesicular transport or pinocytosis. In this process special organelles, called vesicles, are postulated to "ferry" solutes in both directions across the width of endothelial cells (see Fig. 3). For other solutes with appropriate characteristic molecular sizes, an additional route of entry is conceivable. They may either diffuse or be convected through the slit created by the junction between two endothelial cells. The effective junction width is thought to be approximately 80Å. Thus, a molecule the size of LDL (characteristic dimension = 195Å) would be sterically excluded from the junctions. If intact LDL is transported across the endothelium, the most likely mechanism is vesicular transport. However, it remains to be proven that LDL is actually transported in this manner.

What happens after solute has crossed the intimal endothelium is also poorly understood. Presumably it either diffuses or is convected toward the adventitia. The effective diffusion coefficient
Figure 3: POSSIBLE TRANSPORT MODES

- Pinocytosis
- Exchange-Diffusion
- Pore Diffusion and/or Convection

Lumen
Endothelium
Media
is likely to be smaller than in aqueous solutions because of the heterogenous structure (70% water) of the media. Interactions with wall components such as elastin and acid mucopolysaccharides may also influence the transport rate. No studies have been reported on the rate of diffusive LDL transport across arterial tissue. What has been shown is that LDL reacts with certain mucopolysaccharides and elastin in \textit{in vitro} systems. This has led to the speculation that these components might bind LDL in the aortic wall (77, 78). If so, this might explain the propensity for cholesterol to accumulate.

Just as little is known about the diffusive transport of LDL through aortic tissue, so research on the convective movement of solute is limited. A pressure gradient exists across the aortic wall, from 100 mm Hg on the intimal side to near 0 mm Hg on the adventitial side. Transmural hydraulic fluxes on the order of 0.01 ml/cm²-hr have been measured \textit{in vitro} on the human iliac artery (106) and the rabbit aorta (112). Because of the nonphysiological conditions of these experiments, however, controversy exists as to whether this hydraulic flow actually exists \textit{in vivo}. Moreover, no one has studied how this hydraulic flow might affect the intramural movement of LDL nor has the relative importance of diffusive and convective transport been assessed.

Besides the entry and movement of solute across the wall, also important in the transport process is how solute is cleared from the tissue. Inasmuch as accumulation is the net effect of influx and efflux, the malfunction of the removal mechanism(s) may be a contributing factor in the propagation of the atherosclerotic process.
In most tissue, proteins which pass from the blood stream into the extravascular space are returned to the circulation via the lymphatic system. In the human aorta, lymphatic terminals exist in the outer media and in the adventitia. In other species, such as the rabbit, the exact location of these terminals relative to the media-adventitia interface is not known. The mechanism whereby lymphatic vessels remove interstitial fluid is both convective and diffusive. Solute-containing fluid is thought to pass through the junctions of the lymphatic endothelium as well as gain entry by vesicular transport. Thus, the LDL, which convects and/or diffuses across the aortic media, is most likely removed by the lymphatic system. Unfortunately, the rate of lymphatic removal is not known. Thus, its importance in the cholesterol accumulation process has not been assessed.

The only studies which bear on the relative importance of convection and diffusion of macromolecules across aortic tissue are qualitative. The intramural distribution of labeled albumin, labeled cholesterol and a labeled mixture of β- and γ-globulins has been determined for rabbits (128 – 130) and swine (132 – 134) at various intervals ranging up to four days after the injection of the labeled material. The results from the studies are in conflict. In the swine experiments, cholesterol and albumin entered the wall primarily through the endothelium. The rabbit studies suggest that albumin and β- and γ-globulins (LDL is a β-globulin) enter mainly from the adventitial side. However, questionable experimental techniques, such as failure to safeguard against post mortem diffusion and poor tissue sectioning procedures, may render some or all of the conclusions from these
studies of little value from a quantitative standpoint. Moreover, the
data presented are insufficient to permit theoretical analysis in
terms of the relative contributions of diffusion and convection in the
movement of solute within the media.

In summary, none of the previous research in the field has
approached the problem of LDL transport across the aortic wall from an
integrated point of view, considering at the same time the rates of
influx, efflux and the solute transport mechanisms within the wall.
The approach taken in this thesis was to improve experimental methods
of previous investigators and to determine the intramural distribution
of labeled LDL and labeled albumin across the aortic wall of the
rabbit as a function of time. A mathematical transport model including
diffusion, convection and solute interaction with wall constituents
was derived to permit interpretation of the results in terms of
potential factors affecting the transport of macromolecules into and
across blood vessel walls.

B. Experimental Methods

The overall procedure was to inject radio-iodinated LDL or
albumin into rabbits, and after a predetermined interval (1/6, 1/2, 4,
24 or 67 hours) sacrifice the animal and excise the aorta. The
intramural radial distribution of radioactivity was established by
counting serial tissue sections prepared on a cryotome. Multiple
experiments were carried out with each solute at each time interval.

A summary of the procedures used to isolate LDL from human plasma
is provided on Fig. 13. LDL in the density range 1.025 - 1.050 gm/ml
was prepared by ultracentrifugal fractionation of freshly drawn plasma.
Citrated Plasma ($\rho_s = 1.010$)

Ultracentrifugation-$\rho_s = 1.025$
(20 hr; 40,000 rpm)

$\rho < 1.025$ discard

High Density KBr-NaCl ($\rho_s = 1.35$)

Ultracentrifugation-$\rho_s = 1.050$
(20 hr; 40,000 rpm)

$\rho > 1.050$ discard

LDL ($1.025 < \rho < 1.050$)

Figure 131 Preparation of LDL—Experimental Procedures
Iodination of both LDL and albumin was performed by the iodine monochloride method. Radioactive iodine is substituted into tyrosine rings along the protein polypeptide backbone. Thus, in the case of LDL, only the protein moiety was labeled. Almost none of the label was associated with the LDL lipids. The purity of the labeled preparations was confirmed by immunoelectrophoresis, immunodiffusion, paper electrophoresis and gel permeation chromatography. No contaminating labeled protein could be detected. Virtually all of the nonprotein-bound labeled compounds (presumably inorganic iodide) were removed prior to injection by dialysis. Thus, the injectates consisted of labeled LDL or labeled albumin free of any detectable contamination.

The injectates were administered to rabbits (New Zealand white) via the ear vein. Blood samples were taken throughout the experiment to establish the change in plasma isotope concentrations over time. The animal was sacrificed with an overdose of anesthetic, and the aorta quickly isolated and removed. To prevent post mortem diffusion of the solutes within the aortic wall, the tissue was immediately frozen after having been gently sandwiched flat between two glass microscope slides. This freezing step represents a significant improvement over the techniques used by previous investigators.

The distribution of solute across the wall was determined by counting successive radial sections prepared on a cryotome. In the sectioning technique (see Fig. 10) small (~0.7 cm²) pieces of the aorta were mounted endothelial side face-down on frozen tissue embedding medium. The surface of the embedding medium was parallel to
Summary of Experimental Procedures

Figure 10.
the knife cutting plane so as to ensure that each section was taken in a plane parallel to the endothelial surface. Sections were cut at 20μ and all media and near-media adventitial slices transferred to precooled test tubes and set aside for subsequent counting. Special note was made of any slices which were visibly contaminated with blood. Blood contamination was sometimes prevalent in slices taken near the media-adventitia interface. The results from these slices were not used in the final data analysis. On average the rabbit aortic intima plus media resulted in 12 sections of 20μ each. As many as nine profiles were determined from one aorta.

The tissue samples were counted on a gamma well-spectrometer. Before counting, all nonprotein-bound radioactivity was removed by successive washes with 10% trichloracetic acid. Thus, the radioactivity which was counted represented only isotope which was still bound to protein. The results were expressed as the concentration of isotope in each tissue sample relative to the initial plasma isotope concentration. All profiles for a given solute at a particular time interval were grouped according to their position relative to the aortic intima and media-adventitia interface and then averaged. This resulted in a grand average profile for both albumin and labeled LDL at each time interval studied.

C. Experimental Results

The grand average profiles for LDL are presented on Figs. 31 and 32. The ten minute distribution indicates the presence of labeled LDL at low concentrations throughout the entire aorta. The shape of the profile suggests that labeled solute entered the media across the
Figure 31 Grand Summary of LDL Data—10 Minutes Through 4 Hours

- INTIMA
- TIME AFTER INJECTION
  - 10 MIN
  - 30 MIN
  - 4 HR

- MEDIA - ADVENTITIA BORDER

Relative Tissue Concentration, \( \frac{c}{c_p}(0) \times 10^3 \)

Relative Position, \( x/L \)
Figure 32  Grand Summary of LDL Distribution Data-4 Hours Through 67 Hours

TIME AFTER INJECTION

- ▲ 4 HRS
- ■ 24 HRS
- ● 67 HRS

Relative Tissue Concentration, $c/c_p(0) \times 10^3$

Relative Position $x/L$
intima and from the adventitia. These results are in accord with experiments performed on swine but at odds with previous rabbit studies. At subsequent times up to four hours the tissue concentration was higher, but a characteristic gradient over the inner 30% of the media was maintained. At still later times (24 and 67 hours), the concentration near the intima declined. However, the level near the media-adventitia interface remained relatively constant. The removal of labeled solute from the vessel wall is due to the decay in plasma isotope concentration during the course of the experiment. As shown on Fig. 24, the plasma isotope concentration fell to approximately 25% of its initial level after 24 hours. This decay could be approximated by the double exponential function shown on Fig. 24. After 67 hours, 4 - 15% of the initial concentration remained.

The labeled albumin grand average profiles are presented on Fig. 4D. Just as in the LDL studies, labeled solute penetrated the entire 240µ thick media in less than ten minutes, entering both through the intima and from the adventitia. Labeled solute concentrations increased at each subsequent time interval up to 24 hours. No 67 hour studies were performed with labeled albumin. In contrast to the LDL profiles, the albumin distributions tended to be more U-shaped with much higher concentrations found at all relative positions. However, due to scatter in the data, only near the intima were the differences between the albumin and LDL results statistically significant ($p < 0.05$). The plasma isotope decay rate was less in the albumin experiments. This is shown on Fig. 24. After 24 hours
Least Squares Regression:
\[
\left[ \frac{C_p(t)}{C_p(0)} \right] = 0.25 e^{-1.557t} + 0.75 e^{-0.0254t}
\]
\[(125\text{I} - \text{Albumin})\]

Least Squares Regression:
\[
\left[ \frac{C_p(t)}{C_p(0)} \right] = 0.29 e^{-0.777t} + 0.71 e^{-0.0434t}
\]
\[(125\text{I} - \text{LDL})\]

Figure 24 Double Exponential Decay of Labeled Albumin and LDL
Figure 40  Grand Summary of Albumin Distribution Data
the plasma isotope concentration was approximately 40% of its initial level.

The difference in the accumulation rates of both solutes is best appreciated on Fig. 41 where the average relative tissue concentration, determined by integrating the grand average profiles, is higher at every time interval. Note also that LDL accumulation goes through a maximum between $\frac{1}{2}$ and 24 hours whereas the albumin accumulation is monotonically increasing over this same interval. This difference is due in part to the faster LDL plasma isotope decay rate. Considering just the short term accumulation data during which time the relative plasma isotope decay rates are not a factor, one concludes that albumin uptake is greater than for LDL.

The significance of the experimental results is that they represent a more reliable estimate of the intramural distribution of labeled albumin and LDL in rabbit aorta than previous studies provided due to improvements in experimental technique. More importantly, they demonstrate how rapidly sudden changes in plasma solute concentration affect the solute concentration in the aortic wall. While labeled solute concentrations were quite small relative to plasma levels, the profiles indicate that the intimal endothelium does permit passage of protein-labeled LDL. Hence, LDL levels in plasma influence the LDL concentration in aortic tissue.

D. Theory

To interpret the LDL profiles from a more fundamental point of view, a theoretical model was developed. The aortic wall was treated
Figure 41: Comparison of Labeled LDL and Labeled Albumin Average Net Accumulation Expressed Relative to the Initial Plasma Isotope Concentration.
as a continuum and transport within the media was assumed to be by both convection and diffusion with the possible interaction of solute and wall components taken into account. Labeled solute was taken to cross the endothelium solely by vesicular transport. At the media-adventitia interface, both convection and diffusion were allowed. This is shown schematically on Fig. 9. The interaction between LDL and wall components was modeled as follows:

\[ a + b \xrightarrow{\text{vesicular transport}} c \]  

(2.1)

where \(a\) is the mobile LDL, \(b\), the binding substrate (e.g., acid mucopolysaccharides), and \(c\), the LDL-substrate complex. The kinetics were taken to be of the form:

\[
\frac{\partial C}{\partial t} = k_1 a b - \frac{k_2}{c} = k_1 c - k_2 c
\]

(2.2)

for the binding substrate concentration, \(C_b\), in large excess. This model was selected arbitrarily in that the true reaction mechanism and kinetics are not known.

Because the thickness of the rabbit aorta is roughly 10% of its internal radius, the problem could be cast in Cartesian rather than cylindrical coordinates without significant loss in accuracy. The overall governing partial differential equation for diffusion and convection across aortic media of width, \(L\), is:

\[
\frac{\partial}{\partial x} \left\{ \frac{D}{\text{eff}} \frac{\partial C}{\partial x} \right\} - \frac{J_c}{D} \frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} + \frac{\partial C}{\partial t}
\]

(2.3)
Figure 9. An Integrated View of Transport Processes within the Aortic Wall.
where the subscript, a, has been dropped for convenience. The boundary conditions are:

(1) at the endothelium, $x = 0$:

$$J_f C_p(t)(1-R_1) + \frac{K_1}{\varepsilon}(C_p(t) - C) = \frac{J_f}{\varepsilon} C - D \frac{\partial C}{\partial x}$$  \hspace{1cm} (2.4a)

(2) at the media-adventitia interface, $x = L$:

$$\frac{K_1 A_c}{A_m} (C - C_p(t)) + \frac{K_1 A_c}{A_m} \frac{J_f}{\varepsilon} C(1-R_2) = \frac{J_f}{\varepsilon} C - D \frac{\partial C}{\partial x}$$  \hspace{1cm} (2.4b)

$$t = 0, \text{ all } x: \quad C = C_c = 0$$  \hspace{1cm} (2.4c)

where $D_{\text{eff}}$ is the effective solute diffusion coefficient; $J_f$, the convective superficial velocity; $C_p(t)$, the plasma isotope concentration; $R_1$ and $R_2$, the solute rejection coefficients at $x = 0$ and $x = L$; $K_1$, $K_c$ and $K_L$ are mass transfer coefficients (discussed below); $\varepsilon$, the tissue void fraction available for LDL accumulation; and $A_c$, $A_L$ and $A_m$, the surface areas of the capillary, lymphatic and luminal endothelium, respectively. The plasma isotope concentration was assumed to decay as described by:

$$\frac{C_p(t)}{C_p(0)} = \alpha_1 e^{-\beta_1 t} + \alpha_2 e^{-\beta_2 t}$$  \hspace{1cm} (2.5)

where the constants $\alpha_i$ and $\beta_i$ were determined from experimental data ($\alpha_1 + \alpha_2 = 1$).
The boundary condition at the endothelial surface \((x = 0)\) is written in its most general form to take into account the possibility that some solutes may enter the vessel wall by diffusion and convection through the endothelial junctions (i.e., \(R_1 \neq 1\)). For LDL, total solute rejection is assumed \((R_1 = 1)\). The endothelial mass transfer coefficient, \(K_1\), is directly proportional to the flux of vesicles across the endothelium.

\[ K_1 = S_v V_v \quad (2.6) \]

where \(S_v\) is the flux of vesicles successfully traversing the width of the endothelium (in either direction) and \(V_v\) is the average volume of a vesicle.

The boundary condition at the media-adventitia interface \((x = L)\) assumes vesicular transport across the capillary endothelium in the adventitia, and solute disappearance into the lymph by diffusive and convective means. The formulation of this boundary condition can be appreciated with reference to Fig. 55. The capillaries in the adventitia are assumed to allow the passage of LDL into the extravascular space just outside the media. While there is a convective flow out of the capillary as well \((J_c)\), only microsolutes are assumed capable of passing through the capillary endothelium in this fashion. Likewise, there is a convective flow in the inward direction at the venous end of the capillary \((J_v)\) which is also assumed to carry no solutes the size of LDL. Thus, only diffusive transport of solute across the capillaries is considered. The removal of LDL is taken
Figure 55 Material Balance Over Extravascular Adventitial Space

Mathematical Boundary Describing Extravascular Adventitial Space Adjacent to Media
to occur exclusively into the lymph both by convection and by diffusion. $J_f$ is used in the convective removal term (as opposed to $J_L$), thus implying that the inward and outward convective flows across the capillary endothelium nearly cancel (i.e., $J_C \approx J_V$) and that the remaining net convective flow from the capillaries has no effect on the solute distribution in the media. Although the capillaries and lymphatic terminals only exist outside the media in the rabbit aorta, their net effect is assumed to be exerted right at the media-adventitia interface. All assumptions in this analysis are consistent with current concepts of solute transport in capillary beds.

In addition to the boundary condition posed in (2.4b), a limiting case was also investigated:

$$\text{at } x = L: \quad C = KC_p(t) \quad (2.7)$$

This equilibrium condition is approached for large $K_C$ and $K_L$ in (2.4b). Physically speaking, if the transcapillary solute flux and lymphatic removal flux are large relative to the rate of transmedial diffusion, the media-adventitia interface concentration will be maintained at an apparent equilibrium concentration determined by the ratio of solute flux from the capillary to the flux back into the lymph.

Before solving, the problem was cast in dimensionless form by means of the following transformations:
The solution, \( N + \Psi \), which is the sum of the bound and mobile relative tissue concentrations, is expressed as a function of the remaining dimensionless groups. \( P \) is the wall Peclet number; \( \tau \), dimensionless time; \( T_1 \), \( T_2 \) and \( T_L \) are mass transfer Biot numbers; \( \Theta \), a Thiele modulus; and \( m \), the reaction equilibrium constant. With the exception of \( \tau \) and \( m \), these dimensionless groups describe the ratio of transport or reaction rates relative to rate of solute diffusion through the media.

An analytical solution for \( N + \Psi \) can be obtained for \( D_{\text{eff}} \neq f(x) \). Calculations were initially made assuming no internal reaction \((\Theta=0)\). Estimates for the various constants \((J_f, D_{\text{eff}}, K_1, \varepsilon, K_A / A_m \) and \( K_L / A_m \)) were based on data in the literature where available. Variations in the parameters were studied in an attempt to bring the calculated profiles into reasonable agreement with the experimental LDL data. However, all attempts using this simplified model were unsuccessful.
The problem in bringing the experimental data and theoretical predictions (without internal reaction) into accord centered on the rapid influx of labeled solute noted after ten minutes experimentally. In order to account for this influx theoretically, a $D_{\text{eff}}$ on the order of $3 \times 10^{-8} \text{ cm}^2/\text{sec}$ is required (for reasonable values of $T_1$). This is shown on Figs. 66 - 68. While a reasonable fit can be obtained for both the ten minute and 30 minute data, at four hours and longer the theory is at considerable variance with experimental observations. Theory predicts solute distribution profiles which are practically linear (pseudo steady state) across the media at four hours (Fig. 68). The experimental data possessed upward concavity at this time interval. If a smaller $D_{\text{eff}}$ were assumed, a better fit could be obtained at four hours, but only at the expense of the agreement at the ten minute and 30 minute intervals. Note that the equilibrium boundary condition (2.7) was used in these calculations. However, similar behavior is observed when the more complex condition (2.4b) is tested. Thus, one can conclude that, regardless of the boundary condition specified for $\eta = x/L = 1$, a convection-diffusion model without internal reaction is not an adequate representation of the experimental situation.

Including the reaction term in the model does not provide any better agreement for the slow to moderate binding rates investigated. The net effect of the binding model posed here is simply to amplify the profile which would be predicted without binding. This is readily deduced from the form of the analytical solution. The steady state solution to the problem takes the form
Figure 66: Effect of $D_{\text{eff}}$ -Equilibrium Boundary Condition

$t = 0.167$ hours $L = 0.024$ cm

$p = 1.0$

$T_1 = 0.05$

$\varepsilon = 0.42$

$\kappa = 0.0040$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041\tau}$

$C_p(0)$

$\tau = 0.0696 \quad D_{\text{eff}} = 6.67 \times 10^{-8}$ cm$^2$/sec

$\tau = 0.0348 \quad D_{\text{eff}} = 3.33 \times 10^{-8}$ cm$^2$/sec

$\tau = 0.0058 \quad D_{\text{eff}} = 5.6 \times 10^{-9}$ cm$^2$/sec

Relative Position, $\eta = x/L$
Figure 67: Effect of $D_{\text{eff}}$-Equilibrium Boundary Condition

$t = 0.5$ hours
$P = 1.0$
$T_1 = 0.05$
$\varepsilon = 0.42$
$\kappa = 0.0040$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.04lt}$
$\frac{C_p(t)}{C_p(0)}$
$L = 0.024 \text{ cm}$

$\tau = 0.208$, $D_{\text{eff}} = 6.67 \times 10^{-8} \text{ cm}^2/\text{sec}$

$\tau = 0.104$, $D_{\text{eff}} = 3.33 \times 10^{-8} \text{ cm}^2/\text{sec}$

$\tau = 0.018$, $D_{\text{eff}} = 5.6 \times 10^{-9} \text{ cm}^2/\text{sec}$

Relative Concentration, $\psi = C/C_p(0)$

Relative Position, $\eta = x/L$
Figure 68: Effect of $D_{\text{eff}}$—Equilibrium Boundary Condition

- $t = 4$ hours
- $P = 1.0$
- $T = 0.05$
- $\varepsilon = 0.42$
- $\kappa = 0.0040$
- $C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
- $\frac{C_p(t)}{C_p(0)}$
- $L = 0.024 \text{ cm}$

Relative Concentration, $\psi = C/C_p(0)$

- $\tau = 0.8325, 1.67 \quad D_{\text{eff}} = 6.67 \times 10^{-8} \text{ cm}^2/\text{sec}$, $D_{\text{eff}} = 3.33 \times 10^{-8} \text{ cm}^2/\text{sec}$

- $\tau = 0.140 \quad D_{\text{eff}} = 5.6 \times 10^{-9} \text{ cm}^2/\text{sec}$

Relative Position, $\eta = x/L$
\[(N + \psi)_{s.s.} = A(\eta)B(\eta)\left(1 + \frac{1}{m}\right)\]  \hspace{1cm} (2.8)

For the case where binding is neglected, the steady state solution is given as:

\[\psi_{s.s.} = A(\eta)B(\eta)\]  \hspace{1cm} (2.9)

Comparison of (2.8) and (2.9) demonstrates that, at equilibrium, the total solute concentration in the wall is increased a factor of \(1/m\) by the binding reaction. Recall that \(m\) is the ratio of reverse to forward binding rates, or the binding reaction equilibrium constant. Therefore, except at early times for very fast binding rates, the shapes of the predicted profiles are similar to the no binding case, only at a higher concentration. Thus, a theoretical model which assumes slow-to-moderate reaction rates within the arterial wall is not appropriate either.

The basis for postulating internal interaction of LDL with wall constituents are studies which demonstrate that certain mucopolysaccharides and elastin react with LDL in vitro to form complexes. Since the reaction mechanism and the kinetics are unknown, the failure of the kinetic model chosen here might conceivably be attributable to choosing the wrong kinetic model. Equally plausible is the possibility that the binding sites are nonuniformly distributed within the aortic wall and/or that the wall is anisotropic. Hodara, et al. (89) have found chondroitin sulfate, a particular mucopolysaccharide, in higher concentrations near the intima than near the
adventitia in the media of bovine aorta. Not knowing the exact
distribution of binding sites in rabbit aorta, a number of different
types were studied. The net result was that for the binding site
distribution \( (C_b) \) shown in Fig. 105 by the function \( g(\eta) \) and
assuming that \( D_{eff} \) was spatially variant also as shown on Fig. 105
by the function \( f(\eta) \), theory and experiment could be brought into
reasonable agreement.

Because of the complex functionality of the binding distribution
chosen, numerical solution techniques were required to approximate the
solution to the problem. The comparison of the predicted profiles
with the experimental data is presented on Figs. 106 and 107. While
reasonable agreement is seen at all time intervals of interest, this
by no means implies that the model employed is truly the appropriate
one. Rather, it simply suggests that spatially nonuniform reaction of
LDL with wall components and variable \( D_{eff} \) is one possible explanation
for the experimental results. Clearly, experiments designed, first
of all, to test whether binding of LDL actually takes place in vivo,
and secondly, to determine the mechanism and kinetics of this process
are required before a more rigorous analysis of the data is possible.
It is also possible that other sets of parameters, outside the domain
of those investigated in this study, would also provide a reasonable
fit of the data.

Although the model presented here is highly speculative, a
number of interesting observations can be made with regard to the
relative importance of the endothelium and intramural diffusion and
convection of solute across the vessel wall. With regard to the
Figure 105:

Assumed Variation in Effective LDL Diffusion Coefficient and AMPS Binding Sites

\[ D_{\text{eff}}^*(\eta) = D_{\text{eff}} f(\eta) \]

\[ \Theta^*(\eta) = \Theta g(\eta) \]

\[ f(\eta) = 1.002236 - 0.902236e^{-6\eta} \]

\[ g(\eta) = 1.0114e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.0116 \]
Figure 106 Comparison of Theoretical Predictions and LDL Experimental Data (t = 0.167, 0.5, and 4 Hours)

\[
\begin{align*}
T_1 &= 0.0288 \\
\rho &= 0.6 \\
\epsilon &= 0.42 \\
T_2 &= 0.1 \\
T_L &= 12.0 \\
R_2 &= 0.0 \\
\Theta &= 0.7 \\
m &= 0.6 \\
\end{align*}
\]

\[
\frac{\partial \Psi}{\partial \eta} = T_2 (\Psi - \epsilon \frac{C_p(t)}{C_p(0)}) + T_L \Psi + \Psi (1 - R_2)_{\eta=1}
\]

- ○ LDL 0.167 Hour Average Data
- ■ LDL 0.5 Hour Average Data
- ▲ LDL 4 Hour Average Data

Relative Tissue Concentration, \( C/C_p(0) \)

Relative Position, \( x/L \)
Figure 107  Comparison of Theoretical Predictions and LDL Experimental Data (t = 24 and 67 Hours)

\[ T_1 = 0.0288 \]
\[ p = 0.6 \]
\[ \varepsilon = 0.42 \]
\[ T_2 = 0.1 \]
\[ T_L = 12.0 \]
\[ R_2 = 0.0 \]
\[ \Theta = 0.7, m = 0.6 \]

- LDL 24 Hour Average Data
- LDL 67 Hour Average Data

24 Hour Theory \((\tau = 13.97)\)
67 Hour Theory \((\tau = 37.24)\)
endothelium, the best fit of the data was obtained for values of $K_1$, the endothelial mass transfer coefficient, within a factor of three of the value one would predict based on the best estimates for vesicular transport rates: Thus, the results are consistent with the concept that LDL crosses the endothelium exclusively in this fashion. Moreover, the magnitude of the dimensionless parameter, $T_1 (=0.0288)$ which indicates the relative rate of transendothelial transport compared with intramural diffusion, is quite small. In physical terms this implies that the endothelium is the rate limiting barrier in the intimal uptake process. Any factor, such as chemical or mechanical (hemodynamic) stress, which alters the integrity of the endothelium, is likely to result in marked enhancement of solute into the vessel wall.

With regard to the hydraulic permeability, the value of $J_f$ used ($=0.0035 \text{ ml/cm}^2\cdot\text{hr}$) in the calculations shown on Figs. 106 and 107 is 3x lower than the aortic hydraulic flux measured \textit{in vitro}. This is a reasonable deviation since the results of the \textit{in vitro} studies, due to the unavoidable unphysiological nature of the experiments, undoubtedly represent an overestimate of the true flux. Of even more significance is the fact that the model grossly overpredicts solute concentration when $J_f$ is chosen close to zero. This suggests that a hydraulic flux is required to promote solute clearance from the wall to the lymphatic terminals. Moreover, the wall Peclet number ($=J_f L/D_{eff}$) for $J_f = 0.0035 \text{ ml/cm}^2\cdot\text{hr}$ is of order unity. Since the Peclet number describes the ratio of convective to diffusive transport, a value of unity is indicative of the fact that the transport process
is not dominated by either mechanism. Both are equally important in moving solute through the aortic media.

The $D_{\text{eff}}$ used in the model was assumed to increase from $9.3 \times 10^{-9}$ cm$^2$/sec near the intima to $9.3 \times 10^{-8}$ cm$^2$/sec near the adventitia. While one is justified in questioning whether such a large variation actually exists, it should be pointed out that a $D_{\text{eff}}$ in this range is not unreasonable for LDL in light of its aqueous diffusion coefficient, which is reported to be $2.25 \times 10^{-7}$ cm$^2$/sec (235). One would a priori anticipate that tortuosity effects associated with the complex structure of the aortic wall would increase the effective diffusion path length. For glucose and norepinephrine aortic $D_{\text{eff}}$ five to ten fold smaller than the aqueous diffusivities have been reported. Thus, the range of $D_{\text{eff}}$ for LDL used in these calculations most likely encompasses the true value. In short, the theoretical model, albeit quite speculative as concerns the nature of any binding processes which might be occurring, is consistent with reasonable estimates of endothelial vesicular transport rates, hydraulic flux and aortic effective diffusion coefficients for LDL.

The major conclusions of this study can be summarized as follows:

1. Labeled LDL and labeled albumin enter and accumulate within the rabbit aortic wall.

2. Entry is gained both through the intima and across the media-adventitia interface.

3. The rate of influx is greater for labeled albumin than for labeled LDL, suggesting that the uptake mechanism may be, in part, dependent on molecular size.
(4) The influx of labeled LDL is consistent with the concept of transendothelial passage solely by vesicular transport.

(5) Solute accumulation and distribution cannot be explained in terms of convection and diffusion of solute across an isotropic medium.

(6) Hydraulic convection may be required to promote solute clearance into the lymphatics.

(7) The LDL distribution data are in accord with theoretical predictions which assume convective and diffusive LDL transport with the existence of spatially nonuniform interaction of LDL with wall components.
III. INTRODUCTION

A. Scope and Purpose

The purpose of this thesis is to gain a more fundamental understanding of the factors affecting the transport of macromolecules into and across large blood vessel walls. The transport properties of blood vessel walls and factors affecting these properties have broad implications on atherosclerosis. Atherosclerosis, broadly defined, is the disease state characterized by the accumulation of fatty and fibrous materials within blood vessel walls. It is generally agreed that the accumulated solute originates in the blood plasma. The mechanisms and causes of this intramural solute transport and accumulation are poorly understood. Many theories have been offered. They involve all possible contributing factors such as blood chemistry, hemodynamics, arterial wall permeability and the biochemistry of the arterial wall, to name a few. Evidence has been presented in support of all the current theories (see APPENDIX A). However, most theories have at least one common basis—they have to account for the transport of blood lipids, perhaps in the form of intact lipoproteins, into and across the blood vessel wall. Thus, the knowledge of factors affecting the movement of biological macromolecules (such as lipoproteins) into and across the vessel wall is central to understanding the dynamic development of atherosclerotic lesions.

B. Relevance

Atherosclerosis, and its associated complications, is the single most important cause of mortality in the United States. In 1967,
over 50% of all deaths were attributed to cardiovascular disease (1). Effective treatment of the disease is hampered by an inadequate understanding of its causes. Thus, studies such as this one, are of importance in that, taken together, they should help define the factors responsible, and hopefully, lead to a diminution of mortality due to cardiovascular disease.

C. Pathology of Atherosclerosis

In this section and the sections that follow, the pathology of atherosclerotic lesions and events which may account for this pathology will be discussed. That will be followed by an analysis of the literature pertinent to the transport properties of arterial tissue.

As mentioned previously, the disease is characterized by the focal accumulation of fatty and fibrous material within the vasculature. For purposes of comparison and terminology, a schematic representation of the normal aorta is presented in Fig. 1. Note that the arterial wall is composed of three distinct concentric regions. The smallest of the three regions is the intima. The intima is defined as the region between the lumen-vessel wall interface and the first elastic lamina. It is composed of a single layer of cells, known as the endothelium, which separate the flowing blood from the rest of the vessel wall. The endothelium is supported by the basement membrane. The region between the basement membrane and internal elastic lamina contains ground substance (collagen, elastin fibers, glycosaminoglycans) and occasional smooth muscle-like cells. The endothelial cell layer is continuous, and viewed en face, takes on a cobblestone appearance. The cells are ellipsoidal in
Figure 1. Cross-sectional view of aortic wall (2).
shape and are thought to be held together by mucopolysaccharide-like substances (3). They possess non-thrombogenic properties and serve to regulate the transfer of blood micro- and macro- solutes into the subendothelial space. This regulatory function can be viewed in light of the detailed morphology of adjoining endothelial cells (Fig. 2). Note that each cell contains a nucleus and numerous intracytoplasmic organelles. Allegedly important in the transport regulatory function are a particular type of organelle called vesicles. These vesicles are the vehicle in a process (pinocytosis) wherein material on the surface of the luminal side of the cell is imbibed and carried across to the abluminal side and discharged. The details of this process will be discussed later in this section (III. D. a.). A second possible mechanism whereby the endothelium regulates transport involves the regions between two adjacent cells known as the junctions or intercellular clefts. Some substances in the blood are thought to either diffuse or be convected to the subendothelial space via these junctions. Steric exclusion effects due to the critical dimension of these junctions are thought to determine the nature of solutes transported in this manner (see III. D.). A third possible mechanism for transendothelial transport for hydrophobic material would be by solute dissolution into the cell membrane followed by diffusion around the periphery of the cell. Alternatively, hydrophilic material could gain access to the endothelial cytoplasm and simply diffuse directly to the subendothelial space. The relative importance of each of the aforementioned transport paths varies with the molecular species involved. However, the importance of the regulatory function
Figure 2: Morphology of Adjoining Endothelial Cells
of the endothelium can not be underestimated. The remaining parts of the intima (the basement membrane and the ground substance) might also be important in transport regulation. Their possible role will be discussed later in this section (III. D.).

The central annular region of the arterial wall is the media. As seen in Fig. 1, it contains concentric layers of elastic lamina separated by ground substance and smooth muscle cells. Depending on animal species and the specific vessel (i.e., aorta, carotid, etc.) anywhere from 20-80 elastic laminae may be found in the media. The elastic laminae are not continuous. Gaps, or fenestrae, allow for communication across laminae. In blood vessels with more than 25-30 laminae, a capillary network (vasa vasorum) penetrates the outer part of the media (4). These capillaries provide nutrients for the smooth muscle cells in the outer two-thirds of the large vessels. The inner one-third is thought to be nourished by solute transport from the vessel lumen (5). The main function of the media is to provide structural support to enable the vessel to withstand stress induced by pulsatile blood flow.

The outermost region (Fig. 1) is the adventitia. It is characterized by loose connective tissue, with no organized elastic layers. A peripheral capillary bed is present in the aortic adventitia of most species. Not shown in Fig. 1 is the adventitial lymphatic system. As in most capillary beds, the lymphatics drain the extracellular space of the vessel wall, thereby removing cellular waste products. In summary, the blood vessel wall is composed of intima, media, and adventitia; all three of which play a role in determining
the nature of solute transport into and across the blood vessel wall.

Given the above broad overview of the normal blood vessel wall, the characteristics of diseased tissue will be summarized. The earliest manifestation of the disease is thought to be the formation of ribbon-like fatty streaks, nonuniformly distributed primarily in the vessels nearest the heart, i.e., the coronaries and the aorta. No proof has been offered, however, which convincingly demonstrates that these fatty streaks are precursors to the more advanced plaques. The fatty streaks are confined to the intima which is usually abnormally thickened. The majority of the fat in these lesions is found inside foam cells which are either specifically adapted smooth muscle cells (6) or macrophages which originated in the blood (7). These fatty streaks by themselves are not thought to be of any pathological consequence. However, they may develop into more advanced forms of lesions.

Another type of lesion found predominantly in adults has been called the gelatinous lesion (8). It is different from the fatty streak in that most of the lipid is extracellular. Moreover, the chemical composition of the lipid is quite similar to blood plasma lipid. Such is not the case with the lipid found in fatty streaks. The differences in composition are presented in Table I. As with the fatty streak, the gelatinous lesion in its early stages is generally confined to the intima. Only in more advanced lesions has degenerated elastic tissue in the media below the lesion been observed.

The most advanced lesion is the fibrous plaque. Great quantities of fibrin and lipid are found in the intima. Most of the lipid is
**TABLE I**

Comparison of Serum LDL*, Perifibrous Lipid in Normal Intima and Early Fibrous Lesions, and the Intracellular Lipid in Fatty Streaks (8)

<table>
<thead>
<tr>
<th></th>
<th>Serum LDL</th>
<th>Intima with Perifibrous lipid</th>
<th>Fatty streaks with numerous fat-filled cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>'Normal'</td>
<td>'Young'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ages 40-59</td>
<td>fibrous plaques</td>
</tr>
<tr>
<td>Total lipid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/100 mg dry tissue</td>
<td></td>
<td>10.8</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>38.7</td>
</tr>
<tr>
<td>% composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>58.2</td>
<td>42.3</td>
<td>59.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>67.9</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>11.6</td>
<td>13.0</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.8</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>20.0</td>
<td>29.3</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15.8</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>10.2</td>
<td>15.4</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>% of total CEFA**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 : 1</td>
<td>24.1</td>
<td>28.0</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50.3</td>
</tr>
<tr>
<td>18 : 2</td>
<td>46.8</td>
<td>38.6</td>
<td>43.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14.0</td>
</tr>
<tr>
<td>20 : 3</td>
<td>trace</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>20 : 4</td>
<td>5.2</td>
<td>5.3</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Low-density lipoprotein  

** Cholesteryl ester fatty acid
extracellular and similar in composition to plasma lipids (see Table I), leading some to speculate that the gelatinous lesion is a precursor to the fibrous plaque (8). These fibrous plaques are sometimes so extensive that they compromise flow through the blood vessel lumen. For example, plaques could cause coronary obstruction (insufficiency), the direct result being a heart attack. Another pathological consequence of fibrous plaque formation is a stroke. A portion of the plaque may break free forming an embolus which has the potential to obstruct flow in a peripheral vessel of the brain, thereby causing partial or total paralysis. Finally, the plaque may weaken the vessel wall to the point that it ruptures. This event is known as a dissecting aneurysm. Alternatively, the plaque may simply calcify with age, rendering the vessel wall rigid. This so called hardening of the arteries is of consequence only in that it tends to elevate systemic blood pressures. Thus, vessel lesions can lead to terminal events or simply a relatively benign hypertensive state.

The perplexing part of lesion growth and development is that no one knows for sure what initiates the process. Moreover, no one knows whether fatty streaks definitely develop into the complicated fibrous plaques. Most of the evidence suggests that fatty streaks are totally unrelated to fibrous plaques. For example, fatty streaks tend to be most prevalent in the upper thoracic aorta whereas plaques are found more often in the abdominal aorta (9). Also, the mouths of the intercostal arteries are not spared of plaques as they are of fatty streaks (9). Thus, the mechanisms for fatty streak formation and fibrous plaque formation may be totally different.
Some of the best evidence that bears on the causes of human lesion formation is found in the chemical analysis of the lesion material and how it compares with the plasma chemical composition. Most of the plasma lipids are carried with protein in complexes called lipoproteins. These lipoproteins are classified by their relative densities and their electrophoretic mobilities (Table II). As can be seen in Table II, very low-density lipoproteins (VLDL) have a pre-β electrophoretic mobility and consist mainly of triglycerides. Low-density lipoproteins (LDL) have β-mobility and contain cholesterol as the predominant lipid component. High-density lipoproteins (HDL) have α-mobility and are relatively high in protein with the lipid split evenly between cholesterol and phospholipid. The typical concentrations in the plasma of nonfasting males shown for VLDL, LDL, and HDL indicate that plasma LDL carries the bulk of plasma cholesterol. This fact is significant in that both free cholesterol and ester cholesterol levels are extremely high in atheromatous lesions (Table I). While arteries do have the ability to synthesize cholesterol from smaller substrates, the bulk of the evidence from animal and human studies suggests that the majority of accumulated lesion cholesterol is derived from the plasma (11, 12). Consequently, an integral part of the disease process, if not also the cause, involves the transfer of cholesterol and other plasma solutes into the vessel wall.

The chemical form in which cholesterol might be transferred has been a question of extreme interest. Two separate processes have been postulated: (1) Uptake of cholesterol in the form of intact β-lipoprotein, or (2) Uptake of the cholesterol but not the protein
### TABLE II

The Plasma Lipoprotein Spectrum (10)

<table>
<thead>
<tr>
<th>Lipoprotein Properties</th>
<th>VLDL</th>
<th>HDL₃</th>
<th>HDL₂</th>
<th>LDL</th>
<th>VLDL</th>
<th>Endogenous Particles</th>
<th>Chylomicrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrated Density-gm/ml</td>
<td>1.20-1.15</td>
<td>1.16-1.12</td>
<td>1.12-1.08</td>
<td>1.05-1.01</td>
<td>1.01-0.93</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>Diameter-Å</td>
<td>74</td>
<td>79</td>
<td>103</td>
<td>190</td>
<td>210-760</td>
<td>400-2600</td>
<td>1200-11,000</td>
</tr>
<tr>
<td>Molecular Weight-gm/mol x10⁻⁶</td>
<td>0.15</td>
<td>0.18</td>
<td>0.38</td>
<td>2.2</td>
<td>3-128</td>
<td>20-5000</td>
<td>500-430,000</td>
</tr>
<tr>
<td>Paper</td>
<td>?</td>
<td>Pre-α</td>
<td>Pre-α</td>
<td>β</td>
<td>Pre-β and trail</td>
<td>Pre-β and trail</td>
<td>Origin</td>
</tr>
<tr>
<td>Electrophorisis-mobility</td>
<td>?</td>
<td>Pre-α</td>
<td>Pre-α</td>
<td>β</td>
<td>Pre-β and trail</td>
<td>Pre-β and trail</td>
<td>Origin</td>
</tr>
</tbody>
</table>

#### Composition-% by Weight

<table>
<thead>
<tr>
<th></th>
<th>VLDL</th>
<th>HDL₃</th>
<th>HDL₂</th>
<th>LDL</th>
<th>VLDL</th>
<th>Endogenous Particles</th>
<th>Chylomicrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Cholesterol</td>
<td>0.3</td>
<td>-</td>
<td>2</td>
<td>10</td>
<td>3-5</td>
<td>4.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Cholesterol Ester</td>
<td>3</td>
<td>-</td>
<td>20</td>
<td>36</td>
<td>10-13</td>
<td>15</td>
<td>3.9</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>28</td>
<td>-</td>
<td>24</td>
<td>20</td>
<td>13-20</td>
<td>10</td>
<td>4.3</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>5</td>
<td>-</td>
<td>4</td>
<td>12</td>
<td>50-60</td>
<td>67</td>
<td>87</td>
</tr>
<tr>
<td>Protein</td>
<td>62</td>
<td>-</td>
<td>50</td>
<td>22</td>
<td>5-12</td>
<td>2-5</td>
<td>2</td>
</tr>
</tbody>
</table>

#### Normal Levels

<table>
<thead>
<tr>
<th></th>
<th>Males-mg%</th>
<th>Females-mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>222±31</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>264±59</td>
</tr>
<tr>
<td></td>
<td>53±48</td>
<td>172±81</td>
</tr>
<tr>
<td></td>
<td>369±81</td>
<td>313±36</td>
</tr>
<tr>
<td></td>
<td>159±116</td>
<td>56±60</td>
</tr>
<tr>
<td></td>
<td>12±13</td>
<td>2±3</td>
</tr>
</tbody>
</table>
moiety of β-lipoprotein (the details of these possible processes will be discussed later in III. D.). Based on recent immunological evidence, the first possibility has been supported. Smith and Slater (8, 11) found β-lipoprotein activity in both normal and atheromatous human aorta. Their techniques indicated that lipid was still bound to protein, but the composition of this lipid could not be determined. Prior to these studies, others had confirmed the presence of the β-lipoprotein protein moiety in atheromatous tissue (13-21), both by immunological and radiochemical methods. Their studies failed to demonstrate, however, that the protein was still bound to lipid in a lipoprotein complex. Others have found that lipid material extracted from atheromatous aortic homogenates was similar to plasma lipoproteins in its ultracentrifugal flotational properties (22-24). Thus, the recent results of Smith and Slater strongly suggest that intact lipoprotein accumulates in both normal and atheromatous tissue. Moreover, other plasma solutes have been isolated as well. Positive identification has been made for albumin, fibrin, and α-lipoproteins (8, 13, 14). Since Smith and Slater (8) have found fibrin activity in normal tissue, one speculates that plasma macromolecular solutes are continually infiltrating the blood vessel wall. Thus, the high concentration of these solutes found in lesions may simply be an exaggerated end result of a normally occurring process. What factors are involved in this exaggerated solute accumulation remains the focus of much of the experimental work in atherosclerosis.

Given the uncertainty in how the disease starts and proliferates, many theories of atherogenesis pervade the literature. Most of
these theories are based on correlations of the incidence of the disease with observable parameters. These parameters are commonly called risk factors. Among the most important risk factors are blood pressure levels, blood cholesterol levels, smoking, sex, age, obesity, and heredity. The theories reviewed in APPENDIX A indicate how some of these factors might be involved. Whether any or all of the risk factors are causal is a matter of controversy. It is safe to assume, however, that atherosclerosis is caused by multiple factors acting either additively or synergistically and that an essential feature of the disease process, if not also the cause, involves the transfer of blood solutes into the vessel wall. Consequently, an understanding of the dynamics of transmural macromolecular transport and the properties of the arterial wall which affect this transport is an important objective in atherosclerosis research.

D. Possible Factors Involved in Blood-Artery Macromolecular Transport

For plasma solutes to accumulate in the normal blood vessel wall, a mechanism for transport through the endothelial cell layer must be operative. The extent of intramural accumulation will be dictated not only by the factors controlling this transendothelial transport, but also by the driving forces and arterial transport properties operative in the media and adventitia of the blood vessel wall. The following sections review studies relevant to these transport processes.

1. Transendothelial Transport Mechanism

As mentioned in III. C., the main functions of the endothelium are to provide a non-thrombogenic interface between blood and the arterial wall and, perhaps, to regulate the transmural transport of
blood solutes. Three distinct transport pathways have been hypothesized for molecules such as LDL. These are shown in Fig. 3. Intact LDL might be transported exclusively by vesicular transport (pinocytosis), or in combination with diffusion, and/or convection through endothelial junctions. A third superimposable possibility involves transfer by dissolution of just part of the LDL molecule. It is conceivable that only the lipid moiety is taken up by the endothelial cell membrane, and that it subsequently diffuses to the subendothelial space either in the cell membrane or across the cell cytoplasm in conjunction with a hydrophilic carrier. Any of these three mechanisms would result in net uptake of cholesterol. A fourth mode of cholesterol transfer is also possible. It is known, for example, that the free cholesterol (unesterified) moiety of plasma LDL exchanges with free cholesterol in the arterial wall (12). This exchange mechanism, however, does not result in net cholesterol uptake.

The most recent evidence available suggests that normal transendothelial cholesterol transport occurs both by pinocytosis of intact LDL and perhaps by dissolution of part of the LDL. In the absence of intact endothelium, direct leakage of plasma solutes directly into the media is likely. For a detailed review of this subject, the reader is referred to a paper by French (3).

a. Transendothelial Vesicular Transport

Electron microscopy studies performed on capillary and arterial endothelial transport indicate that relatively small solutes are transported primarily through endothelial pores (junctions?) while larger solutes are carried almost exclusively by vesicles.
Figure 3: POSSIBLE TRANSPORT MODES

- Pinocytosis
- Exchange-Diffusion
- Pore Diffusion and/or Convection
- Lumen
- Endothelium
- Media
The physical dimensions of the two pathways relative to the size of the solute involved seem to dictate the route of entry. For example, horseradish peroxidase (characteristic dimension 20-30 Å) and saccharated iron oxide (polydisperse with characteristic dimensions 20-70 Å) are found in both junctions and vesicles of both capillary (25-32) and arterial (33-40) endothelium. However, when the larger probe molecules ferritin (characteristic dimension 100 Å), colloidal carbon (characteristic mean dimension 200 Å), and colloidal gold have been used, uptake by only vesicles was observed (41-49). Vesicles have an internal diameter on the order of 500 Å (50). The critical junction dimension, based on capillary permeability studies, is thought to be on the order of 60-90 Å (51-52). Hence, the probe uptake studies are consistent with molecules <45 Å diffusing and/or being convected through junctions while larger molecules are probably transported primarily by vesicles.

The relative importance of junction-transport and vesicular transport has been estimated (53) for albumin and dextrans (range of Stokes Radii = 20-130 Å). They concluded that large molecules are transported mainly by vesicles whereas 50% of the transcapillary albumin (Stokes radius ≈ 36 Å) transport and 99.9% of the hydraulic clearance is through "pores" (junctions?).

These studies have obvious relevance to the transendothelial transport of LDL. From electron microscopy studies, LDL is thought to be spherical in shape with an average molecular radius of 97 Å (54, 55). This would suggest that transendothelial LDL movement would be accomplished exclusively by vesicular transport. Only recently
has any evidence been found to support this speculation (20). Immunological LDL activity was found associated with human endothelial vesicle. Taken by itself, this observation does not prove that LDL is transported in this manner. For example, it may simply be stored, not transported, in vesicles. However, the probe studies previously cited add additional indirect proof for LDL transport in this manner. In addition, in vitro heart perfusion studies (on rats) with protein-labeled LDL, VLDL, and HDL (56, 57) suggest the presence of at least the protein moieties of the lipoproteins (if not intact lipoproteins) in endothelial vesicles but not in junctions. Apo-HDL (the protein moiety of HDL) uptake, on the other hand, was greater than for any of the lipoproteins studied (including intact HDL). This result is in accord with small molecule transport through junctions being faster than large molecule transport by vesicles. The fact that the work was performed in vitro leaves its physiological significance open to question. However, recent double-label LDL uptake studies (one label on the cholesterol moiety and one label on the protein) performed in vivo with rabbits strongly suggest that lipid transport is accomplished via intact LDL (58). The ratio of labeled protein to lipid in the aorta was similar to that for the plasma. These studies, unfortunately, do not rule out the possibility that cholesterol transport is effected by some sort of physiochemical exchange mechanism and that the similarity in protein-lipid ratios was merely coincidental. Both free and esterified cholesterol may have been labeled. Thus, free cholesterol exchange without net cholesterol transfer may have
occurred. The evidence to suggest that physiochemical exchange of cholesterol between the blood and vessel wall may be significant will be discussed later (III.D.1.c).

b. **Selectivity of Vesicular Transport**

One of the crucial questions concerning vesicular transport is how is it influenced by the nature of events which occur on the cell surface? In some systems, pinocytosis is known to be a selective process (59). That is, only certain molecules are capable of triggering pinocytosis. The first step involved in pinocytosis is thought to be the binding of the molecule to the cell surface. In amoeba and tumor cells, this binding is dependent on the net charge of the proteins to be transported (59,60).

With isolated human and animal endothelial cells in tissue culture, lipoprotein uptake could be inhibited by anionic compounds and by heparin suggesting that in endothelial cells as well, net surface charge is important. Other binding mechanisms are possible. Lipase enzyme activity has been demonstrated on endothelial cells in mammary glands (62). It is conceivable that VLDL, because of its high triglyceride content, is enzymatically degraded to LDL while bound to the endothelial cell surface. LDL transport by vesicles may be the reaction product removal mechanism (63).

While there is very little evidence to suggest preferential transport of LDL due to surface selectivity effects, this possibility can not be ignored when discussing the transport properties of arterial tissue.
c. Physiochemical Exchange and Dissolution Mechanism for Cholesterol Transport

As shown in Fig. 3, LDL may give up part of its cholesterol load to the endothelial cell surface. If this transfer is not a one-for-one exchange, net cholesterol uptake would result. For clarity, when net uptake is inferred, the process will be called "dissolution." Transfer with no net uptake will be termed "exchange." The importance of the "exchange" and "dissolution" concepts was first recognized during the 1960's as a result of aortic lipid uptake studies performed with animals. It was shown that the ratio of cholesteryl ester to free cholesterol in rabbit and cockerel lesions was different than for the plasma lipids (12, 64, 65). These results were presented as an argument against simple infiltration of plasma lipids into the arterial wall (12). Subsequent studies have shown that free cholesterol and esterified cholesterol may be transported by different mechanisms (66-68). Since labeled free cholesterol uptake was found to be greater than labeled-cholesteryl ester uptake, one may infer that mainly free cholesterol undergoes "dissolution" and/or "exchange." Perfusion studies with pigeon aortas suggest that cholesteryl esters are transported only as part of intact LDL. In vivo studies also support this tenet (70, 71). Hence, "exchange" and/or "dissolution" of labeled LDL free cholesterol superimposed over vesicular transport of intact LDL may account for cholesterol uptake by the arterial wall. Whether "dissolution" of free cholesterol actually occurs and is a significant determinant of arterial cholesterol accumulation can not be
ascertained from these studies since the uptake of labeled free cholesterol does not allow one to differentiate between "dissolution" and "exchange." This problem confounds the interpretation of all cholesterol uptake studies where the label is on free cholesterol. Further studies are required to determine if free cholesterol "dissolution" is a significant factor in arterial cholesterol accumulation processes.

To summarize this section on transendothelial transport mechanisms, the most reasonable interpretation of all of the arterial transport studies is that vesicular transport is probably the primary mechanism for the entry of intact LDL into the normal arterial wall. LDL free cholesterol "dissolution" at the arterial surface may or may not be important in the overall cholesterol transport process. Very little is known about what selectivity effects, if any, may be exerted by events occurring on the endothelial surface. Thus, the transport of cholesterol into the arterial wall remains a poorly understood phenomenon.

2. Effect of the Basement Membrane on Transmural Solute Transport

Despite the fact that the endothelium is the initial barrier, it may not represent the limiting resistance to transmural solute transport. As mentioned earlier, the endothelium is supported by a continuous basement membrane. This membrane could act as an additional solute transport resistance. French (3) reviews the function of the basement membrane and concludes that particles larger than 100 Å in diameter may be retained by it. Hence, the passage of LDL (Stokes diameter = 193 Å) could be restricted
in this manner. However, the recent studies showing LDL penetration into the media of perfused rat arteries (56, 57) indicate that not all of the incident LDL is rejected by the basement membrane. No studies are available, however, which characterize its rejection properties more precisely.

3. Effects of Elastic Laminae on Transmural Transport

As with the basement membrane, very little is known about the binding and retention properties of the aortic elastic laminae. This is surprising in light of the fact that elastin is the single most abundant chemical entity in the walls of large blood vessels. Chemically, it is protein with a fairly well defined amino acid composition. It can not be hydrolyzed in strong alkalai. In fact, this characteristic serves as the basis for one method of isolating arterial elastin. In its native state, it generally forms a cross-linked fibrous network intimately entwined with collagen, proteoglycans, and structural glycoproteins.

The role elastin plays in lesion development is unclear. Histological studies have demonstrated lipid deposition, in early atherosclerotic lesions, near the internal elastic lamina (IEL). The implication is that the IEL either restricts the passage of lipids or physiochemically binds them. It is known that elastin will bind lipids. In fact, in vitro studies on human arteries have demonstrated a propensity for atheromatous elastin to bind cholesteryl esters significantly more than normal elastin (72, 73). These lipid-elastin bonds could not be broken by hot alkalai treatment suggesting that the process is irreversible. The binding involved only the lipid moieties of VLDL and LDL. No
HDL lipid was bound. The amino acid composition of "diseased" elastin was altered from normal suggesting that the protein composition of the elastin is a factor in determining its binding affinity. These results imply that conditions which influence the synthesis of arterial elastin may predispose the artery to excessive lipid accumulation. One such condition may be hypertension. Hypertensive rat aortas are characterized by a strikingly increased elastin content (74). Stretched elastin, which is undoubtedly a feature of hypertension, also binds more lipid in vitro than normal unstretched elastin (75). Likewise, X-irradiation treatment, which probably affects synthesis of elastin, results in elastin which tends to bind excessive quantities of horseradish peroxidase in vivo (76). Thus, LDL and/or VLDL transport across the arterial wall could be affected quite dramatically by the state of the elastin. Since no kinetic data are available and since these binding studies were performed in vitro, one can only speculate as to the physiological significance of this binding reaction in vivo.

4. Effect of Ground Substance on Transmural Transport

The ground substance of arteries is composed mainly of collagen and a class of compounds known as acid mucopolysaccharides (AMPS). Mucopolysaccharides are highly hydrated, jelly like substances which provide intercellular lubrication. As the name implies, they are unbranched polymers with a repeating disaccharide monomer. The most abundant AMPS in the arterial wall is hyaluronic acid (37%). Heparin sulfate (27%), dermatan sulfate (17%) and chondroitin sulfate A and C (19%) are present in lesser amounts.
As with elastin, the effect of ground substance on macromolecular transmural transport is not clear. Some have hypothesized, however, that acid mucopolysaccharides act as a sieve preferentially retaining certain large macromolecules. The basis for this hypothesis has recently been reviewed (77, 78). The evidence is largely circumstantial. Extracts of human atheromatous plaques have been shown to contain complexes which electrophoretically and calorimetrically behave as complexes of LDL protein and mucopolysaccharide (79-83). Histological examination of atheromatous human plaques have demonstrated a close association between the locale of lipid deposits and areas staining positively for AMPS (18, 84). Moreover, animals which experience spontaneous atherosclerosis most readily, with some exceptions, have the highest relative AMPS content in their arteries (85). In vitro studies also confirm the propensity of VLDL and LDL to bind with certain AMPS (86). One theory of the binding mechanism is that Ca++ ions crosslink the protein moiety of lipoproteins with sulfate groups on the AMPS (77). Because both LDL and AMPS have a net negative charge at physiological pH, hydrogen binding and hydrophobic interactions may also be involved. The degree of binding has been shown to be dependent both on the concentration of LDL and the amount of "unreacted" AMPS available (82). Hence, in cases where arterial injury has induced a reparative process and a concomitant increased synthesis of AMPS, LDL binding may be significant (87, 88). Unlike elastin binding, AMPS binding is reversed by high ionic strength (77-79). Thus, local cation concentrations may determine the extent of binding in vivo. The distribution
of the various AMPS across the arterial wall also would result in regional differences in binding. Hodara, et al. (89) found the highest levels of AMPS near the intima of bovine aorta with a uniform decline toward the media. Hence, if AMPS-LDL complexes distribute the same way, this might give cause for the excessive lipid accumulation found in diseased arterial intima. Implied herein is that AMPS-LDL binding is detrimental. However, complex formation may provide an immobilizing function allowing enzymatic catabolism of the lipid (90), and, therefore, be beneficial. Clearly, further studies are required on the rate, mechanism, and distribution of AMPS-LDL interactions before its physiological significance can be ascertained. However, based on current work, one can conclude that AMPS-LDL interactions should affect LDL movement in the arterial wall.

5. Effect of the Lymphatic System on Transmural Transport

Since lipid accumulation is localized in and near the arterial intima in diseased vessels, primary importance has been placed on the role of the endothelium in macromolecular transport regulation. However, perhaps equally important in determining the extent of accumulation may be the nature of the clearance mechanisms which remove substances from the interstitial space of the arterial wall. The lymphatic system is intimately involved in this process. The lymphatics are thought to remove unused protein and other solutes from the extracellular space. In fact, 50% or more of the circulatory plasma protein escapes daily from the blood stream and is returned via the lymphatic system (91). Most of this transfer occurs across capillary beds. However, small amounts also "leak" through large
blood vessels (92). In capillaries, large proteins like albumin and to a limited extent lipoproteins and injected dextrans (ranging in size from 20-130 Å) are carried from the capillary into the lymph (93-96). Lipoprotein transfer is enhanced by thermally injuring the capillaries (97, 98) and by anoxia (99). This is thought to be due to increased endothelial permeability. However, the basement membrane or some other subendothelial factor further restricts clearance since lipoprotein rejection could be correlated with the relative molecular size even after injury (96). Lymph removal studies on large vessels have been hampered due to technical problems. Techniques have not been developed to isolate lymph originating solely from arteries. However, indirect evidence is available to suggest that lipoproteins are cleared from large vessels in much the same manner as in capillaries. Immunofluorescent methods were employed to show that LDL apo-protein moves from the intima to the adventitia in less than four hours (100). Others have demonstrated that lymph duct ligation causes ingested colloidal iron to accumulate in coronary arteries (101). Furthermore, electron microscopy studies have allowed visualization of lipid particles entering aortic lymphatic vessels of hypercholesterolemic rats (102). In addition, stripping the adventitia (presumably including the lymphatics) from the abdominal aorta of rabbits impedes the removal of horseradish peroxidase (103). It is interesting that large molecules can gain access to the lymphatic vessel by either passing through endothelial cell junction or by pinocytosis (104). Even more intriguing is the fact that once inside the lymphatic, proteins
do not escape (92). Therefore, the lymphatics possess a very efficient protein removal mechanism. If the removal is compromised, significant intramural solute accumulation may result. Only with further experimentation, however, can the full importance of this process relative to atherosclerosis be assessed.

6. Transmural Transport Driving Forces

If lipoproteins and other macromolecules are cleared by the arterial lymphatic system, some mechanism must bring the solute to the lymphatics in the adventitia of the blood vessel wall. On theoretical grounds one can hypothesize this transport to be due to diffusion and/or bulk fluid movement through the tissue extracellular space. No studies have been conducted to differentiate between these two possible mechanisms. However, diffusive transport and hydraulic conductance properties of arterial tissue have been studied separately in in vitro systems. A review of these experiments will serve as a basis for the analysis of arterial solute uptake studies reported in the literature.

a. Hydraulic Conductance of Arterial Tissue

One of the original hypotheses of atherogenesis is the filtration theory (105). Blood plasma solutes are thought to infiltrate the arterial wall under the influence of the transmural pressure gradient. Once inside the wall, large solutes, such as LDL, are trapped. This sieving effect is thought to be responsible for an excessive intimal lipid accumulation and subsequent lesion development. As originally postulated, the theory left unanswered questions concerning how LDL negotiated intact endothelium and what
interactions caused the sieving effect. Even whether or not a hydraulic flow exists is still a matter of controversy. The only studies on arterial hydraulic conductance have been performed in vitro, and, thus are subject to the usual questions concerning in vivo physiological relevance.

In the early 1950's, the hydraulic flux of plasma across excised human iliac arteries was determined (106, 107). Their results are presented as a function of the applied pressure (Fig. 4). Note that the permeation flow varied linearly with pressure and that at physiological pressures (100 mm Hg) a flux of ~0.01 ml/cm²-hr was observed. While this flux is of small magnitude, it most likely represents an overestimate because of the condition of the arterial tissue during the experiment. Most studies were conducted for 60 hours in vitro. Based on recent observations by Fry (108), the endothelium was probably not intact during most of this period. Furthermore, no compelling evidence was presented that proved the media was unaffected by their procedures. The authors also failed to correct for vessel "creep". They simply measured fluid level changes in a graduated capillary situated upstream of the ligated vessel. Thus, any changes due to distension of the vessel were erroneously interpreted as transarterial fluid flow. Also shown on Fig. 4 are the data of Boughner, et al. (109-111). These flux estimates were taken in a manner similar to that of Wilens and McCluskey with the additional feature that vessel "creep" corrections were made. However, the reliability of the data is suspect of the grounds that the surface area available for filtration was only estimated and not measured. Thus, the fluxes could be in error by as much as 100-200%.
Boughner, Burton, et al. (109-111)

Yamartino (112)

Wilens (106-107)

Figure 4: Aortic Hydraulic Permeability
More recent experiments (112), performed under more physiological in vitro conditions on rabbit aorta, also demonstrated the existence of a hydraulic flow sensitive to the applied pressure gradient (Fig. 4). It is intriguing that at physiological pressures, the flux (0.015 ml/cm²-hr) across the rabbit aorta was very similar to that observed by Wilens and McCluskey using human iliac arteries. One would have expected higher fluxes with the rabbit artery since it is much thinner (0.025 cm) than the human iliac artery (>0.1 cm). Of course, species and tissue differences in addition to differences in technique, make a direct comparison of the results risky. Since the endothelium was not completely intact even in Yamartino's work, his results probably also represent an upper bound for the true hydraulic conductance of arterial tissue. Besides degenerated tissue morphology, the fact that steady pressures were used in these studies may also affect the applicability of the results. Some have suggested that pulsing pressures may cause transmural transport by a "milking" mechanism (3, 113). However, there is no experimental evidence to support claims that the hydraulic conductance would be different under pulsing pressures. Hence, all one can safely conclude from these in vitro studies is that arterial tissue partially deficient of endothelium has a small, but measurable, hydraulic conductance.

If the hydraulic flow is a significant factor in macromolecular transport, solute uptake and accumulation should increase with the applied pressure gradient. As summarized in APPENDIX A, a good correlation exists between hypertension and the incidence of
atherosclerosis. Some *in vitro* studies also suggest that a transmural pressure gradient accelerates the movement of cholesterol across arterial tissue (80, 107, 114-116). However, in most of these studies the endothelium was not intact. Also, since the arterial wall distended with increased pressure gradients, the effect of increased permeability due to distension could not be separated from the effect of the increased driving force. More recent *in vitro* studies (108, 117, 118) suggest that increased stretch, not increased driving force, accounts for the augmented transport under high pressure drop conditions. Still others have found no relationship between perfusion pressure and cholesterol uptake in *in vitro* studies (119, 120).

In the work of McCullagh and Ehrhardt (120), reported in abstract form only, just two pressures were studied, (0 and 50 mm Hg), both of which were below the normal physiological range. Hence, the true effect of pressure was not adequately tested. Taken as a group, these *in vitro* solute transport studies indicate (with some exceptions) that solute movement across arterial tissue lacking intact endothelium is accelerated by an increase in the transmural pressure drop. Thus, neglecting the effect of the endothelium, one can conclude that solute transport can in part occur by bulk fluid movement *in vitro*.

b. **Diffusive Transport Studies**

The only quantitative studies concerning the diffusion of solutes across arterial tissue involved substances associated with arterial wall metabolism (glucose, oxygen, carbon dioxide, etc.). No measurements have been made on macromolecular diffusion rates.
It was shown that the glucose diffusion coefficient was higher 
($1.73 \times 10^{-6}$ cm$^2$/sec) in the "intima-subintima" than in the media 
($1.27 \times 10^{-6}$ cm$^2$/sec) (5). Values of $7.3 \times 10^{-7}$ cm$^2$/sec and $10^{-6}$ cm$^2$/sec 
have been reported for the diffusivity of norepinephrine across 
rabbit arterial tissue (121). Since the molecular weight of norepi-
nephrine (169) is similar to that of glucose (180), the similarity 
in arterial diffusion coefficients is not surprising. The aqueous 
diffusion coefficient of glucose at $37^\circ$ C has been reported as 
$9.1 \times 10^{-6}$ cm$^2$/sec (122). Hence, the tortuosity effects of arterial 
tissue reduce the diffusivity of glucose and norepinephrine five to 
ten fold.

7. The Relative Importance of Diffusion and Convection

The only evidence which bears on the relative importance of 
the hypothesized driving forces is qualitative. These studies 
have involved the determination of labeled solute distributions 
across the vessel wall. Hence, they are of extreme importance in 
providing insight into the origin of the accumulated solute and the 
direction in which it is being transported. However, they are 
inclusive with regard to the relative importance of the proposed 
transport driving forces.

The first experiments of this type were conducted by Duncan, 
et al. (123-126). They studied isotopically labeled LDL (labeled in 
separate experiments either on the free and ester cholesterol or 
protein moiety) and albumin aortic distributions after an in vivo 
exposure period of up to seven days. The labeled proteins were 
administered in a single dose. After sacrifice, the aortic wall
was subdivided into three layers: inner, middle, and outer media. Their results for $^{125}$I-LDL (protein labeled) are presented in Fig. 5. Since the relative radioactivity at all time intervals studied was highest in the inner media, they concluded that the primary mode of lipoprotein apo-protein entry into the wall was through the intima. The fact that the gradient disappears slowly, if at all, suggested that clearance is slow relative to the rate of entry. Adams, et al., (127-130) conducted similar experiments using the rabbit as the experimental animal. They refined the approach of Duncan and co-workers by preparing 40μ frozen serial sections of the arterial wall on a refrigerated microtome. Thus, a more detailed distribution was obtained. Their results for $^3$H-cholesterol (as lipoptotein?), $^{125}$I-albumin, and $^{125}$I-globulin are presented on Fig. 6. The results suggest that cholesterol may enter the blood vessel wall from the adventitia as well as across the intima adventitia. Albumin and globulin distributions suggest that transendothelial transport may not be as important as transport from the adventitia for these solutes. Thus, the nature of the solute and the species would seem to dictate the primary entry point. Adams' data are subject to criticism, however, on the grounds that the isotopic labels may have entered the tissue in a different form than in the original injectate. That is to say, the distribution could represent free $^{125}$I which was liberated during the catabolism of the injected material. Duncan, et al., on the other hand, corrected for this possibility.
Figure 5: Distribution of $^{125}$I - Low Density Lipoprotein Across Canine Aorta (126)
Figure 6: RABBIT AORTIC DISTRIBUTIONS (128)
The most recent experiments of this type have involved yet another animal species (131-134). These investigators studied the transmural distribution of cholesterol-labeled lipoproteins, labeled albumin, and labeled fibrinogen in hogs. Their results are presented in Fig. 7&8. Note that the gradients for all three solutes suggest primary entry through the intima. Also, the radioactivity in the intima rises between one and four hours suggesting that the endothelium and/or intima may be a rate limiting resistance. These albumin results are in direct conflict with those of Adams, et al. This discrepancy may be attributable to the species difference. However, a more likely explanation is the failure of Adams to properly correct his data for free iodide-125 whereas Schwartz, et al. did.

The only other solute distribution studies reported in the literature involve oxygen (135, 136). Using a micro-oxygen electrode, these investigators demonstrated a steep oxygen gradient from the intima across normal rabbit aorta. Thus, if diffusion is operative, arterial oxygen is transported through the intima towards the adventitia. In atheromatous vessels, the gradient was not nearly as steep. This suggests a much higher overall permeability to oxygen. In this regard, it is interesting to note that Adams, et al. found the albumin gradient in atheromatous rabbit vessels indicative of infiltration from the vessel lumen whereas in normal vessels, albumin penetrated primarily from the adventitia (84, 129). Thus, the conditions of the arterial wall may affect how solutes are transported.
Figure 7: Distribution of $^{131}$I-Fibrinogen and $^{131}$I-Albumin Across Hog Aortic Wall (133, 134)
Figure 8  Distribution of $^{3}$H Cholesterol Across Hog Aortic Wall (132)
Only limited conclusions can be reached based on these solute distribution studies. In the dog, rabbit, and hog, lipoprotein cholesterol apparently enters the vessel wall predominantly through the intima. On the other hand, protein distribution in the rabbit aorta seems to reflect a more important adventitial contribution. Thus, protein and lipid may be transported in a different manner in the rabbit aorta. No such difference is apparent from results with the dog or hog, however. Thus, the manner by which cholesterol and protein are transported through aortic tissues is unresolved. Nonetheless, these studies do not rule out the possibility that both diffusion and/or convection may be involved.

A concise summary of the factors potentially involved in the transport and accumulation of blood lipids in the arterial wall is afforded by Fig. 9. Blood lipid, perhaps as intact LDL, enters the normal arterial wall across the intima and the adventitia by means of transendothelial vesicular transport. Physicochemical "dissolution" of just the free cholesterol moiety of the LDL may also be a factor. Stress induced endothelial damage may enhance convection and/or diffusion through endothelial junctions. Intramural solute movement is most likely the result of diffusion and convection with local binding to acid mucopolysaccharides and elastin affecting net accumulation. LDL removal is in part effected by the lymphatic system. While this model is obviously a simplified representation of a very complex process, it incorporates into a concise form most of the experimental observations summarized in this section. Hence, its utility is as a framework upon which experimental strategies can be constructed.
Figure 9. An Integrated View of Transport Processes within the Aortic Wall
E. **Specific Purposes of This Study**

The objectives of the studies summarized in this thesis are as follows:

1. To determine the relative importance of diffusion and convection in the transport of blood macromolecules across the blood vessel wall.
2. To develop a more precise understanding of all the parameters which may govern this process.

Two approaches were taken toward these ends. Hydraulic permeabilities of both normal and atheromatous rabbit aorta were measured in vitro. Secondly, the transmural distribution of labeled albumin and LDL across rabbit aorta was studied as a function of time. An unsteady state mathematical transport model, which accounts for vesicular transport across the endothelium, convection and diffusion within the media, and solute interaction with constituents in the vessel wall was developed to interpret the data and provide a means for estimating the wall transport parameters.
IV. APPARATUS AND PROCEDURES

A. Solute Distribution Studies

Radioisotopically labeled rabbit serum albumin (RSA) and/or human low-density lipoprotein (LDL) were given intravenously to 3-6 kg New Zealand white rabbits. Ten minutes to 67 hours later the animals were sacrificed and their aorta quickly removed and frozen. The transmural radioactivity distribution was determined by counting serial tissue sections prepared with a refrigerated microtome. Measures were taken to ensure that only protein-bound radioactivity was counted. The details of the procedures used are described below.

1. Preparation of Proteins

The human LDL used in these studies was obtained from Red Cross plasma (Massachusetts Blood Program, 812 Huntington Avenue, Boston, Massachusetts). The LDL separation procedure was a modification of that of Hatch and Lees (10). The plasma salt density was raised to 1.025 gm/ml by adding a calculated amount of KBr-NaCl solution (density 1.349 gm/ml, pH 8.0) as indicated by Havel, et al. (224). An initial salt density of 1.010 gm/ml was assumed for the citrated plasma. A Beckman L2-65B ultracentrifuge was used (40,000 rpm for 20 hours; 40.3 rotor) to separate all blood proteins with an aqueous density less than 1.025 gm/ml. These proteins were discarded, and the salt density of the remaining proteins ($\rho_s > 1.025$) was raised to 1.050 gm/ml by again adding a calculated amount of NaCl-KBr solution. A second 20 hour ultracentrifugation was performed (40.3 rotor; 40,000 rpm). The yellow supernatant was pooled and
the infranatant discarded. Thus, LDL with an aqueous density between 1.025 and 1.050 gm/ml was isolated. Usually two units of plasma resulted in 6-10 ml of LDL solution with 3-8 mg protein per ml. In most cases the purity of the preparation was checked by immunoelectrophoresis (10). Without exception the LDL solution so prepared was immunologically reactive with only rabbit anti-human LDL antiserum. It was tested with rabbit anti-human HDL and anti-human albumin antisera as well. The antisera were prepared and tested to ensure their monospecificity as described by Schonfeld, et al. (225). The final LDL preparation was dialyzed at 4°C against at least 1000 ml of saline (0.85% (w/v) NaCl, 0.1 M disodium (ethylenedinitrilo) tetraacetate (EDTA), pH 8.6) prior to further processing. In one study rabbit LDL was used. It was isolated in the same manner as above from freshly-drawn hypercholesterolemic rabbit plasma. No immunological testing was conducted to determine the purity of this rabbit LDL. However, paper electrophoresis of the labeled protein was performed to determine its electrophoretic properties. This procedure is described in the next section. The bovine serum albumin (Pentex, Cat. No. 81-001, Miles Laboratory, Elkhart, Indiana) and rabbit serum albumin (Cat. No. 3000R, Nutritional Biochemicals Corporation, Cleveland, Ohio) used were the purest fractions commercially available.

2. Radioisotope Protein Labeling

The method of McFarlane (40) as modified by Billheimer, et al. (241) was used to prepare $^{125}\text{I}$-LDL and $^{125}\text{I}$-albumin. The relative proportion of the reagents was calculated based on no more than one
molecule of iodide per molecule of LDL or albumin protein. The molecular weight of the LDL-apoprotein was assumed to be 500,000 (242) in the initial studies and 100,000 (241) in later experiments. For albumin, \( \sim 5\) mCi of \(^{125}\text{I}\) was used; for LDL, \( \sim 10\) mCi. Labeling efficiencies were determined by precipitation of the labeled protein solution (diluted 1:1000) with 1.0 ml of 10% (w/v) trichloracetic acid (TCA). Approximately 0.1 ml of a 6% bovine albumin solution was added to increase the bulk of the precipitate. All precipitable radioactivity was assumed to represent \(^{125}\text{I}\) bound to the protein. Thus, counting the precipitate and supernatant separately allowed calculation of the percentage of the label which was bound, i.e., the efficiency of labeling. The unbound \(^{125}\text{I}\) (free \(^{125}\text{I}\)) was removed by dialysis overnight at 4°C against at least four changes of physiological saline (500 volumes relative to the volume of labeled protein solution).

LDL solutions were further purified in some cases by ultracentrifugation. The LDL salt density was adjusted to 1.050 gm/ml by dialysis against 8.7% (w/v) NaCl (0.1% (w/v) disodium EDTA, pH 8.6). Centrifugation was carried out in a Beckman swinging bucket rotor (SW 41) for at least 30 hours at 41,000 rpm. The LDL supernatant was pipetted off and the remaining solution pooled. The volumes of the LDL supernatant and pooled remainder were measured in graduated cylinders and aliquots were assayed to determine the relative proportions of TCA-precipitable radioactivity in each sample.

Labeled LDL solution was also tested for homogeneity by gel permeation chromatography. Sephadex G-200 was used in a 1.5 cm
diameter silicone coated (Siliclad, Clay Adams, Division of Becton Dickinson and Company, Parsippany, New Jersey) glass column operated at 4°C. The Sephadex gel was hydrated in TRIS buffer (0.001 M tris (hydroxymethyl) aminomethane (TRIS), 0.001 M EDTA, 0.02% (w/v) NaN₃, pH 8.6) according to procedure described by the manufacturer (Pharmacia Fine Chemicals, Piscataway, New Jersey). After deaeration under vacuum, the gel was allowed to settle in the column by gravity. Visual inspection insured the absence of any discernible air bubbles in the column. The flow rate was adjusted to ~0.1 ml/min and the column allowed to equilibrate for two days under flowing conditions. ¹²⁵I-LDL solution, previously dialyzed against TRIS buffer, was introduced in an 0.1 ml aliquot with care exercised not to incorporate air into the system in the process. A fraction collector (7000 Ultrarac, LKB-Producter, Bromma, Sweden) was used to collect the first 100-1 ml fractions. The radioactivity in each fraction was determined by counting procedures described later.

Just as with the unlabeled LDL preparations, immunoelectrophoresis was employed to test the immunological reactivity of labeled LDL. The details of this procedure are given by Hatch and Lees (10) and will not be discussed here. The labeled LDL solutions were tested against rabbit anti-human LDL and anti-human whole serum.

In addition to immunoelectrophoresis, paper electrophoresis of some labeled LDL and albumin injectates as well as plasma from the recipient animals was conducted. Again, the details of the procedure are given by Hatch and Lees (10). Briefly summarized, 0.010 ml of radioactive sample was applied to a cellulose acetate
paper strip, saturated with a barbital buffer. An electrical potential was applied across the strip (110 v, 0.010 amp) for 16 hours. The paper strips were then fixed and stained with bromphenol blue or Oil Red O to demarcate the proteins or lipids, respectively. After drying, the strips were subdivided into equal sized pieces of 0.5-1 cm width. Each piece was transferred to a test tube for assay of the radioactivity. Results of the radioactivity as a function of migration distance were thus obtained.

3. Injection of Labeled Proteins

The procedures involved starting with the injection of the labeled protein into the rabbit and concluding with the sectioning of the radioactive aorta are illustrated in Fig. 10. The details are discussed in the next three sections. Rabbits were weighed to within 0.05 kgm and placed in a restraining box with their head exposed. A catheter (Intracath, Cat. No. 3116, Deseret Pharmaceutical Company, Sandy, Utah) was inserted in the lateral ear vein and held in position with a bulldog clamp. The ear vein was first dilated by heating with a 25 watt light bulb to facilitate insertion of the catheter. To insure that the catheter was in the vein, <1.0 ml of filtered (Millex Disposable Filter Unit, Sterile 0.22 µ, Millipore Corporation, Bedford, Massachusetts) saline (0.85% (w/v) NaCl, 0.1% (w/v) EDTA, pH 8.6) was given through the catheter. The labeled protein solutions (2-5 ml) were also filtered during the injection process, and the filter was rinsed with ~1 ml of saline to minimize the loss of labeled protein. The activity of the injectate ranged between 1 and 5 mCi. This corresponded to a
Summary of Experimental Procedures

Figure 10. Summary of Experimental Procedures
specific activity of approximately 0.125 mCi/mg LDL protein and 0.625 mCi/mg albumin. Protein determinations were made by the method of Lowry, et al. (43). The concentration of protein in the injectate was approximately 5 mg/ml for albumin and 2.5 mg/ml for LDL. Blood samples were collected in tubes containing 10 mg sodium EDTA before and at intervals after the isotope injection by venous puncture of the contralateral ear vein. The plasma was separated from the red cells by centrifugation at 2000 rpm for 10 minutes. In experiments of greater than four hour duration, the rabbit was removed from the restraining box and placed in a cage. Food and water were then taken ad lib. During the entire experiment, all isotope handling was performed in an exhaust hood.

4. Sacrifice of Animal and Removal of the Aorta

After a predetermined length of time ranging from 10 minutes to 67 hours, the animal was sacrificed by giving intravenously an overdose of sodium pentobarbital (Somnethol, J. A. Webster, Inc., North Billerica, Massachusetts). The thoracic cavity was quickly opened and the thoracic aorta exposed. Care was taken to prevent blood drainage into the cavity by clamping the inferior vena cava. The entire aorta from the arch to the diaphragm was recovered and flushed immediately with physiological saline (0.9% (w/v) NaCl) until all visible traces of blood disappeared. The aorta was dissected longitudinally between the branching intercostal vessels and the aorta rinsed again. In some experiments, the aorta was separated into six or seven equal length pieces. Each tissue segment was gently flattened between two glass cover slips and
frozen (Cryokwik, IEC, Damon/IEC Division, Needham Heights, Massachusetts). In most experiments, the entire aorta was flattened all at once between two glass microscope slides and frozen. In this case, the aorta was subdivided into smaller lengths just prior to the sectioning procedures (see next section). Between the time the animal was sacrificed and the entire aorta frozen, 5 - 8 minutes elapsed. The aorta was kept frozen anywhere from 1 - 7 days before further processing.

5. Sectioning and Preparation of Aorta

The frozen aortic sections were serially sectioned using a refrigerated (-10°C to -14°C) microtome (Cryo-Cut, American Optical Instrument Corporation, Buffalo, New York). In preparation for sectioning, a flattened frozen section (41cm in length) or aorta was first trimmed to eliminate the intercostal vessels. The frozen tissue was next gently thawed by breathing on the endothelial surface (the adventitial surface was frozen to a glass cover slip) and immediately blotted to remove all excess surface moisture. As quickly as possible, the tissue was mounted endothelial-side down on the cryotome chuck which was covered with frozen tissue mounting medium (Ames 0. C. T. Compound, Ames Company, Division, Miles Laboratories, Inc., Elkhart, Indiana). The mounting medium was presaturated with Evans Blue (approximately 3.5% (w/w)) and transferred onto the metal chuck precooled to the cutting temperature. While the blued mounting medium was freezing, a wood applicator stick was employed to draw out the medium on the chuck in such a manner that fibrous strands formed. These strands were wrapped over the
surface of the partly frozen medium so as to incorporate small air pockets in the frozen medium. Once the entire medium had solidified, the chuck was passed under the knife cutting edge until a \( \sqrt{5} \) cm\(^2\) planar area was made available for mounting the tissue. Thus, the surface of the medium, onto which the endothelial surface of the aorta was placed, was precisely parallel to the cutting plane of the microtome knife. In the process of mounting, some adventitia would cling to the blued mounting surface. This was scrupulously trimmed away with a scalpel. This entire process is shown schematically in Fig.10. Further tissue mounting medium was used to buttress the tissue on all four sides. This was quickly frozen with Cryokwik. After a short period of time (5 to 10 minutes) to allow for partial temperature equilibration, sectioning of the adventitia at 20\(\mu\) was started. Of the initial adventitial slices, all but the last few were discarded. The abrupt transition from adventitia to media was readily apparent and so noted. The adventitial slices were considerably more deformable, and visually, they were less opaque than the media sections. Any evidence of blood contamination in a slice was recorded. These slices were excluded in the final determinations. Each media section was placed in an individual precooled test tube and set aside for later radioactivity counting. In some cases, especially as the sections neared the endothelium, portions in excess of 20\(\mu\) thickness would be removed with an individual slice. Therefore, subsequent slices would represent only some fraction of the original section area. Slices that took more than 30% extra were not included in the data analysis,
while corrections were made for those which took less than 30% extra. These corrections were based on a revised estimate of the total slice volume, taking into account thickness and cross sectional area of the normal slice plus the thickness and area of the extra portion of tissue. For example, if the third slice from the end (i.e., the portion of tissue 40-60 μ distant from the mounting media-endothelial surface interface; see Fig.11) represented the entire original cross sectional area plus 15% of the area from the subjacent tissue in the 0-40 μ range, the total volume of tissue taken was assumed to be the normal volume, V (= area x 20 μ), plus the volume of the subjacent tissue, 2 x 0.15 V. This corrected total volume of 1.3 V was used to calculate the concentration of radioactivity in this slice instead of the normal volume, V. This correction, of course, introduced significant errors if the thickness and area of the extra tissue taken were substantial. Hence, only in the 80 μ closest to the endothelial surface, and only for those cases which involved less than 30% extra tissue, was this correction applied.

The cross sectional area of the mounted tissue was determined by outlining the shape of the tissue perimeter on a transparent plastic sheet. The weight per cm² of the sheet was determined by preparing four and 16 cm² square pieces and weighing them. Hence, the weight of the plastic tissue outline could be converted into area through use of this calibration factor.

Carrying the example further, the two subjacent tissue sections would in this case comprise only 85% of the original tissue area,
Figure 11. Tissue Slicing Techniques - Area Corrections
and, therefore, result in tissue slices with but 85% of the normal tissue volume. Consequently, the volume of these slices was assumed to be 0.85V for purposes of calculating the concentration of radioactivity in each slice. If ever less than 40% of the tissue area remained due to prior sections taking extra, the radioactivity results were ignored in all further data analysis.

The results from the tissue slice nearest the intima were from time to time discarded for other reasons as well. Since the advance of the chuck into the cutting knife was in increments of 20μ, there was no way to guarantee that the last section would be an entire 20μ thickness. With no good way of estimating the thickness of this terminal slice, one could not calculate its volume. Hence, the results from this section were not used. However, for purposes of determining the total thickness of tissue between the endothelium and the media-adventitia junction, this slice was assumed to be of 20μ thickness. This introduced roughly a 5% error in the total thickness determination.

The slice-to-slice reproducibility of this sectioning technique was tested. Tritiated water (3H2O) was frozen in a plastic test tube mold and mounted on the blued mounting medium in exactly the same manner as described above for aortic tissue. Successive 20μ sections were quantitatively collected and assayed for radioactivity, using a Packard Tri-Carb liquid scintillation counter (model no. 3385). Insta-Gel (Packard Instrument Company, Downers Grover, Illinois) was used as the scintillator fluid. The variation in slice-to-slice 3H radioactivity was assumed to be attributable to variation
in slice thickness since each section was of identically the same cross sectional area. The results (summarized in APPENDIX D) indicated a 6.2% standard deviation about the mean $^3$H radioactivity over the 24 slices sectioned. Thus, the slicing equipment afforded fairly precise results.

The procedure was not without frustrating aspects. For some unknown reason or reasons, the mounted aortic tissue would not always adhere well to the surface of the mounting medium. Sometimes, the entire 200μ thickness of tissue would break off of the mount before any sections could be cut and retained. While the exact cause or causes of the problem were never ascertained, it was sufficiently infrequent in occurrence to obviate any changes in the procedures. Critically important in optimizing the success of the procedure was the nature of the frozen mounting medium. Only solutions of Ames O.C.T. compound just barely saturated with Evans Blue (≈3.4% (w/w)) gave the proper tissue-mount adhesive quality. Too low a concentration of Evans Blue or too large an excess both gave undesirable results. The microtome knife cutting angle and the sharpness of the knife also seemed to be important. The knife angle was set empirically and the knife sharpened by conventional means at weekly intervals. Microtome temperatures outside of the −10 to −14°C range were also detrimental to the successful application of the methods described here. However, while the procedure was not successful 100% of the time, with practice one could develop the necessary skills to guarantee good results most of the time.
6. **Radioactivity Determinations**

All radioactivity counting was performed on a dual-channel gamma well spectrometer (model no. 1085, Nuclear-Chicago Corporation, Des Plaines, Illinois) precalibrated to give maximum $^{125}$I and $^{131}$I counting efficiencies. All tissue and plasma samples were treated with 10% TCA prior to counting. LDL protein should have precipitated under these conditions. A small aliquot (v 0.1 ml) of 6% albumin solution was added to each sample to increase the bulk of the precipitate. Low speed centrifugation (2000 rpm for 10 min) was employed to separate the precipitated proteins from the aqueous phase. This procedure was repeated two-to-three times to wash any entrained aqueous phase radioactivity from the pellets. In this manner all non-protein radioactivity associated with the liquid phase (e.g., free ionic $^{125}$I and small iodinated polypeptides) was removed from the tissue and plasma samples before radioactivity counting. Hence, all radioactivity determinations were performed on only "washed" protein precipitates.

In some cases, for purposes of testing the exhaustiveness of the TCA washing procedure, the supernatants were also assayed. The radioactivity in the third wash was generally not distinguishable from background counting rates. All washed tissue samples were counted for 10 minutes and all plasma samples for one minute. Generally, tissue sample radioactivities ranged from zero to 300 counts/min above background, with most falling in the 20-80 counts/min range. Plasma samples were first diluted 1:1010 in 0.9% (w/v) NaCl before counting. Typically, these dilutions resulted in
counting rates of 20,000 - 30,000 counts/min above background for each aliquot.

7. **Expressing Results**

   Each tissue radioactivity was normalized as follows:

   \[
   \psi = \frac{C}{C_p(0)} = \frac{\{ \text{counts/min-ml} \}_\text{tissue}}{\{ \text{counts/min-ml} \}_\text{initial plasma}}
   \]

   The volume of tissue in each slice was taken as the product of its area and its 20\(\mu\) thickness. The initial plasma sample, taken within five minutes of the isotope injection, was used in the denominator above. See APPENDIX B for sample calculations. By normalizing with respect to the initial plasma concentration, comparisons of data could be made between experiments. The radial position of each slice was also normalized relative to the total thickness of the media plus intima. This thickness was estimated from the number of consecutive 20\(\mu\) sections that were obtained between the first section which was totally media (no adventitia) and the last section nearest the endothelium. On average 12.1±1.0 slices (244\(\mu\)) were so recovered. The number ranged from eight to 15 with most tissues resulting in 11, 12, or 13 consecutive sections. Data within any one experiment were then grouped by dimensionless radial position and the average dimensionless concentration determined. As a generalization, the dimensionless radioactivity concentrations could be calculated to within 11\% when no area corrections were involved and to within 16\% allowing for area corrections. See APPENDIX B for the details of the sample calculations.
8. **Plasma Chemical Analysis**

In some experiments total plasma cholesterol was determined using a standard automated method (Autoanalyzer, Technicon, Inc., Tarrytown, New York).

9. **Special Experiments**

One experiment was designed to determine the extent to which any blood contamination influenced the total radioactivity of labeled solute in the media. For this experiment $^{51}$Cr-labeled red cells were prepared by standard methods (226) from blood collected the same day as the experiment. The cells were washed three times with aqueous 0.9% (w/v) NaCl. This removed over 98% of the unbound $^{51}$Cr as determined by comparing the relative amounts of red cell-bound and unbound radioactivities in the washed preparation. Labeling efficiencies were approximately 50%. Thus, the final 15 ml volume of cells contained ~5 mCi of $^{51}$Cr. It was administered to the animal just prior to the labeled protein ($^{125}$I-LDL). Blood, plasma, and tissue radioactivities were determined in the usual manner using the gamma well-spectrometer. Corrections were made (see APPENDIX B: Sample Calculations) for $^{51}$Cr radioactivity which registered in the $^{125}$I channel.

In a separate experiment the aortic distribution of free $^{125}$I (as Na $^{125}$I) was determined. The $^{125}$I was taken from the dialysate of one of the labeled protein solutions. The administration of the isotope and tissue preparation was the same as the cases where labeled proteins were given.
Two other variations were performed. In one, the rabbit was anesthetized with sodium pentobarbital (30 mg/kg) prior to the isotope injection. The animal was maintained unconscious through the duration of the experiment. In the second variation, $^{125}$I-LDL prepared as described above was given to a small (0.6 kg) rabbit to prescreen the labeled material in vivo. It was assumed that the screening rabbit removed any aggregated or denatured protein which may have been present in the injectate. After 17 minutes, this donor rabbit was exsanguinated and the blood plasma (~15 ml) recovered by centrifugation. This $^{125}$I-LDL plasma along with unscreened $^{131}$I-LDL was given to a second recipient rabbit. The recipient animal was later sacrificed in the usual manner. Radioisotope counting on plasma and tissue samples took into account the overlap of the $^{131}$I radioactivity into the $^{125}$I counting channel (APPENDIX B). All samples were also corrected for $^{131}$I decay.

10. Aortic Tissue Homogenate Analysis

A number of different attempts were made to identify the chemical nature of the radioactive solute in the blood vessel wall. The abdominal aorta was used throughout. An aortic extract was prepared by first scissor mincing the tissue, and in most cases, homogenizing the tissue in a glass tissue homogenizer. This consisted of a mating ground glass or teflon rod and a heavy duty test tube. An electric motor was used to drive the rod. Barbital buffer (10) and in some cases, 15% NaCl (w/v), was used as the extract medium. A pinch of sand was used to facilitate homogenization. After homogenization, extraction of the homogenate was
carried out with gentle agitation for 16 - 24 hours at 4°C. The tissue debris was separated from the extract by centrifugation, and the radioactivity in the homogenate extract and the tissue debris determined separately. The extract from LDL experiments was also tested by immunoelectrophoresis and immunodiffusion for the presence of LDL (10).

B. Hydraulic Permeability Studies

The fluid permeation rate through rabbit aorta was determined \textit{in vitro} as a function of the applied pressure drop. A specially designed filtration cell was used. The details of these experiments are described below.

1. Apparatus

The system used in the experiments is shown in Fig. 12. It consisted of a Plexiglas filtration cell (see APPENDIX C: Details of Experimental Apparatus and Procedures), graduated capillary flow meters sensitive to 0.0001 ml changes, and a mercury manometer. Three-way valves (LV-3, Pharmacia Fine Chemicals, Piscataway, New Jersey) permitted the introduction of liquid into the various lines. The filtration cell and flow meters were maintained at 37°C by a thermoregulated water bath. Steady pressures were employed throughout. In all cases, the aortas were first stretched by a factor of 1.3 - 1.4 (117) in both directions before being clamped into the filtration cell. This returned the aortas to their \textit{in vivo} dimensions. A description of the stretching rack is provided in APPENDIX C: Details of Experimental Apparatus and Procedures.
Figure 12: Hydraulic Permeability Experimental Apparatus
2. Procedures

Rabbit aortas were obtained from freshly sacrificed animals housed in the Massachusetts Institute of Technology Animal Quarters. They were immediately placed in prewarmed (37°C) Krebs Ringer phosphate buffer (224) and transported to the laboratory. The loose adventitial tissue was dissected away and the vessel opened flat by cutting longitudinally along its ventral aspect. With the aorta stretched to its in vivo dimensions, the filtration cell was clamped in position around the aorta. Before clamping, a flexible but impermeable plastic sheet (Parafilm, American Can Company) was placed over the tissue in one of the two chambers. Thus, fluid displacement observed in the flow meters connected to this chamber was attributable to tissue compression only. The fluid displacement in the other flow meter was the result of both tissue compression and hydraulic permeation. Filtered (Swinney Filters, 0.22μ Millipore Corporation, Bedford, Massachusetts) Krebs-Ringer phosphate fortified with glucose (1 mg/ml) was introduced into each chamber and the cell connected to the pressure source and to the flow meters. Each capillary and connecting tubing was prefilled with Krebs-Ringer phosphate - 1% Tween - 0.25% Evans Blue, to eliminate all the gas from the system. The surfactant minimized wetting problems in the flow meters and the blue dye facilitated reading the position of the air bubble. The time which elapsed between death of the animal and the start of the experiment was one to two hours. Throughout the entire procedure, care was taken not to traumatize the aortic endothelium in any way. Pressures up to 260 mm Hg were studied.
Flow readings were taken at regular intervals (usually every three minutes) until steady rates were attained. This usually required 30-40 minutes at a given pressure. Four separate pressures were generally used per experiment. The average experimental duration was four to six hours, not counting preparation time.

At the conclusion of each experiment the endothelial integrity was assessed qualitatively using histological techniques. The aorta was stained (225) by successive 30 second exposures to AgNO₃ (0.25% (w/v)), NaH₂PO₄ (0.1 M, pH 7.4), a bromide solution (1% (w/v) NH₄Br, 3% (w/v) CoBr₂) and a final rinse with Na H₂PO₄ (0.1 M, pH 7.4). Tissues were fixed at 40°F in buffered formalin for at least 24 hours. Microscopic evaluation of endothelial integrity was performed by viewing the surface of the aorta under a light microscope (American Optical Instrument Company, Buffalo, New York). The tissue was held flat between the microscope slide and cover slip by two hair pins. Pictures of the endothelium were taken with the microscope camera attachment.
V. EXPERIMENTAL RESULTS

The presentation of the experimental results is subdivided into sections dealing with the labeled solute distribution studies and the hydraulic permeability studies. Only an overall summary of the results is covered here. The supporting detailed results of individual experiments are discussed in APPENDIX D.

A. Solute Distribution Studies

A number of different procedures were employed to characterize the physiochemical properties of the labeled albumin and LDL preparations which were given to the experimental animals. The results of these procedures are presented first, followed by a summary of the labeled LDL and albumin transmural distributions.

1. Testing of the Injected Labeled Proteins
   a. Immunoelectrophoresis

   Immunoelectrophoretic techniques were applied to the labeled LDL preparations to determine if the labeling procedure in any way altered the electrophoretic mobility of the antigenic moiety of LDL. An electric field induced the migration of the LDL preparation through agarose gel which was precoated onto a glass slide. Subsequent to the protein migration, antisera specific against one or more plasma proteins were allowed to diffuse throughout the gel. Rabbit anti-human LDL and anti-human whole serum were used. Where antigenic identity existed, a precipitin line would form. Shown in Fig. 13 is a typical immunoelectrophoretic pattern for one of the $^{125}$I-LDL preparations. The single precipitin lines are indicative of a β-globulin reactive with anti-LDL and anti-whole
Figure 13: Immunelectrophoresis of Iodinated LDL
serum. The arrowhead-shaped precipitin zone near the center well is due to inorganic salt ion interaction with the agarose gel and has nothing to do with the immunological reactivity of the LDL preparation. This, therefore, demonstrates that only β-protein was present and that the labeling procedures had not altered its electrophoretic mobility.

b. Immunodiffusion

Another immunological test was also performed as an additional check for unwanted protein contamination in the LDL preparation. The details of the procedure are based on the work of Ouchterlony (227). Labeled LDL is placed in a circular center well on an agarose coated glass slide. Antisera specific against various plasma proteins are situated in the six surrounding wells. As the antisera and labeled LDL diffuse through the gel, precipitin lines form where immunological identity exists. This is illustrated on Fig.14. Note that a single precipitin arc is found between the LDL well and the wells containing anti-whole serum, anti-VLDL, and anti-LDL. No reaction zones are present near the anti-HDL, anti-albumin, nor the anti-globulin wells. Since LDL and VLDL have an apo-protein in common, one would expect LDL to react with both anti-LDL and anti-VLDL in the manner shown on Fig.14. The absence of immunological reactivity with the other three antisera is indicative of the purity of the LDL injectate.

c. Gel Permeation Chromatography

The results from the foregoing procedures suggested the lack of any detectable non-LDL plasma protein contamination in
Figure 14: Immundiffusion of Iodinated LDL

A - anti-albumin
B - anti-whole serum
C - anti-LDL
D - anti-VLDL
E - anti-HDL
F - anti-γ-globulin
the labeled preparations. The evidence that the labeled material was primarily LDL and not smaller polypeptides was provided by gel permeation chromatography (GPC). Labeled albumin was studied as well as preparations of labeled human and rabbit LDL. The results for the human LDL are shown on Fig. 15; the rabbit LDL results are given on Fig. 16. The peak radioactivity for both samples was found in the 18th ml elution fraction. Note that the void volume of the column was 18 ml. Thus, the labeled proteins were excluded from the G-200 Sephadex gel used in these studies just as one would expect for a molecule of 2 x 10^6 molecular weight. (The exclusion molecular weight for Sephadex G-200 is 800,000). No other radioactive peaks were detectable until 45-50 ml had been eluted. This minor second peak was largely unbound ^{125}I as determined by measuring the TCA precipitable radioactivity (=29.4%). The results for labeled albumin are shown on Fig. 17. Only one broad radioactive peak was found centered about an elution volume of 29 ml. As with the LDL preparations, the presence of a small free ^{125}I peak was observed near an elution volume of 47 ml. Since all free ^{125}I was removed by dialysis just prior to the administration of the labeled preparations to the animals, this minor impurity was of no significant experimental consequence. Note that no peaks were found for any of the preparations between 35 and 45 ml. Thus, the presence of significant quantities of small labeled polypeptides is contra-indicated.

d. Paper Electrophoresis

Paper electrophoresis was performed on both labeled LDL and albumin. The migration patterns were typical for each
Figure 15  Gel Permeation Chromatography
Human $^{125}$I - LDL Elution Through G-200 Sephadex

free $^{131}$I peak (fraction 47: 29.4% TCA-precipitable)
Figure 16 Gel Permeation Chromatography
Rabbit $^{125}\text{I}$-LDL Elution Through G-200 Sephadex
Figure 17 Gel Permeation Chromatography
Rabbit $^{125}$I-Albumin Elution Through G-200 Sephadex
protein. Pictures of representative electrophoretic patterns of plasma proteins are shown in Fig. 18. The four strips are for four plasma samples taken from a rabbit at different intervals after the injection of radioactive albumin. Note that the protein-stained band most readily visible demarcates the albumin migration distance. The $\beta$-proteins are only faintly visible, migrating a distance equal to roughly 15% of the albumin migration distance. The quantitative distribution of rabbit and human LDL radioactivity found in injectate solutions is presented as a function of migration distance on Figs. 19 and 20. Note that no significant amount of radioactivity migrated more than three centimeters. As is shown on Fig. 21, the radioactivity associated with labeled albumin migrated 7-9 cm. Thus, the migration distances of the LDL radioactivity and the albumin radioactivity were consistent with the relative electrophoretic mobility of the two proteins. The broadness of the LDL radioactivity distribution over the 0-3 cm range could possibly be attributable to trailing effects--the physical absorption of the labeled protein onto the paper. Another possibility would be the presence of aggregated labeled material, such as denatured protein, in the preparations. The procedure used to minimize the potential consequences of this possibility are discussed in the next section. Note that low levels of radioactivity were detectable in the 0-6 cm range on the labeled albumin electrophoretic pattern (Fig. 21). This pre-peak radioactivity might be attributable trailing effects, labeled aggregates, and/or free $^{125}$I which was inadvertently introduced during the process of staining the paper.
Figure 18: Paper Electrophoresis of $^{125}$I-LDL Rabbit Plasma
(Bromphenol Blue Used To Stain For Protein)
Figure 19 Paper Electrophoresis of Rabbit $^{125}$I-LDL Injectates: Radioactivity Distribution
Figure 20  Paper Electrophoresis of Human $^{125}$I-LDL

Injectates: Radioactivity Distribution
Figure 21 Paper Electrophoresis of Rabbit $^{125}$I-Albumin Plasma: Radioactivity Distribution
strip. The ramifications of these possibilities on the aortic uptake results will be discussed later (VII. DISCUSSION OF RESULTS).

e. Ultracentrifugational Analysis

In most cases the labeled LDL solutions were subjected to an additional ultracentrifugation step prior to administration to the animal. Not only did this provide a test for the aqueous density of the labeled material, but it also served as a final purification step. The fraction of the radioactivity which still floated with LDL (1.025<p<1.050) was determined. Results from six preparations are given in Table III. With one exception, better than 80% of the radioactivity was recovered in the LDL density fraction. The other fractions were discarded and only the LDL fraction was injected into the animal. It should be noted that this ultracentrifugational "washing" does not guarantee the homogeneity of the labeled LDL. For example, aggregated material was often detected floating with the LDL. This presumably represented denatured proteins. These aggregates were removed by filtration prior to injection into the animal as evidenced by the results of an in vivo labeled LDL screening experiment discussed later (see A3aii.). Thus, the material injected into the animal was not detectably contaminated with foreign labeled compounds.

f. Summary of Injectate Testing

With the exception of analytical ultracentrifugation, the test results described in the preceding paragraphs were obtained on one or two randomly chosen labeled protein preparations. Taken as a whole, they indicate no detectable amount of labeled foreign
TABLE III.
Distribution of Radioactivity (\textsuperscript{125}I-LDL) After Analytical Ultracentrifugation in SW41 Rotor

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Solute</th>
<th>(%) of Total Radioactivity</th>
<th>Density Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>167</td>
<td>\textsuperscript{131}I-LDL*</td>
<td>6.7</td>
<td>80.3</td>
</tr>
<tr>
<td>167</td>
<td>\textsuperscript{125}I-LDL*</td>
<td>5.7</td>
<td>66.7</td>
</tr>
<tr>
<td>160</td>
<td>\textsuperscript{125}I-LDL*</td>
<td>-</td>
<td>95.7</td>
</tr>
<tr>
<td>156, 157</td>
<td>\textsuperscript{125}I-LDL*</td>
<td>-</td>
<td>81.3</td>
</tr>
<tr>
<td>154, 155</td>
<td>\textsuperscript{125}I-LDL*</td>
<td>-</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>\textsuperscript{125}I-LDL+</td>
<td>-</td>
<td>96.9</td>
</tr>
</tbody>
</table>

* Human LDL
+ Rabbit LDL
material was present in the injectates given to the experimental animals. The characterization of the labeled solute circulating in the plasma of the recipient rabbits is summarized in the next section.

2. Plasma Isotope Analysis

Plasma samples were taken at intervals during each experiment in order to characterize the change in driving force for labeled solute transport into the vessel wall. The rate of removal of labeled solute from the plasma varied from animal to animal. The range of the plasma isotope decay curves as well as an individual decay curve is shown on Figs. 22 and 23. Note that after 24 hours, the LDL radioactivity was but 17-43% of the initial level (Fig. 22). Whereas, with labeled albumin, 41-49% remained after 24 hours (Fig. 23). The plasma isotope half-lives ranged from 4-20 hours (average = 10.8) for LDL and 16.5-22.8 hours (average = 20.1) for albumin (Table IV.). Only the LDL experiments were extended to 67 hours. Plasma isotope concentrations at this time had fallen to 4-11% of the initial level (Fig. 22).

As with most labeled proteins, the plasma isotope levels fell in exponential fashion. This is shown for two particular experiments on Fig. 24. Both LDL and albumin data were statistically fit with a two component exponential function. Others have used this two pool model to describe LDL and albumin turnover. The first accounted for the observed rapid rate of isotope disappearance during the initial three hours. The second term allowed for the gradual decline at longer times. The half-lives of the two components
Figure 22 \(^{125}\)I-LDL Plasma Decay: Range Over All Experiments

\[ \frac{C_p(t)}{C_p(0)} \]

TIME, HOURS
Figure 23 $^{125}$I-Albumin Plasma Decay: Range Over All Experiments

Exp. 170
TABLE IV.

Plasma Isotope Half-life *

<table>
<thead>
<tr>
<th>LDL Experiment No.</th>
<th>Duration (hr)</th>
<th>t_1/2 (hr)</th>
<th>Albumin Experiment No.</th>
<th>Duration (hr)</th>
<th>t_1/2 (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>160</td>
<td>24</td>
<td>3.98</td>
<td>170</td>
<td>24</td>
<td>16.5</td>
</tr>
<tr>
<td>137</td>
<td>67</td>
<td>6.16</td>
<td>158</td>
<td>24</td>
<td>21.0</td>
</tr>
<tr>
<td>142</td>
<td>24</td>
<td>8.33</td>
<td>136</td>
<td>24</td>
<td>22.8</td>
</tr>
<tr>
<td>165</td>
<td>24</td>
<td>10.63</td>
<td></td>
<td></td>
<td></td>
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<td>166</td>
<td>67</td>
<td>15.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>163</td>
<td>67</td>
<td>20.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average = 10.8 ± 6.0

Average = 20.1 ± 3.2

* Defined as the time required for the plasma isotope concentration to fall to 50% of its initial value as estimated from interpolation of semi-logarithmic plasma decay curves.
Figure 24 Double Exponential Decay of Labeled Albumin and LDL

Least Squares Regression:

\[
\left[ \frac{C_p(t)}{C_p(0)} \right] = 0.25 e^{-1.557t} + 0.75 e^{-0.0254t}
\]

\((^{125}\text{I-Albumin})\)

\[
\left[ \frac{C_p(t)}{C_p(0)} \right] = 0.29 e^{-0.777t} + 0.71 e^{-0.0434t}
\]

\((^{125}\text{I-LDL})\)
were 0.89 and 15.97 hours for LDL and 0.45 and 27.29 hours for albumin. The fits are quite good for these two particular experiments. However, due to the variability among experiments, these functions are not universally applicable to all albumin and LDL data. A least squares analysis was performed to fit all of the albumin and all of the LDL data for experiments of at least four hour duration (Figs. 25 and 26). The resulting functions are reasonable approximations of the data for times less than two hours. At longer times the deviation between the functions and the experimental data is more pronounced. The half-lives for the two component decay rates are 0.89 and 16.91 hours for LDL and 1.07 and 36.48 hours for albumin. These two plasma decay functions were used in conjunction with the analysis of the labeled LDL and albumin intramural distributions. The specific manner in which the functions were utilized will be discussed later (see VI. THEORY).

In two experiments (Experiments 142 and 143), the distribution of the plasma radioactivity in the terminal plasma sample was studied. The plasma lipoproteins were separated by ultracentrifugation (see IV. APPARATUS AND PROCEDURES) and the protein-bound radioactivity in each fraction determined. Experiment 142 was a 24 hour double isotope study. Four hours prior to the end of the experiment, a second dose of labeled LDL ($^{131}$I-LDL) was administered to the animal. $^{125}$I-LDL was given at the start of the experiment. Experiment 143 was a 30 minute single isotope study. Thus, the two experiments represent three different deviations, viz., 30 minutes, four hours, and 24 hours. The distribution of the isotope among
Figure 25 Double Exponential Decay for all Albumin Experiments 4 Hours and Longer - Least Squares Analysis

For $t$ in hours,

$$f_1(t) = \frac{C_p(t)}{C_p(0)} = 0.25 e^{-0.647 t} + 0.75 e^{-0.019 t}$$

Without $\Phi$,

$$f_2(t) = \frac{C_p(t)}{C_p(0)} = 0.29 e^{-0.574 t} + 0.71 e^{-0.0199 t}$$
For $t$ in hours,
\[ \frac{C_p(t)}{C_p(0)} = 0.33 e^{-0.775t} + 0.67 e^{0.041t} \]

Figure 26 Double Exponential Decay for all LDL Experiments 4 Hours and Longer - Least Squares Analysis
the lipoprotein fractions in the terminal plasma samples is presented in Fig. 27. The LDL fraction contained 93.8 - 95.1% of the plasma protein-bound radioactivity. Less than 3.6% of the radioactivity was associated with proteins with an aqueous density greater than 1.063 gm/ml.

Paper electrophoresis was also used to characterize the radioactive plasma proteins. The results for rabbit and human \(^{125}\)I-LDL experiments (Figs. 28 and 29) are similar to the radioactivity distributions in the original injectates (Fig. 19 and 20). The majority of radioactivity migrated with \(\beta\)-proteins. The electrophoretic distribution of \(^{125}\)I-albumin in rabbit plasma was already presented (Fig. 21) showing practically all the radioactivity in the albumin fraction.

To test the possibility that some of the radioactive LDL injectates contained aggregated material, an in vivo screening experiment was conducted. \(^{131}\)I-LDL, prepared in the usual manner, was screened in a donor rabbit (0.63 kg). After 17 minutes, the donor animal was exsanguinated and the blood plasma (containing "screened" \(^{131}\)I-LDL) along with a dose of "unscreened" \(^{125}\)I-LDL, was given to a second recipient rabbit. The ratio of "screened" to "unscreened" LDL radioactivity in the recipient rabbit plasma was followed over the four hour duration of the experiment. The ratio was found not to change during the four hours (Fig. 30). Both labeled lipoproteins disappeared from the plasma at the same rate. The comparison of the intramural distribution of the "screened" and "unscreened" labeled LDL is presented at the end of the next section.
Figure 27 Distribution of Label in Terminal Rabbit Plasma Ultracentrifugation Fractions
Figure 28 Paper Electrophoresis Human $^{125}$I-LDL Exp. 154 - Terminal Plasma (30 Minutes)
Figure 29 Paper Electrophoresis Rabbit $^{125}$I-LDL Exp 155 - Terminal Plasma Sample (30 Minutes)
Figure 30 Effect of Prescreening on the Clearance of Labeled Lipoproteins from Rabbit Plasma
3. **Intramural Distribution of LDL and Albumin**

As discussed previously (IV. **APPARATUS AND PROCEDURE**), the distribution of labeled solute across the aortic wall was determined by counting successive 20μ thick sections prepared on a refrigerated microtome. Only the tissue radioactivity which precipitated in 10% TCA was counted. Thus, the results were expressed as the protein-bound radioactivity (counts/min) per ml of tissue relative to the radioactivity per ml in the initial plasma sample. The volume of each tissue slice was taken as the product of its area and 20μ-thickness. Each experiment resulted in up to 10 separate profiles of the normalized tissue radioactivity as a function of positions within the wall. Any tissue slices which were visibly contaminated with blood (usually a few of the slices near the media-adventitia junctions) were excluded from the data analysis, as were all adventitia slices, since invariably, these were highly contaminated with blood. A special experiment using ^{51}Cr-labeled erythrocytes verified that, except for the adventitia slices and a few random slices just inside the media, blood contamination was negligible (see **APPENDIX D**). Therefore, since only protein-bound (TCA precipitable) radioactivity was counted, the profiles represented extravascular labeled solute which had accumulated in the aortic wall. The raw data from each profile were combined into one average profile by grouping slices according to their position relative to the intima and media-adventitia junction (see **APPENDIX B: SAMPLE CALCULATIONS**). Thus, each experiment was expressed in terms of a single average profile. To compare profiles resulting from experiments of different duration,
all profile raw data resulting from experiments of the same duration were averaged to form the grand average profile. For LDL, 10 minute, 30 minute, 4 hour, 24 hour, and 67 hour experiments were conducted. With the exception of 67 hours, albumin data were taken at the same intervals. In addition to the labeled solute distribution across the wall, an estimate of the total solute accumulation in the wall after a given time was made by numerically integrating the grand average profiles. The overall average results are discussed below. Individual experiment results are covered in APPENDIX D.

a. Labeled LDL Distribution and Net Accumulation

The grand average distribution of labeled LDL after 10 minutes, 30 minutes, and 4 hour experiments is shown on Fig. 31. The profiles reflect a rapid entry and penetration of labeled solute with the labeled solute penetrating the entire thickness of the wall in less than 10 minutes. At all three times the concentration of solute was highest near the intima and lowest in the center part of the media. The absolute levels in the wall were quite low. On a per volume basis the tissue contained less than 1% of the labeled solute activity in plasma. However, the concentration in the tissue increased with increasing time. The change in total accumulation with time will be discussed in more detail later, but note that the shape of the profiles up to four hours remains largely the same regardless of the level of labeled solute in the wall. The difference between the 10 minute and 30 minute profiles in mean level at all radial positions is statistically significant (p < 0.05) (Table V. ). However, not all of the four hour profile means are
Figure 31  Grand Summary of LDL Data-10 Minutes Through 4 Hours

TIME AFTER INJECTION

- 10 MIN
- 30 MIN
- 4 HR

Relative Position, x/L

Relative Tissue Concentration, C/Cp(0) x 10^3

INTIMA

4 HR

30 MIN

10 MIN

MEDIA - ADVENTITIA BORDER

0 0.2 0.4 0.6 0.8 1.0
Relative Position, x/L

10 8 6 4 2 0

(a)
<table>
<thead>
<tr>
<th>Range of Depth (cm)</th>
<th><strong>10 Minutes</strong></th>
<th><em>p</em></th>
<th><strong>30 Minutes</strong></th>
<th><em>p</em></th>
<th><strong>4 Hours</strong></th>
<th><em>p</em></th>
<th><strong>24 Hours</strong></th>
<th><em>p</em></th>
<th><strong>67 Hours</strong></th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 - 0.05</td>
<td>0.0053 ± 0.0023</td>
<td>&lt;0.010</td>
<td>0.0104 ± 0.0040</td>
<td>N.S.</td>
<td>0.0111 ± 0.0019</td>
<td>N.S.</td>
<td>0.0119 ± 0.0091</td>
<td>&lt;0.05</td>
<td>0.0028 ± 0.0012</td>
<td>(8)</td>
</tr>
<tr>
<td>0.051 - 0.15</td>
<td>0.0038 ± 0.0036</td>
<td>&lt;0.050</td>
<td>0.0070 ± 0.0034</td>
<td>&lt;0.02</td>
<td>0.0107 ± 0.0039</td>
<td>&lt;0.02</td>
<td>0.0062 ± 0.0035</td>
<td>&lt;0.05</td>
<td>0.0034 ± 0.0025</td>
<td>(13)</td>
</tr>
<tr>
<td>0.151 - 0.25</td>
<td>0.0024 ± 0.0014</td>
<td>&lt;0.001</td>
<td>0.0058 ± 0.0022</td>
<td>&lt;0.05</td>
<td>0.0087 ± 0.0044</td>
<td>N.S.</td>
<td>0.0059 ± 0.0036</td>
<td>&lt;0.02</td>
<td>0.0035 ± 0.0022</td>
<td>(22)</td>
</tr>
<tr>
<td>0.251 - 0.35</td>
<td>0.0015 ± 0.0008</td>
<td>&lt;0.001</td>
<td>0.0035 ± 0.0017</td>
<td>N.S.</td>
<td>0.0051 ± 0.0029</td>
<td>N.S.</td>
<td>0.0015 ± 0.0030</td>
<td>&lt;0.02</td>
<td>0.0026 ± 0.0021</td>
<td>(22)</td>
</tr>
<tr>
<td>0.351 - 0.45</td>
<td>0.0014 ± 0.0009</td>
<td>&lt;0.001</td>
<td>0.0029 ± 0.0012</td>
<td>N.S.</td>
<td>0.0040 ± 0.0024</td>
<td>N.S.</td>
<td>0.0040 ± 0.0021</td>
<td>N.S.</td>
<td>0.0033 ± 0.0023</td>
<td>(22)</td>
</tr>
<tr>
<td>0.451 - 0.55</td>
<td>0.0009 ± 0.0007</td>
<td>&lt;0.001</td>
<td>0.0026 ± 0.0014</td>
<td>N.S.</td>
<td>0.0036 ± 0.0019</td>
<td>N.S.</td>
<td>0.0030 ± 0.0016</td>
<td>N.S.</td>
<td>0.0025 ± 0.0022</td>
<td>(22)</td>
</tr>
<tr>
<td>0.551 - 0.65</td>
<td>0.0007 ± 0.0004</td>
<td>&lt;0.001</td>
<td>0.0023 ± 0.0011</td>
<td>&lt;0.05</td>
<td>0.0034 ± 0.0016</td>
<td>N.S.</td>
<td>0.0029 ± 0.0014</td>
<td>N.S.</td>
<td>0.0033 ± 0.0023</td>
<td>(23)</td>
</tr>
<tr>
<td>0.651 - 0.75</td>
<td>0.0008 ± 0.0006</td>
<td>&lt;0.001</td>
<td>0.0025 ± 0.0016</td>
<td>&lt;0.05</td>
<td>0.0032 ± 0.0013</td>
<td>N.S.</td>
<td>0.0031 ± 0.0017</td>
<td>N.S.</td>
<td>0.0025 ± 0.0020</td>
<td>(22)</td>
</tr>
<tr>
<td>0.751 - 0.85</td>
<td>0.0011 ± 0.0010</td>
<td>&lt;0.010</td>
<td>0.0031 ± 0.0018</td>
<td>&lt;0.05</td>
<td>0.0042 ± 0.0015</td>
<td>N.S.</td>
<td>0.0038 ± 0.0015</td>
<td>N.S.</td>
<td>0.0038 ± 0.0025</td>
<td>(21)</td>
</tr>
<tr>
<td>0.851 - 0.95</td>
<td>0.0016 ± 0.0014</td>
<td>&lt;0.050</td>
<td>0.0044 ± 0.0034</td>
<td>N.S.</td>
<td>0.0041 ± 0.0018</td>
<td>N.S.</td>
<td>0.0048 ± 0.0017</td>
<td>&lt;0.05</td>
<td>0.0030 ± 0.0020</td>
<td>(13)</td>
</tr>
<tr>
<td>0.951 - 1.00</td>
<td>0.0029 ± 0.0022</td>
<td>&lt;0.020</td>
<td>0.0052 ± 0.0021</td>
<td>N.S.</td>
<td>0.0046 ± 0.0023</td>
<td>N.S.</td>
<td>0.0046 ± 0.0023</td>
<td>N.S.</td>
<td>0.0034 ± 0.0021</td>
<td>(13)</td>
</tr>
</tbody>
</table>

**Average Net Accumulation**

| Average Net Accumulation | 0.0018 ± 0.0005 | 0.0042 ± 0.0007 | 0.0055 ± 0.0008 | 0.0046 ± 0.0010 | 0.0031 ± 0.0007 |

**Expressed as (cpm/ml) tis / (cpm/ml) initial plasma ± Standard Deviation; Number of observations shown in parentheses**

**Average Net Accumulation**

| Average Net Accumulation | 0.0018 ± 0.0005 | 0.0044 ± 0.0008 | 0.0073 ± 0.0012 | 0.0110 ± 0.0024 | 0.0112 ± 0.0026 |

**Expressed as (cpm/ml) tis / (cpm/ml) average plasma ± Standard Deviation**

* Students t-test between two adjacent means; *p* < 0.05 considered statistically significant; N.S. = not significant

**Determined by numerically integrating each plasma decay curve and averaging all results from experiments of the same duration**
statistically greater than those for 30 minutes. This is in part attributable to greater variation within the four hour experimental profiles. Note, too, that variation about the means for all times is greatest near the intima and also near the media-adventitia junction. The possible reasons for this will be covered later (VII. DISCUSSION OF RESULTS).

The results from the longer-term experiments are illustrated on Fig. 32. The four hour curve is included to facilitate the comparison with the short-term profiles. Unlike the short-term profiles, the 24 hour and 67 hour profiles are dissimilar in shape. The 24 and four hour profiles are virtually the same with the level highest near the intima and lowest in the center media. However, after 67 hours, the gradient no longer persists. In addition, the absolute level of labeled solute in the wall after 67 hours is lower in the intima than at four and 24 hours. Interestingly enough, the level in the outer media remains the same at longer times. Thus, only at 67 hours does the shape vary. Changes in absolute level of the profiles are most pronounced up to four hours.

This variation in absolute level of the profiles appreciated in terms of changes in net accumulation. The tabular data (Table V.) for average accumulation are presented on Fig. 33. The rate of accumulation is highest up to 30 minutes. Thereafter, it diminishes to the point that somewhere between 30 minutes and 24 hours, influx is completely offset by efflux resulting in no net accumulation of labeled solute. Between 24 and 67 hours depletion of labeled solute in the wall is observed. Thus, net accumulation goes through a
Figure 32  Grand Summary of LDL Distribution Data—4 Hours Through 67 Hours

**TIME AFTER INJECTION**
- ▲ 4 HRS
- ■ 24 HRS
- ○ 67 HRS

Relative Tissue Concentration, \( C / C_p(0) \times 10^3 \)

**Relative Position** \( x/L \)

**Intima**

- 4 HR
- 24 HR
- 67 HR

(b)
Figure 33 Average Net Accumulation of Labeled LDL Expressed Relative to Initial Plasma Isotope Concentration
maximum. This is undoubtedly a reflection of the fact that the plasma
isotope levels decline in an exponential fashion throughout the
experiment. As is shown on Fig.34, if the accumulation is expressed
relative to the time-averaged plasma isotope concentrations, no such
maximum occurs. However, presentation of the data in this manner
is misleading in that it suggests that even after 24 hours, the aorta
is continuing to accumulate labeled LDL when, in actuality, it is not.
This point will be more thoroughly discussed later (VII. DISCUSSION
OF RESULTS) when comparisons are made between these results and those
reported previously in the literature.

1. **Justification of Averaging Procedures**

The trends in labeled solute distribution and net
accumulation shown thus far are the result of averaging all the
experimental data for a given time. Certain assumptions are implied
by analyzing the data in this fashion. First of all, it is assumed
that any variation in the intramural profile with position from the
aortic arch to near the diaphragm can be neglected. Secondly, the
intima-media thickness (L) of all tissue samples analyzed is taken
to be not widely different. Thirdly, it is assumed that within a
given experiment, profile-to-profile variation is random such that
the average of all profiles represents the best estimate of the true
profile for that experiment. And finally, the method of data analysis
used here assumes that animal-to-animal variation is also random.
Thus, the average of the raw data from all experiments for a given
time is taken to be the best estimate of the true solute distribution
for that particular time interval. The validity of these assumptions
will be discussed in detail later (see VII. DISCUSSION OF RESULTS).
Figure 34 Average Net Accumulation of Labeled LDL Expressed Relative to the Time-Averaged Plasma Isotope Concentration

- Calculated value based on averaged experimental profiles and averaged plasma isotope concentrations (+ S.D.)
For the moment, suffice it to say that the averaging procedure was justified on the grounds that it afforded a concise method for presenting the data that allowed for comparison of the profiles at different experimental times.

To demonstrate that the variation among profiles from a single experiment was essentially random, individual profiles from ten minute and four hour $^{125}$I-LDL studies are compared with the experimental average from each experiment (Figs.35 and 36). Similar comparisons for other time intervals are found in APPENDIX D. Note that the average of all profiles from the same experiment represented a smoothed distribution. Thus, the random effects, introduced by factors such as slice thickness variation and the non-isotropic nature of the tissue, were damped out by averaging. It should also be pointed out that the variation in the absolute level of dimensionless tissue concentration between profiles was greater at four hours than at 10 minutes. As a generalization, variation in the profile levels was the largest at the four and 24 hour time intervals. However, the shapes of the individual profiles were generally the same as the individual experimental average regardless of the time interval.

The relatively good agreement between individual profiles and the average profile for a given experiment is especially significant when one considers that the individual profiles were not distinguished according to position along the thoracic aorta. Others have shown that net labeled solute uptake indeed does vary with position in the aorta of dogs (179). Unfortunately, the data presented here were not recorded in such a fashion to permit such a comparison.
Figure 35 Comparison of Raw Profile Data and the Experimental Average Profile (Exp 157) \( t = 10 \) Minutes
Figure 36  Comparison of Individual Profile Data With the Experimental Average-LDL (Exp. 145-\( t = 4 \) hours)

- Individual Profiles
- Experiment Average
The variation between average profiles for individual experiments at the same time interval is illustrated on Fig. 37 for the four hour LDL studies. All average profiles indicated the greatest solute accumulation near the intima with a fairly uniform lower-level retention in the outer two-thirds of the wall. Although not large, the biggest differences between experimental averages were found near the intima. This observation held for most all of the time intervals. Since the animal-to-animal variation in the profiles was not great, the averaging of the raw data from individual profiles to form a grand average profile could be justified.

ii. Results of Special LDL Experiments

In all but one experiment human labeled LDL was used as opposed to rabbit labeled LDL. No differences in the uptake or distribution of the labeled LDL could be attributed to the source of the LDL. This is shown on Fig. 38 where the distribution of rabbit LDL after 30 minutes is compared with the overall average of all 30 minute LDL experiments. The profiles were virtually identical.

To test the possibility that some of the injected LDL may have been in an aggregated state, a screening experiment (previously described; V. A3) was conducted. The ratio of unscreened to screened labeled LDL in the plasma of the recipient animal did not change during the four hour exposure period (Fig. 30). The relative distribution of the "screened" and "unscreened" LDL in the aorta of the recipient rabbit was also similar (Fig. 39). Although the level of unscreened labeled solute is slightly higher near the intima, this difference is well within experimental
Figure 37 Comparison of Experimental Average Profiles for 4 Hour Labeled LDL Penetration
Figure 38 Comparison of Labeled Rabbit LDL and Human LDL Distributions (± Standard Error of the Mean) (t = 30 Minutes)

- O--Rabbit LDL (Exp. 155)
- Grand Average of all 30 Min LDL Data (N=5)

Relative Tissue Radioactivity C/Cp(o)

Relative Position X/L
Figure 39 Comparison of Penetration of Screened and Unscreened Labeled LDL in the Same Animal, t = 4 hours
variation for typical four hour experiments. The standard error of the means is not shown for the screening experiment results since these results represent radioactivity in pooled tissue samples as opposed to the usual average of 6-7 separately determined tissue profiles. The lower isotope levels used in the screening experiment prevented accurate analysis of the individual profiles. Pooling was done by quantitatively combining frozen TCA-precipitated tissue residues in single tubes according to the relative position of each slice. Note that the screening experiment results were similar to the overall average of all four hour LDL experiments. This suggests that the labeled LDL was not aggregated to any significant degree in any of the four hour LDL studies.

One other LDL experiment was conducted with $^{51}$Cr-labeled red blood cells to insure that none of the intramural labeled LDL was due to blood contamination. The results as this experiment as well as an experiment on the distribution of free $^{125}$I are covered in APPENDIX D: Details of Experimental Results. The results from an anesthetized rabbit experiment are also given in APPENDIX D.

b. Intramural Labeled Albumin Distribution

Albumin distribution studies were performed identically to the LDL experiments. The overall average profiles are presented graphically on Fig. 40 and in tabular form in Table VI. The shape of the profiles were generally the same at all time periods studied. A minimum in labeled solute level was observed near the middle of each average distribution. The levels near the intima were generally higher than near the media-adventitia junction. However, there
Figure 40  Grand Summary of Albumin Distribution Data
**TABLE VI.**

<table>
<thead>
<tr>
<th>Range of Depth (n)</th>
<th>10 Minutes</th>
<th>30 Minutes</th>
<th>4 Hours</th>
<th>24 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 - 0.05</td>
<td>0.0158 ± 0.0006</td>
<td>N.S.</td>
<td>0.0149 ± 0.0027</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.051 - 0.15</td>
<td>0.0083 ± 0.0030</td>
<td>&lt;0.05</td>
<td>0.0133 ± 0.0038</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.151 - 0.25</td>
<td>0.0049 ± 0.0017</td>
<td>&lt;0.02</td>
<td>0.0091 ± 0.0036</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.251 - 0.35</td>
<td>0.0029 ± 0.0013</td>
<td>&lt;0.01</td>
<td>0.0063 ± 0.0027</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.351 - 0.45</td>
<td>0.0028 ± 0.0017</td>
<td>N.S.</td>
<td>0.0038 ± 0.0017</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.451 - 0.55</td>
<td>0.0018 ± 0.0008</td>
<td>&lt;0.01</td>
<td>0.0036 ± 0.0008</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.551 - 0.65</td>
<td>0.0020 ± 0.0007</td>
<td>&lt;0.02</td>
<td>0.0040 ± 0.0023</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.651 - 0.75</td>
<td>0.0013 ± 0.0007</td>
<td>&lt;0.01</td>
<td>0.0033 ± 0.0026</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>0.751 - 0.85</td>
<td>0.0036 ± 0.0043</td>
<td>N.S.</td>
<td>0.0050 ± 0.0042</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.851 - 0.95</td>
<td>0.0065 ± 0.0061</td>
<td>N.S.</td>
<td>0.0060 ± 0.0068</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.951 - 1.00</td>
<td>0.0047 ± 0.0044</td>
<td>N.S.</td>
<td>0.0060 ± 0.0040</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Average Net Accumulation †

<table>
<thead>
<tr>
<th></th>
<th>0.0044 ± 0.0010</th>
<th>0.0065 ± 0.0012</th>
<th>0.0078 ± 0.0014</th>
<th>0.0091 ± 0.0014</th>
</tr>
</thead>
</table>

\[ \frac{C}{C_0} \] **

<table>
<thead>
<tr>
<th></th>
<th>1.0000</th>
<th>0.9810 ± 0.0031</th>
<th>0.7710 ± 0.0020</th>
<th>0.6580 ± 0.0012</th>
</tr>
</thead>
</table>

Average Net Accumulation ‡

<table>
<thead>
<tr>
<th></th>
<th>0.0044 ± 0.0010</th>
<th>0.0074 ± 0.0014</th>
<th>0.0101 ± 0.0019</th>
<th>0.0143 ± 0.0024</th>
</tr>
</thead>
</table>

† Expressed as (cpm/ml)_{tis} / (cpm/ml)_{initial plasma} ± Standard Deviation; Number of observations shown in parentheses

‡ Expressed as (cpm/ml)_{tis} / (cpm/ml)_{average plasma} ± Standard Deviation

* Students t-test between two adjacent means; p < 0.05 considered statistically significant; N.S. = not significant

** Determined by numerically integrating each plasma decay curve \( \int_0^t \frac{C}{P} dt \) and averaging all results from experiments of the same duration

\[ \frac{C}{C_0} \]
existed considerable variation of the data in these regions as evidenced by the magnitudes of the standard deviations (Table VI.). Note that, as in the LDL studies, labeled albumin penetrated the entire aortic wall in less than 10 minutes. Unlike the LDL studies, the 24 hour profile level was slightly higher than at four hours.

c. Comparison of LDL and Albumin Labeled Solute Distributions

At all time intervals studied up to 24 hours, the dimensionless solute concentrations were higher for albumin than for LDL at most relative depths across the arterial wall. The comparison between mean levels at each time interval is presented in Table VII. At 10 minutes, the albumin dimensionless concentrations were statistically (p < 0.05) greater than those for LDL at all but two relative depths. Similar statistical differences were observed at the other time intervals with the exception of four hours. At this time the albumin dimensionless concentrations were significantly higher only in the outer 45% of the vessel wall.

The average net albumin accumulation, as determined by numerically integrating each grand average distribution, increased with increasing time. A comparison between albumin and LDL labeled solute accumulations is given on Fig. 41. At all time intervals studied, the dimensionless labeled albumin accumulation exceeded that for LDL. Both solute accumulation curves reflected the rapid influx of labeled material at early times followed by a period between 0.5 and 24 hours where the rate of accumulation diminished. Part of the differences in accumulation at four and 24 hours may be attributable to the faster plasma isotope disappearance rate in the LDL studies.
### TABLE VII.

**Comparison of Grand Average Albumin and LDL Distribution Data**

**t = 10 minutes**

<table>
<thead>
<tr>
<th>Range of Depth (mm)</th>
<th>LDL (C/(_0)P) S.D.</th>
<th>Albumin (C/(_0)P) S.D.</th>
<th>p +</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 - 0.05</td>
<td>0.0053 ± 0.0023</td>
<td>0.0158 ± 0.0106</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>0.051 - 0.15</td>
<td>0.0038 ± 0.0030</td>
<td>0.0083 ± 0.0003</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.151 - 0.25</td>
<td>0.0024 ± 0.0014</td>
<td>0.0049 ± 0.0017</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.251 - 0.35</td>
<td>0.0015 ± 0.0008</td>
<td>0.0029 ± 0.0013</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.351 - 0.45</td>
<td>0.0014 ± 0.0009</td>
<td>0.0028 ± 0.0017</td>
<td>&lt;0.02*</td>
</tr>
<tr>
<td>0.451 - 0.55</td>
<td>0.0009 ± 0.0007</td>
<td>0.0018 ± 0.0008</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.551 - 0.65</td>
<td>0.0007 ± 0.0004</td>
<td>0.0020 ± 0.0007</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.651 - 0.75</td>
<td>0.0008 ± 0.0006</td>
<td>0.0013 ± 0.0007</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>0.751 - 0.85</td>
<td>0.0011 ± 0.0010</td>
<td>0.0036 ± 0.0045</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>0.851 - 0.95</td>
<td>0.0016 ± 0.0014</td>
<td>0.0065 ± 0.0061</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>0.951 - 1.00</td>
<td>0.0029 ± 0.0022</td>
<td>0.0047 ± 0.0044</td>
<td>&lt;0.50</td>
</tr>
</tbody>
</table>

**t = 4 hours**

<table>
<thead>
<tr>
<th>Range of Depth (mm)</th>
<th>LDL (C/(_0)P) S.D.</th>
<th>Albumin (C/(_0)P) S.D.</th>
<th>p +</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 - 0.05</td>
<td>0.0111 ± 0.0019</td>
<td>0.0125 ± 0.0058</td>
<td>&lt;0.70</td>
</tr>
<tr>
<td>0.051 - 0.15</td>
<td>0.0107 ± 0.0039</td>
<td>0.0103 ± 0.0061</td>
<td>&lt;0.90</td>
</tr>
<tr>
<td>0.151 - 0.25</td>
<td>0.0087 ± 0.0044</td>
<td>0.0081 ± 0.0045</td>
<td>&lt;0.90</td>
</tr>
<tr>
<td>0.251 - 0.35</td>
<td>0.0051 ± 0.0029</td>
<td>0.0070 ± 0.0029</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>0.351 - 0.45</td>
<td>0.0040 ± 0.0024</td>
<td>0.0056 ± 0.0025</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>0.451 - 0.55</td>
<td>0.0036 ± 0.0019</td>
<td>0.0053 ± 0.0030</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>0.551 - 0.65</td>
<td>0.0034 ± 0.0016</td>
<td>0.0051 ± 0.0024</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>0.651 - 0.75</td>
<td>0.0032 ± 0.0013</td>
<td>0.0062 ± 0.0032</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.751 - 0.85</td>
<td>0.0042 ± 0.0015</td>
<td>0.0087 ± 0.0059</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.851 - 0.95</td>
<td>0.0041 ± 0.0018</td>
<td>0.0096 ± 0.0061</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.951 - 1.00</td>
<td>0.0046 ± 0.0023</td>
<td>0.0112 ± 0.0051</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

**t = 30 minutes**

<table>
<thead>
<tr>
<th>Range of Depth (mm)</th>
<th>LDL (C/(_0)P) S.D.</th>
<th>Albumin (C/(_0)P) S.D.</th>
<th>p +</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 - 0.05</td>
<td>0.0104 ± 0.0040</td>
<td>0.0149 ± 0.0027</td>
<td>&lt;0.300</td>
</tr>
<tr>
<td>0.051 - 0.15</td>
<td>0.0070 ± 0.0034</td>
<td>0.0133 ± 0.0038</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.151 - 0.25</td>
<td>0.0058 ± 0.0022</td>
<td>0.0091 ± 0.0036</td>
<td>&lt;0.020*</td>
</tr>
<tr>
<td>0.251 - 0.35</td>
<td>0.0035 ± 0.0017</td>
<td>0.0063 ± 0.0027</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>0.351 - 0.45</td>
<td>0.0029 ± 0.0012</td>
<td>0.0038 ± 0.0017</td>
<td>&lt;0.200</td>
</tr>
<tr>
<td>0.451 - 0.55</td>
<td>0.0026 ± 0.0014</td>
<td>0.0036 ± 0.0008</td>
<td>&lt;0.050*</td>
</tr>
<tr>
<td>0.551 - 0.65</td>
<td>0.0023 ± 0.0011</td>
<td>0.0040 ± 0.0023</td>
<td>&lt;0.010*</td>
</tr>
<tr>
<td>0.651 - 0.75</td>
<td>0.0025 ± 0.0016</td>
<td>0.0033 ± 0.0026</td>
<td>&lt;0.300</td>
</tr>
<tr>
<td>0.751 - 0.85</td>
<td>0.0031 ± 0.0018</td>
<td>0.0050 ± 0.0042</td>
<td>&lt;0.050*</td>
</tr>
<tr>
<td>0.851 - 0.95</td>
<td>0.0044 ± 0.0034</td>
<td>0.0060 ± 0.0068</td>
<td>&lt;0.500*</td>
</tr>
<tr>
<td>0.951 - 1.00</td>
<td>0.0052 ± 0.0021</td>
<td>0.0060 ± 0.0040</td>
<td>&lt;0.500*</td>
</tr>
</tbody>
</table>

**t = 24 hours**

<table>
<thead>
<tr>
<th>Range of Depth (mm)</th>
<th>LDL (C/(_0)P) S.D.</th>
<th>Albumin (C/(_0)P) S.D.</th>
<th>p +</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 - 0.05</td>
<td>0.0119 ± 0.0091</td>
<td>0.0136 ± 0.0032</td>
<td>&lt;0.900</td>
</tr>
<tr>
<td>0.051 - 0.15</td>
<td>0.0106 ± 0.0035</td>
<td>0.0120 ± 0.0046</td>
<td>&lt;0.010*</td>
</tr>
<tr>
<td>0.151 - 0.25</td>
<td>0.0059 ± 0.0036</td>
<td>0.0094 ± 0.0046</td>
<td>&lt;0.050*</td>
</tr>
<tr>
<td>0.251 - 0.35</td>
<td>0.0065 ± 0.0030</td>
<td>0.0080 ± 0.0038</td>
<td>&lt;0.010*</td>
</tr>
<tr>
<td>0.351 - 0.45</td>
<td>0.0060 ± 0.0021</td>
<td>0.0073 ± 0.0028</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>0.451 - 0.55</td>
<td>0.0030 ± 0.0016</td>
<td>0.0065 ± 0.0025</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>0.551 - 0.65</td>
<td>0.0029 ± 0.0014</td>
<td>0.0066 ± 0.0026</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>0.651 - 0.75</td>
<td>0.0031 ± 0.0017</td>
<td>0.0071 ± 0.0022</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>0.751 - 0.85</td>
<td>0.0038 ± 0.0015</td>
<td>0.0082 ± 0.0026</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>0.851 - 0.95</td>
<td>0.0048 ± 0.0017</td>
<td>0.0128 ± 0.0061</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>0.951 - 1.00</td>
<td>0.0046 ± 0.0023</td>
<td>0.0125 ± 0.0052</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* Students t-test between adjacent means

**++** Results expressed as (cpm/ml)_tissue / (cpm/ml)_initial plasma

* Statistically significant difference exists between mean values at the 95% confidence level.

Number of observations shown in parentheses.
Figure 41 Comparison of Labeled LDL and Labeled Albumin Average Net Accumulation Expressed Relative to the Initial Plasma Isotope Concentration
However, even when accumulation is normalized with respect to the time-averaged plasma isotope concentration, albumin uptake was higher than that for LDL at all time intervals (Fig. 42). Thus, net albumin accumulation, which represents the difference between influx and efflux of labeled material, exceeded that for LDL even when the effect of the decaying plasma isotope concentrations is taken into account.

The foregoing comparisons of net solute accumulation was based on the grand average profile for each solute. One experiment was conducted in which differently labeled albumin and labeled LDL were given to the same animal. Thus, the effect of any animal-to-animal variation was eliminated. The comparison of the average profiles from this 30 minute experiment is presented on Fig. 43. The albumin level was higher than LDL only over the inner two-thirds of the vessel wall. No differences were observed in the outer one-third. These observations are generally consistent with the differences noted between the grand average data presented in Table VII. Thus, it is unlikely that any animal-to-animal variation is responsible for the observed differences in net solute accumulation.

To summarize, the differences between the labeled albumin and LDL distributions are characterized by a more rapid influx of albumin giving rise to greater intramural accumulation. Since, at 10 minutes and at 30 minutes, the albumin dimensionless concentrations are consistently higher over the inner two-thirds of the wall, one may infer that the labeled albumin transintimal flux is greater than that for LDL. In the outer one-third of the wall, the concen-
Figure 42 Comparison of Labeled LDL and Labeled Albumin Average Net Accumulation Expressed Relative to the Time Averaged Plasma Radioactivity

* Average Uptake Determined from Average Profiles
Figure 43 Distribution of $^{131}$I-Albumin and $^{125}$I-LDL After 30 Minutes - Exp. No 149

(+ Standard Error of the Mean)

- $^{131}$I-Albumin
- $^{125}$I-LDL

RELATIVE TISSUE RADIOACTIVITY C/Cp(o)

RELATIVE POSITION X/L

Intima

Media Adventitia Junction
trations are not as uniformly different. Thus, transport processes which result in increased concentration near the media-adventitia junction are apparently less sensitive to the nature of the protein involved than those processes which occur in or near the intima.

4. Chemical Identity of Labeled Solute in the Vessel Wall

Numerous attempts were made to extract the labeled solute from the vessel wall (see IV. APPARATUS AND PROCEDURES) and to confirm immunologically the presence of human LDL. All attempts were unsuccessful. Labeled material could be extracted. However, the extract did not possess immunological reactivity with antiserum for LDL under the conditions employed.

The fraction of the radioactivity which was extractable from the abdominal aorta for both albumin and LDL experiments is summarized on Table VIII. On the average, 52.6% of the total radioactivity in albumin experiments was extractable and could be precipitated with 10% TCA. This compares with only 1.6% for LDL. Thus, albumin radioactivity was more readily recovered from the tissue homogenates. The low level of extractable LDL activity no doubt provides a partial explanation for the failure to identify immunologically any LDL in the extracts. Note that the extracts from the albumin studies were not subjected to immunological testing. Also, no analysis of the non-TCA precipitable radioactivity was attempted for either the albumin or the LDL studies. Presumably it represented mainly free labeled iodide which accumulated in the vessel wall during the course of the experiment.
TABLE VIII.
Radioactivity Extracted From Abdominal Aortic Tissue Homogenates

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Solute</th>
<th>Duration (hr)</th>
<th>Radioactivity Extracted * (%)</th>
<th>Precipitable Radioactivity Extracted * (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>158</td>
<td>Albumin</td>
<td>24.0</td>
<td>50.4</td>
<td>37.5</td>
</tr>
<tr>
<td>159</td>
<td>Albumin</td>
<td>4.0</td>
<td>71.7</td>
<td>67.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average 52.6</td>
<td></td>
</tr>
<tr>
<td>161</td>
<td>H-LDL</td>
<td>0.17</td>
<td>10.7</td>
<td>1.0</td>
</tr>
<tr>
<td>164</td>
<td>H-LDL</td>
<td>0.17</td>
<td>4.6</td>
<td>1.3</td>
</tr>
<tr>
<td>154</td>
<td>H-LDL</td>
<td>0.5</td>
<td>10.6</td>
<td>-</td>
</tr>
<tr>
<td>155</td>
<td>R-LDL</td>
<td>0.5</td>
<td>21.8</td>
<td>-</td>
</tr>
<tr>
<td>162</td>
<td>H-LDL</td>
<td>4.0</td>
<td>12.7</td>
<td>1.6</td>
</tr>
<tr>
<td>165</td>
<td>H-LDL</td>
<td>24.0</td>
<td>20.4</td>
<td>1.6</td>
</tr>
<tr>
<td>163</td>
<td>H-LDL</td>
<td>67.0</td>
<td>23.7</td>
<td>3.2</td>
</tr>
<tr>
<td>166</td>
<td>H-LDL</td>
<td>67.0</td>
<td>21.0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average 1.6</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as a percentage of the total radioactivity in the tissue
B. Hydraulic Permeability Studies

The hydraulic permeability of normal and atherosclerotic rabbit aorta was measured in vitro in a specially designed filtration cell. The transmural hydraulic flux as a function of the applied pressure gradient was determined as previously described (IV. APPARATUS AND PROCEDURES). The results of these experiments are summarized on Fig. 44. For normal tissue, observed fluxes were insensitive to pressure and ranged between 0.00075 and 0.0034 ml/cm²-hr. For atherosclerotic tissue, fluxes increased to a maximum of 0.0020 ml/cm²-hr at 160 mm Hg. At an even higher pressure (232 mm Hg), the flux was unchanged (0.00195 ml/cm²-hr). The procedure for measuring the hydraulic flux deserves mention. The hydraulic flux was determined by calculating the difference in fluid displacement rates from tissue covered with an impermeable plastic sheet and uncovered tissue. This differencing technique corrected for fluid displacement which resulted solely from tissue compression effects. The assumption was made that the rate of fluid displacement due to compression was identical for tissue in both chambers of the filtration cell. An experiment was performed to test this assumption. Tissue in both chambers was covered with the plastic sheet and fluid displacement recordings made as a function of the applied pressure drop. The steady state results are given on Fig. 45 for each of the four flow meters employed. The fluid displacement rate was converted to the "apparent" volumetric flux ($J_{f-blocked}$) by dividing by the cell surface area. Over the 50-250 mm Hg pressure range studied, the upper flow meter $J_{f-blocked}$ increased with increasing pressure and ranged from 0.0007 ml/cm²-hr to 0.0032 ml/cm²-hr.
Figure 44 Comparison of Hydraulic Permeability of Normal and Atherosclerotic Aortas (Based on Upper Flow Meters)
Figure 45
Comparison of Apparent Flows Observed when Both Chambers are Covered (Rabbit Aorta)
At each pressure drop used, the lower flow meter $J_f$-blocked were lower. Overall, they ranged from 0.0001 ml/cm$^2$-hr to 0.0025 ml/cm$^2$-hr. Although the upper flow meters gave higher readings, the difference between the two upper flow meter results was comparable to the difference between the two lower flow meter results. This is shown on Fig. 46. The differences were close to zero over the entire pressure range. Hence, the tissue compression effects were virtually the same for each chamber. Thus, justification was provided for taking the net hydraulic flow as the difference between the individual flow meter results.

The individual flow meter results for the normal tissue studies before differencing are shown on Figs. 47 and 48. The apparent volumetric flux through the "open" tissue ranged between 0.0026 ml/cm$^2$-hr and 0.0081 ml/cm$^2$-hr for both experiments. The range in flux from the blocked chambers was 0.00175 to 0.0071 ml/cm$^2$-hr. Comparison of the upper flow meter results or the lower flow meter results reveals that the flux was always greater from the uncovered chamber. Also, note that the apparent fluxes increased with increasing $\Delta P$. However, the net transmural flux, or difference, did not show the same sensitivity to pressure changes (Fig. 44).

The flux from the individual flow meter readings for the atherosclerotic tissue is illustrated on Figs. 49 and 50. The absolute flux values were similar to those from the normal tissue experiments. The blocked chamber readings ranged from 0.00175 to 0.0060 ml/cm$^2$-hr while the open chamber range was 0.0023 to 0.0080 ml/cm$^2$-hr. As with the normal tissue experiments, the
Figure 46  Difference in Apparent Flow Rates [Both Chambers Blocked]
Comparison of Flux from Covered and Uncovered Chambers
Rabbit Aorta

Figure 47
Figure 48 Apparent Volumetric Flux Through Normal Rabbit Aorta (Exp. 121)
Figure 49
APPARENT VOLUMETRIC FLUX ACROSS Atherosclerotic RABBIT ARTERY
Figure 50 Apparent Volumetric Flux Across Atherosclerotic Rabbit Aorta (Exp. 120)
individual fluxes were sensitive to pressure changes. In addition, the net transmural flux, or difference, also tended to increase slightly with pressure (Fig. 44).

Histology was performed to determine the extent of damage to the endothelial surface during the experiment. The endothelial coverage on the terminal tissue samples was estimated based on a visual microscopic survey of the entire tissue area. The results (Table IX.) indicate coverages ranging from 30-65%. Some damage to the endothelium was unavoidable no matter how careful one was in handling the tissue. "Good" endothelium is demonstrated on Fig. 51. It is characterized by a continuous layer of cells which form a cobblestone-like surface. Denuded endothelium (also demonstrated on Fig. 51) is evidenced by the lack of this cobblestone-like appearance. The degree of atherosclerotic lesion coverage varied in the two animals studied. Shown on Fig. 52a is a picture of the aorta from Experiment 120 at the end of the experiment. The test section (left-hand side) was less than 25% covered by lesions. In Experiment 118, lesion coverage was more extensive. Roughly 30-40% of the tissue surface was involved with lesions (Fig. 52b). Since lesion coverage was not complete in either experiment, conclusions as to the effect of lesions on the hydraulic permeability of rabbit aorta may not be warranted.
# TABLE IX.

Condition of Endothelium at the End of the Hydraulic Permeability Experiments

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Type</th>
<th>Time in Cell (hr)</th>
<th>Time Since Sacrifice (hr)</th>
<th>% &quot;Good&quot; Endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>Normal</td>
<td>10.5</td>
<td>11.25</td>
<td>~65</td>
</tr>
<tr>
<td>121</td>
<td>Normal</td>
<td>3.2</td>
<td>4.5</td>
<td>~60</td>
</tr>
<tr>
<td>124</td>
<td>Normal</td>
<td>6.0</td>
<td>7.5</td>
<td>~50</td>
</tr>
<tr>
<td>118</td>
<td>Athero.</td>
<td>5.9</td>
<td>7.6</td>
<td>~30</td>
</tr>
<tr>
<td>120</td>
<td>Athero.</td>
<td>2.1</td>
<td>3.2</td>
<td>~30</td>
</tr>
</tbody>
</table>
Figure 52: Lesion Coverage of Atherosclerotic Aortas
VI. THEORY-CONVECTION AND DIFFUSION ACROSS AORTIC TISSUE

Based on the experimental evidence presented previously (III. INTRODUCTION), solute transport across the arterial wall may be the result of pressure driven convection and/or diffusion. Solute interactions with constituents of the arterial wall may also be important. A number of mathematical models incorporating these features were developed to aid in the interpretation of the experimental results. The formulation of these models and the resulting solute distribution profiles they predict are discussed below.

A. General Formulation

The transfer of labeled solute from the blood into and across the arterial wall may be influenced by not only the transport properties of the arterial tissue itself, but also by local fluid dynamic events near the blood-wall interface. The development here, however, will be restricted to an analysis of just the intramural aspect of this transport process.

1. Governing Partial Differential Equation

Consider the aorta as the wall of a hollow cylinder with internal radius, a, and external radius, b. All labeled solute which penetrates the arterial wall is assumed to originate either from the lumen of the vessel or from the capillary bed in the vessel adventitia. The rate of solute entry may be governed by the transport properties of the endothelium in the arterial intima and in the peripheral capillary bed. Within the arterial wall itself, solute movement is assumed to be
the result of diffusion and pressure-driven convection. Solute may or may not interact with chemical constituents within the wall. Since the thickness of the arterial wall is 10-15% of the vessel internal radius, the thin wall approximation applies and Cartesian rather than cylindrical coordinates can be used without loss in accuracy. Thus, the governing partial differential equation which applies to this situation can be developed by performing a solute mass balance over an element of tissue with infinitesimal thickness, Δx. The terms which enter into this mass balance are shown schematically on Fig. 53. Mathematically, the mass balance yields:

\[
\frac{J_f}{\varepsilon} A_p \left( C/c - \frac{D}{\tau^*} A_p \frac{\partial (C/c)}{\partial x} \right)_x - \frac{J_f}{\varepsilon} A_p \left( C/c - \frac{D}{\tau^*} A_p \frac{\partial (C/c)}{\partial x} \right)_{x+\Delta x} + A_p \frac{\partial R}{\partial t} = \frac{A_p}{\Delta x} \frac{\partial (C/c)}{\partial t} \tag{6.1}
\]

where \( J_f \) is the hydraulic flux (superficial fluid velocity) in mℓ/cm²·sec; D, the solute aqueous diffusion coefficient in cm²/sec, \( \tau^* \), the tortuosity factor (increase in effective diffusion path length); \( A_p \), the effective void fraction, or "pore", area; and R is the rate per unit pore volume at which solute disappears due to reaction. \( C \) is the solute concentration per mℓ of tissue and \( C/c \) the concentration per mℓ of "pore". Since \( A_p = \varepsilon A_m \), where \( \varepsilon \) is defined as the void fraction of tissue available for solute accumulation, the limit of (6.1) as \( \Delta x \to 0 \) becomes:

\[
\left( \frac{D}{\tau^*} \right) \frac{\partial^2 C}{\partial x^2} - \left( \frac{J_f}{\varepsilon} \right) \frac{\partial C}{\partial x} - cR = \frac{\partial C}{\partial t} \tag{6.2}
\]

This then is the governing differential equation within the wall. Mathematically, a unique solution, \( C(x,t) \), exists provided \( J_f \), \( \left( \frac{D}{\tau^*} \right) \), and R are known for all \( 0 < x < L \) and conditions at the boundaries (\( x=0 \) and \( x=L \)) as well as an initial condition are provided. These boundary specifications
Figure 53  Material Balance on Infinitesimal Element of the Aortic Wall
depend, of course, on the mathematical interpretation of the actual physical process involved.

The initial condition is easiest to define in the experimental work performed in this study. The initial labeled solute concentration was zero at every position, \( x \), within the wall. Mathematically,

\[
C(x, 0) = 0 \quad (6.3)
\]

The conditions which apply at \( x=0 \) and \( x=L \) are not so nearly evident.

2. **Endothelial-Side Boundary Condition**

Consider first the movement of solute into the wall at the endothelial surface \( (x=0) \). For large solutes, such as albumin and β-lipoprotein, the major route of entry is thought to be through endothelial vesicles. Solute flux can then be expressed in terms of the vesicular flux across the endothelium.

\[
\dot{N} = S_v \cdot V_v \left( C_v \bigg|_{x=0} - C_v \bigg|_{x=0^+} \right) \quad (6.4)
\]

where \( S_v \) is the flux of vesicles successfully traversing the endothelial cell in either direction (vesicles/cm²·sec); \( V_v \) is the average volume of each vesicle (cm³); and \( C_v \) denotes the average solute concentration within the vesicles. The subscripts \( x=0 \) and \( x=0^+ \) represent positions on the luminal and abluminal sides of the endothelial cell, respectively. Implicit in this equation is the assumption that vesicular transport accounts for all transendothelial solute movement. If solute transport occurred through the intercellular junctions of the endothelium the foregoing expression (6.4) is incomplete. Both diffusive and convective transport terms describing events within the junctions are required. These contributions can be written in terms of a phenomenological
parameter, $R_1$, which is defined as the fraction of plasma solute rejected by the endothelium. That is, the convective and diffusive solute flux is given by

$$\dot{N}igg|_{x=0} = J_f C_p(t)(1-R_1) \quad (6.5)$$

where $0 < R_1 < 1$. In the case of LDL, which has an average Stokes radius of $\sim 96\text{Å}$, $R_1$ should be very close to unity. This assumption is based on the observation that the critical dimension of the endothelial junctions is thought to be on the order of $80\text{Å}$ \cite{51, 52}. On the other hand, smaller solutes, such as albumin (Stokes radius $\sim 36\text{Å}$), may not be completely rejected by the endothelial junctions. Hence, for albumin, $R_1 < 1$. Thus, with the addition of the vesicular contribution, the total flux is given by:

$$\dot{N}igg|_{x=0} = S_V V V \left[ \left. C_v \right|_{x=0} - \left. C_v \right|_{x=0^+} \right] + J_f C_p(t)(1-R_1) \quad (6.6)$$

Restricting the analysis here to solutes the size of LDL or larger, the second term can be neglected. In other words, LDL penetration into the normal arterial wall will be assumed to result from vesicular transport only.

$$\dot{N}igg|_{x=0} = S_V V V \left[ \left. C_v \right|_{x=0} - \left. C_v \right|_{x=0^+} \right] \quad (6.7)$$

but the rejection term will be included in the subsequent analysis to maintain generality.

The evaluation of $\left. C_v \right|_{x=0}$ depends on phenomena which may occur on the endothelial surface. For example, if the vesicle simply imbibes fluid which has the same solute concentration as in the bulk plasma, $\left. C_v \right|_{x=0} = C_p(t)$,
where $C_p(t)$ is the bulk plasma concentration. However, solute partitioning may arise due to steric effects which result when the solute molecular radius approaches the radius of the vesicle opening (called the vesicle stalk). If solute is preferentially bound to, or excluded from the endothelial surface by reaction or selective adsorption, the surface concentration would be different from the bulk plasma $C_p(t)$. Thus, in general,

$$C_v|_{x=0} = k_p C_p(t) \quad (6.8).$$

For $x=0^+$, similar partitioning may be operable.

$$k_m C_v|_{x=0} = C(0^+, t)/\varepsilon \quad (6.9)$$

where $k_p$ and $k_m$ are partition coefficients. Equation (6.8) assumes that rapid equilibration occurs between the contents of the vesicles communicating with the plasma and the plasma itself. Equation (6.9) likewise assumes that the abluminal vesicle solute concentration instantaneously reflects the solute concentration in the subendothelial space. Thus, equation (6.7) can be viewed as the difference in the rates at which solute is carried to and from the subendothelial space. Depending on the relative magnitudes of $C_v|_{x=0}$ and $C_v|_{x=0^+}$, the net solute flux across the endothelium may be positive or negative.

If there is no net solute accumulation in endothelium (i.e., $0 < x < 0^+$), the solute flow at both surfaces should be identical.

$$A_m N_{x=0} = A_m N_{x=0^+} \quad (6.10)$$

But the solute flow at $x=0^+$ can be given in terms of $C(x, t)$:

$$A_m N_{x=0^+} = \left[ J_f \right] A_p (C/\varepsilon) - \left( \frac{D}{\tau A} \right) A_p \frac{\partial (C/\varepsilon)}{\partial x} \quad (6.11)$$
That is to say, solute in the subendothelial space is carried to and from the abluminal side of the endothelial cell both by convection and diffusion just as is the case across the rest of the arterial wall.

Combination of (6.7) through (6.11) yields:

\[
A_m \left\{ \frac{J_f(1-R_l)}{k_m} C_p(t) + S_V \left( \frac{k_p C_p(t) - C(0^+,t)}{k_m} \right) \right\} = \varepsilon A_m \left\{ \frac{J_f}{\varepsilon} \left( \frac{C(0^+,t)}{\varepsilon} \right) \right\} \\
- \left( \frac{D}{\tau^*} \right) \frac{\partial [C(0^+,t)]}{\partial x}
\]

(6.12)

For \( k_p = k_m = 1 \) and \( S_V V \equiv K_1 \) and neglecting the thickness of the endothelium, this becomes:

\[
J_f(1-R_l) C_p(t) + \frac{K_1}{\varepsilon} (\varepsilon C_p(t) - C(0,t)) = \left( \frac{J_f}{\varepsilon} \right) C(0,t) - \left( \frac{D}{\tau^*} \right) \frac{\partial C(0,t)}{\partial x}
\]

(6.13)

Thus, if \( K_1, \varepsilon, \) and \( C_p(t) \) are known, the concentration of solute at \( x=0 \) is completely specified. This then serves as one of the two boundary conditions required to obtain a solution to (6.2). It should be emphasized, however, that partitioning effects have been neglected. Clearly, this formulation must be suitably modified for cases where surface reaction/adsorption and vesicle exclusion effects are important.

3. Media-Adventitia Interface: Boundary Condition

The other specification (at \( x=L \)) is a priori difficult to define. No experimental evidence is available to indicate what physical mechanisms affect the transfer of solute across the media-adventitia interface. A number of possibilities are theoretically conceivable.

In deriving the boundary condition at \( x=L \), we are assuming that an abrupt change in the transport properties of the rabbit aortic wall occurs at the media-adventitia interface. Since the media has concentric layers of elastin while the adventitia does not, and since capillaries are
found only in the adventitia and not in the media, this is probably not a bad assumption in the case of a rabbit aorta. However, in other species, such as man, where capillaries and lymphatic vessels do penetrate the aortic media, the analysis used here would be inappropriate.

Restricting the discussion to the rabbit aorta, first consider all physical events which might be occurring at the media-adventitia interface. These can be discussed with reference on Fig. 54 which depicts the pertinent anatomy in the vicinity of the media-adventitia interface. First, the adventitia is penetrated by a capillary network, and presumably, lymphatic vessels. In the rabbit, none of these capillaries are thought to actually enter the outer media of the vessel wall. The extent to which lymphatic vessels are present in the media of rabbit aorta, if at all, remains to be shown. Assume, for this analysis, that lymphatic vessels, like capillaries, are present only in the adventitia and that some reside in close proximity, to the media at a distance, $L_a$ (see Fig. 55).

To develop the boundary condition at $x=L$, consider the extravascular tissue space in the adventitia. The extravascular tissue space is defined as the total tissue space excluding the capillary and lymphatic vessels. The solute concentration, $C_a$, within this space will dictate the concentration, $C(L,t)$, at the boundary. A relationship between $C_a$ and $C(L,t)$ can be developed by performing a lumped analysis of the extravascular space neglecting any gradients which may arise near lymphatic or capillary vessels. An alternative approach would be to treat the region defined by $L_a$ as a continuum and solve the convection-diffusion equation with the appropriate boundary conditions. The lumped approach is taken here out of convenience. Writing a solute material
Figure 54  Schematic Diagram of Capillaries and Lymphatic Terminals at Media-Adventitia Interface
Figure 55  Material Balance Over Extravascular Adventitial Space

\[
\frac{\partial}{\partial t} A C / \varepsilon \bigg|_{x=L} - \frac{D}{\tau^*} A \frac{\partial (C / \varepsilon)}{\partial x} \bigg|_{x=L} \rightarrow
\]

\[
K_c A_c \Delta C_c \rightarrow J_c A_c p (1-R_c) \rightarrow J_v A_v C_a (1-R_v) \rightarrow
\]

\[
K_A A_L \Delta C_L + J_c C (1-R_c) A_L \rightarrow
\]

Mathematical Boundary Describing Extravascular Adventitial Space Adjacent to Media
balance about the extravascular space as depicted on Fig. 55 yields:

\[ N_m + N_c - N_L = \frac{\partial C}{\partial t} \]  

(6.14)

where the terms on the left represent the media, capillary, and lymphatic contributions, respectively. The accumulation term is included to emphasize the possibility that the extravascular space may have a significant capacitance.

The solute transfer rate at the media-adventitia interface, \( N_m \), is given by:

\[ N_m = A_p \left[ \left( \frac{J}{c_v} \right) (C/c_v) - \frac{D}{\tau^*} \frac{\partial (C/c_v)}{\partial x} \right]_{x=L} \]  

(6.15)

That is, solute is assumed to be transported at the interface both by convection and diffusion.

The passage of solute from capillaries is assumed due to vesicular transport only. Physiologically speaking, this assumption is probably good only for high molecular weight solutes (>100,000) such as LDL. Low molecular weight solutes diffuse and/or are convected through the endothelial junctions as well. Thus, as shown on Fig. 55, an additional term is required in the flux equation, viz., \( J_c \ C_p(t) \ [1 - R_c] \). As with transport across the luminal endothelium of aorta, a phenomenological coefficient is used to describe diffusion and convection in capillary endothelial "pores." At the venous end of the capillary, fluid is convected into the veinule. However, no proteins are thought to be removed by this process. Strictly speaking, protein transport back into the veinule is given as \( J_v \ C_v(t) \ [1 - R_v] \) where \( R_v = 1 \). Thus, for LDL the only
mechanism for transport across capillary endothelium is assumed to be via the vesicles. Thus,

\[ N_c = A_c K_c \left( \frac{C}{p} - \frac{C_a}{\varepsilon_a} \right) \]  \hspace{1cm} (6.16)

where \( \varepsilon_a \) is the void fraction in the extravascular adventitial space.

The constant, \( K_c \) is the rate constant describing the flux of intracytoplasmic vesicles across the endothelium in either direction.

The lymphatic clearance rate is also assumed to be proportional to a concentration driving force. In addition, the lymphatic vessels are assumed to clear bulk fluid and the associated solutes from the interstitial space by a convective process. Therefore,

\[ N_L = K_L A_L \left( \frac{C_a}{\varepsilon_a} - C_L \right) + J_L A_L \frac{C_a}{\varepsilon_a} (1 - R_2) \]  \hspace{1cm} (6.17)

where \( R_2 \) is the phenomenological solute rejection coefficient at the lymphatic vessel surface; \( J_L \), the fluid velocity into the lymphatic vessels; \( K_L \), the vessel mass transfer coefficient; and \( C_L \) the solute concentration within the lymph.

Thus, the solute material balance over extravascular space is

\[ \frac{J_{L,T}}{\varepsilon} A_m C(L,t) - \frac{D_{ST}}{\tau_x} A_m \frac{\partial C(L,t)}{\partial x} + K A_c \left( \frac{C}{p} - \frac{C_a}{\varepsilon_a} \right) + K_L A_L \left( \frac{C_a}{\varepsilon_a} - C_L \right) - J_L A_L \frac{C_a}{\varepsilon_a} \]

\[ (1 - R_2) = A L \frac{\partial C_a}{\partial t} \]  \hspace{1cm} (6.18)
For large $K_L$ and $K_c$, the response time of $C_a$ to a step change in $C_p$ is short. Thus,

$$\frac{\partial C_a}{\partial t} = 0$$

Physically, this means the boundary region is a thin fluid layer with negligible capacity which is instantaneously mixed. Equation (6.18), with the left hand side equated to zero, therefore provides a relationship between $C(L,t)$ and $C_a$. However, we desire a relationship between $C(L,t)$ and the plasma concentration, $C_p$, which is known. One may argue that $C(L,t)$ is in equilibrium with $C_a$ provided $J_f$ is not large. That is to say, if convective effects do not dominate diffusive effects at $x=L$, $C(L,t)$ can be given by:

$$\frac{C(L,t)}{c} = \frac{C_a}{c_a}$$  \hspace{1cm} (6.19)

Substituting this relationship into (6.18) and assuming $\frac{c}{c_a} = 1$, $C_L = 0$, and $A_m J_f = A_L J_L$, we have,

$$\frac{J_f}{c} A_m C(L,t) - \left( \frac{D}{\tau^2} \right) A_m \frac{\partial^2 C(L,t)}{\partial x^2} = K_L A_L \frac{C(L,t)}{c} + J_f \frac{c}{c_a} A_m C(L,t)(1-R_2)$$

$$- K A_c \left( C_p(t) - \frac{C(L,t)}{c} \right) = 0$$  \hspace{1cm} (6.20)

Note that $A_m J_f = A_L J_L$ only for $A_{V_c} J_{V_c} = A_{C_c} J_{C_c}$ since continuity considerations require that $A_m J_f + A_{C_c} J_{C_c} = A_{V_c} J_{V_c} + A_{L_c} J_{L_c}$. Since $A_{V_c} J_{V_c}$ and $A_{C_c} J_{C_c}$ are likely to be smaller than $J_f A_m$ and since there is no good way to evaluate $A_{L_c} J_{L_c}$, $A_{C_c} J_{C_c}$, and $A_{V_c} J_{V_c}$, the assumption would seem not unreasonable.

Equation (6.20) says that the flux of solute in the media at the boundary, $x=0$, is the net result of a solute diffusive flux from the
capillaries and the lymphatic solute removal flux which is both diffusive and convective.

Intuitively, this relationship seems appropriate. However, a number of assumptions have been made which deserve review. Firstly, all solute is assumed to be removed by the lymph either by diffusive or convective processes. No allowance, other than vesicular transport in the reverse direction, has been made for the recovery of solute from the adventitial extravascular space by capillaries and/or veinules. It is known that in muscle capillaries, fluid is released into and subsequently reabsorbed from the interstitial space depending on the net local pressure driving forces across capillary endothelium. In the case of LDL-sized solutes, the capillaries probably clear very little solute, if any, by convective processes (238). Thus, ignoring convective removal of solute by the capillaries is not unwarranted. Also implicit in the formulation of (6.20) is the assumption that the lymphatic vessels and capillaries are sufficiently near the media-adventitia interface to insure that no significant gradients in \( C_a \) exist. Realistically, one might expect the concentration to vary with position, being highest near a capillary and being lower near a non-rejecting lymphatic vessel. However, the lack of knowledge on the detailed morphology of the vessels in the adventitia precludes a more geometrically rigorous approach. Finally, the solute lymph concentration was taken to be zero. In reality, this is not true; \( C_L \) is likely to be finite. With no good way to estimate \( C_L \), it is assumed to be zero, realizing that this may introduce some error in the analysis. Thus, equation (6.20) provides but a rough description of the solute flux at \( x=L \). Clearly, since most of the constants involved
in (6.20) have not been experimentally determined, only order of magnitude estimates can be made. Possible values for these constants will be discussed later in this section.

In order to test the importance of the boundary condition at \( x=L \), a number of other possible forms were studied. Besides (6.20) it is also conceivable that the boundary specification, written simply in terms of an equilibrium condition, may be equally valid.

\[
C_{(L,t)} = \kappa \frac{C_a}{C_p} = \kappa f(C_p) \quad (6.21)
\]

since \( C_a \) is some function of the plasma concentration, \( C_p \). This equilibrium form is a special limiting case of (6.20). This will be shown later where the results of the mathematical analysis are presented.

The experimental LDL results suggest that the slope of concentration gradient is near zero at \( x=L \). Thus,

\[
\frac{\partial C(L,t)}{\partial x} = 0 \quad (6.22)
\]

is another condition which was investigated.

All three possibilities (6.20 - 6.22) will be used in the models developed below. Comparison of the predicted profiles with actual data will enable the selection of the best boundary condition for \( x=L \).
The problem statement is summarized as:

\[
\frac{\partial}{\partial x} \left[ D_{\text{eff}}(x) \frac{\partial C(x, t)}{\partial x} - \frac{J_f}{\epsilon} C(x, t) \right] - \epsilon R(C, x, t) = \frac{\partial C(x, t)}{\partial t} \quad (6.23)
\]

Furthermore, if the arterial wall is isotropic, \(D_{\text{eff}}(x)\) need not be a function of position \((x)\), nor of \(C\), if only low solute concentrations are allowed. Also, neglecting the reaction term \(R\), one finds:

\[
D_{\text{eff}} \frac{\partial^2 C(x, t)}{\partial x^2} - \frac{J_f}{\epsilon} \frac{\partial C(x, t)}{\partial x} = \frac{\partial C(x, t)}{\partial t} \quad (6.24)
\]

subject to:

\[
C(x, 0) = 0 \quad (6.24a)
\]

for \(x = 0\):

\[
J_f C_p(t) \left[ 1 - R_1 \right] + \frac{K_1}{\epsilon} \left[ \epsilon C_p(t) - C(0, t) \right] = \frac{J_f}{\epsilon} C(0, t) - D_{\text{eff}} \frac{\partial C(0, t)}{\partial x} \quad (6.24b)
\]

for \(x = L\):

\[
J_f C(L, t) \left[ 1 - R_2 \right] + \frac{K A_L}{\epsilon A_m} C(L, t) - \frac{K A c}{A_m} \left[ C_p(t) - \frac{C(L, t)}{\epsilon} \right] = \frac{J_f}{\epsilon} C(L, t) - D_{\text{eff}} \frac{\partial C(L, t)}{\partial x} \quad (6.24c)
\]

or

\[
C(L, t) = \kappa C_p(t) \quad (6.24d)
\]

or

\[
\frac{\partial C(L, t)}{\partial x} = 0 \quad (6.24e)
\]

This formulation represents the simplest case, i.e., convection and diffusion across a finite slab of thickness, \(L\), with no internal reaction and
uniform diffusivity throughout. The solution to this case will be discussed first. The solution to (6.23) will be covered subsequently.

B. Convection and Diffusion-No Reaction

1. Solution

The solution of (6.24) can be obtained using Laplace transforms and Duhamel's superposition integral. The details are provided in APPENDIX E. Before attempting the solution, the equation is put in dimensionless form by defining the following symbols:

\[ \eta = \frac{x}{L} \]
\[ P = \frac{J \sqrt{D}}{L} \]
\[ T_1 = \frac{\sqrt{K}}{L} \]
\[ T_2 = \frac{K A L}{A c m} \]
\[ \tau = \frac{D}{L} t \]
\[ \psi = \frac{C(x,t)}{C_p(0)} \]
\[ T_L = \frac{K A L}{A D} \]

Thus, equation (6.24) becomes:

\[ \frac{\partial^2 \psi(\eta,\tau)}{\partial \eta^2} - P \frac{\partial \psi(\eta,\tau)}{\partial \eta} = \frac{\partial \psi(\eta,\tau)}{\partial \tau} \quad (6.25) \]

subject to:

\[ \psi(\eta,0) = 0 \quad (6.26a) \]

for \( \eta = 0 \):

\[ \epsilon p(1 - R_1) \frac{C_p(t)}{C_p(0)} + T_1 \left[ \frac{\epsilon C_p(t)}{C_p(0)} - \psi(0,\tau) \right] = \psi(0,\tau) - \frac{\partial \psi(0,\tau)}{\partial \eta} \quad (6.25b) \]
for $n = 1$:

\[(T_L + T_2) \psi (1, \tau) + \psi (1, \tau) (1 - R_2) - T_2 \frac{C(t)}{C_p(0)} = \psi (1, \tau) - \frac{\partial \psi}{\partial \eta} (1, \tau)\]

\[(6.25c)\]

or

\[\psi (1, \tau) = \kappa \frac{C(t)}{C_p(0)}\]

\[(6.25d)\]

or

\[\frac{\partial \psi (1, \tau)}{\partial \eta} = 0\]

\[(6.25e)\]

The plasma isotope concentration, $C_p(t)$, is represented by a double exponential decay function:

\[\frac{C_p(t)}{C_p(0)} = \sum_{k=1}^{\infty} \alpha_k e^{-\beta_k \tau}\]

\[(6.26)\]

where $\alpha_1 + \alpha_2 = 1$. The constants used in (6.26) are determined from experimental plasma decay data.

The dimensionless parameters each have special significance. This will be discussed in more detail later. For now, note that $\tau$ is the dimensionless time, otherwise known as the Fourier number; $T_1$, $T_2$, and $T_L$ are all mass transfer Biot numbers -- they represent the transport rates at the boundaries relative to diffusion within the media; and $\mathbf{p}$ is the Peclet number describing the relative rates of convection and diffusion.

Note that the three possible boundary conditions at $x=L$ are special cases of the following general form:

\[K_2 \psi (1, \eta) - K_1 \frac{\partial \psi}{\partial \eta} (1, \tau) = K_3\]

\[(6.27)\]
Such that for (6.25c):

\[ K_1' = 1. \]  \hspace{1cm} (6.28a)
\[ K_2' = \rho_2 \cdot (T_2 + T_L) \]  \hspace{1cm} (6.28b)
\[ K_3' = -T_2 \cdot \frac{C_p(t)}{C_p(0)} \]  \hspace{1cm} (6.28c)

and for (6.25d):

\[ K_1' = 0 \]  \hspace{1cm} (6.29a)
\[ K_2' = 1 \]  \hspace{1cm} (6.29b)
\[ K_3' = \kappa \cdot \frac{C_p(t)}{C_p(0)} \]  \hspace{1cm} (6.29c)

and for (6.25e):

\[ K_1' = 1 \]  \hspace{1cm} (6.30a)
\[ K_2' = 0 \]  \hspace{1cm} (6.30b)
\[ K_3' = 0 \]  \hspace{1cm} (6.30c)

Posing the problem with (6.27) as the \( n = 1 \) boundary condition thus affords a general solution which can be made to apply to each of the three particular cases of interest by simply substituting one of the appropriate sets of equations (6.28) - (6.30). The general solution is given by:

\[ \psi(n, \tau) = A \left( B + \sum_{m=1}^{\infty} Q_m e^{-F_m \tau} \right) + AB \left( \sum_{k=1}^{2} \alpha_k e^{-\beta_k \tau} - 1 \right) + \]
\[2A \sum_{m=1}^{\infty} \sum_{k=1}^{2} \alpha_k \beta_k t Q_m \left[ \begin{array}{c} -e^{-\beta_k t} - e^{-F_m t} \\ \beta_k t - F_m t \end{array} \right] \]

(6.31)

Where:

\[A = \exp \left[ \frac{P}{2} (\eta - 1) \right] \]  
(6.31a)

\[F_m = \lambda_m^2 + P^2/4 \]  
(6.31b)

\[B = \left[ \begin{array}{c} [T_1 e^{-tP(1 - R_1)}] \left[ (K_2 - \frac{PK_1}{2}) \sinh (P/2) - \frac{P}{2} K_1' \cosh (P/2) \right] e^{P/2} + \\
\frac{PK_3'}{2} \cosh (P/2) + \left\{ \frac{P}{2} + T_1 \right\} K_3' - [T_1 e^{-tP(1 - R_1)}] \exp (P/2) \\
\left\{ \frac{PK_3'}{2} \cosh (P/2) - \frac{PK_1'}{2} \sinh (P/2) \right\} \sinh (\eta P/2) \\
\left\{ \frac{PK_3'}{2} \right\} \sinh (P/2) + \left\{ \frac{P}{2} (K_2' - T_1 K_1' - PK_1') \right\} \cosh (P/2) \]  
(6.31c)
\[
Q_m = \lambda_m \left\{ \left[ T_1 \, \varepsilon \, \pi \, P \, (1 - R_1) \right] \exp \left( \frac{P}{2} \right) \left[ \left( K_2' \, - \, \frac{PK_1'}{2} \right) \sin \lambda_m - \lambda_m \frac{K_1'}{2} \cos \lambda_m \right] + \right. \\
\left. \lambda_m K_3' \right\} \cosh(\lambda_m \eta) + \left\{ \left( \frac{P}{2} + T_1 \right) K_3' - \left[ T_1 \, \varepsilon \, \pi \, P \, (1 - R_1) \right] \exp \left( \frac{P}{2} \right) \right. \\
\left. \left( K_2' \, - \, \frac{PK_1'}{2} \right) \cos \lambda_m + \lambda_m K_1' \sin \lambda_m \right\} \sin (\lambda_m \eta)
\]

\[
\left( \lambda_m^2 + \frac{P}{2} \right) \left\{ \left( K_2' \, - \, \frac{PK_1'}{2} \right) \left( \frac{P}{2} + T_1 \right) + \lambda_m^2 K_1' + (K_2' - K_1') \left( P + T_1 \right) \right\} \\
\cos \lambda_m + \left\{ 2 \lambda_m K_1' - \lambda_m \left( K_2' - K_1' \right) \left( T_1 - K_1' \right) P \right\} \sin \lambda_m \right\}
\]

\( (6.31d) \)

and \( \lambda_m \) are to zeroes of:

\[
\tan \lambda_m = \frac{-\lambda_m \left[ K_2' - K_1' \left( P + T_1 \right) \right]}{\left( K_2' - \frac{PK_1'}{2} \right) \left( \frac{P}{2} + T_1 \right) + K_1' \lambda_m^2}
\]

\( (6.31e) \)

Note the general properties of the solution (6.31). First of all, all three terms vanish as \( t \to \infty \) and \( \tau \to \infty \). This is consistent with the constraint that the plasma isotope concentration vanish at long times. In other words, the model predicts that all of the labeled solute in the wall will eventually be eluted. Secondly, the solution agrees with the initial condition of zero concentration at all \( \eta \). Thirdly, as is shown in APPENDIX E, the first of the three terms in (6.31) represents the solution for the case of constant plasma isotope concentration. That is, the second and third terms take into account the effect of the decaying plasma concentration.
As would be expected from the problem formulation $\psi(\eta, \tau)$ is given as a function of characteristic dimensionless groups, $p$, $T_1$, $T_2$, $\tau$ and $\eta$. The terms which describe the plasma decay function, i.e., $a_k$ and $b_k$, also enter into the equation. Neglecting these for the moment, we wish to discuss here the physical meaning of the other dimensionless groups.

$P(=J_f L/D_{eff})$ is the wall Peclet number. It can be considered as the ratio of convective solute transport to diffusive solute transport in the wall. A value of $P \ll 1$ would correspond to a situation where diffusive transport is dominant. Conversely, for $P \gg 1$, the primary mode of solute movement would be by convection. A typical value of $P$ for the problem at hand can be estimated using the permeability data presented previously (see IV. INTRODUCTION) and order of magnitude estimates for $D_{eff}$. For LDL, the aqueous diffusion coefficient has been reported as $2.25 \times 10^{-7} \text{ cm}^2/\text{sec}$ (235). Assuming that diffusion in the arterial wall is retarded 5-10 fold due to tortuosity effects, one would expect $D_{eff}$ to fall somewhere in the range $2.25 \times 10^{-8} \text{ cm}^2/\text{sec}$ to $4.5 \times 10^{-8} \text{ cm}^2/\text{sec}$.

Using $J_f = 0.01 \text{ ml/cm}^2\cdot\text{hr}$ and $L=0.024 \text{ cm}$ (the thickness of the rabbit aorta) we have:

$$P = \frac{J_f L}{D_{eff}} = \frac{(0.01) (\frac{1}{3600}) (0.024)}{(3.75 \times 10^{-8})(0.42)} = 4.23$$

Thus, depending on the precise value of the effective LDL diffusion coefficient, one would anticipate a priori that solute movement within the wall is affected about equally by convection and diffusion. This also suggests the magnitude of $P$ which will be of interest in the theoretical model.
Another important dimensionless group in the solution, $\psi(\eta, \tau)$, is $T_1$. Recall that $T_1$, the Biot number, is defined as $K_1 L / D_{eff}$ where $K_1$ is a parameter proportional to $S_v$, the flux of vesicles successfully traversing the endothelium in either direction ($K_1 = S_v V_v$). Hence, in a sense the dimensionless term, $T_1$, represents the ratio of solute transport across the endothelium to solute transport by diffusion within the rest of the wall. For $T_1 \gg 1$, the endothelium does not present a major resistance to the movement of solute into and across the vessel wall. For $T_1 \ll 1$, one would anticipate that the surface endothelium would play a controlling role in the overall transport process.

As was the case for $p$, an order of magnitude estimate can be made for $T_1(=S_v V_v L / \epsilon D_{eff})$. The flux of vesicles across endothelium $S_v$, has been estimated to be $\sim 9$ vesicles/$\mu^2$-sec (237). The average vesicle volume can be deduced from the data of Bruns and Palade (50) where an average vesicle radius is given as 300$\mu$. Taking the inulin space of rabbit aorta to be representative of the space available for LDL movement, we have $\epsilon = 0.42$ (239). Using the same rough value for $D_{eff}$ as before, $T_1$ is estimated as:

$$T_1 = \frac{S_v V_v L}{\epsilon D_{eff}} \approx \frac{(9) \left(\frac{4}{3} (.03)^3\right) (10^{-4}) (.024)}{(0.42) (3.75 \times 10^{-8})} = 0.155$$

The fact that this estimated value is an order of magnitude less than unity suggests that the endothelium presents a major resistance in the overall LDL transport process. For solutes with larger diffusion coefficients, such as albumin, one would expect even smaller values for $T_1$ if transendothelial transport were exclusively by pinocytosis.
However, as was noted previously (see III. INTRODUCTION), albumin is thought to reach the subendothelial space both by vesicular transport and by diffusion and/or convection through the intercellular clefts. This latter pathway is thought to account for ~50% of the transendothelial albumin flux (53). Therefore, an alternate formulation of the boundary condition at \( \eta = 0 \) is necessary when albumin is considered.

The other dimensionless groups besides \( \eta \) and \( \tau \), which enter into the solution for \( \psi(\eta, \tau) \) are introduced in the \( \eta = 1 \) boundary condition. When this condition is written to include the capillary and lymphatic effects, \( T_L \) and \( T_2 \) appear in the final solution. \( T_2 \) is defined as \( \frac{K_C L A_C}{D_{\text{eff}} e A_m} \) and therefore, describes the ratio of transcapillary solute movement relative to intramural diffusive transport. Assuming that solute which penetrates the outer media originates from the adventitia capillary network, and assuming that the rate of entry from the adventitia into the media is controlled by the rate of transendothelial LDL transfer in this peripheral capillary bed, one would anticipate values for \( T_2 \) different in magnitude than for \( T_1 \) due to the difference in surface area available for transendothelial transfer in the capillary bed. How much different \( T_2 \) might be than \( T_1 \) cannot be estimated with any certainty.

However, using numbers quoted in the literature for the density of capillaries per \( \text{mm}^2 \) in muscle tissue (200 - 5000 capillaries/\( \text{mm}^2 \))(221), and assuming an effective capillary length of 100\( \mu \), one can reason that the capillaries in the adventitia have a surface area anywhere from 0.5 to 12.5 times the luminal surface area. Thus \( T_2 \) is probably of the same order of magnitude as \( T_1 \) or higher, i.e. \( \approx 0.1 \) to 1.0.
The other dimensionless parameter, $T_L$, introduced by the far side boundary condition can also be estimated to within an order of magnitude. $T_L$, defined as $K_{L}A_{L}/A_{D_{m}}\varepsilon$, is seen to involve the ratio of the diffusive lymphatic removal constant, $K_{L}$, to the effective solute diffusion coefficient in the media, $D_{eff}$. In a sense therefore, $T_L$ represents the relative lymphatic removal rate while $T_2$ depicts the relative capillary input rate. Since protein accumulation rates within the media are likely to be small compared with the influx and efflux rates, the relative removal rate, $T_L\psi(L,\tau)$, must be able to account not only for the input solute from the capillaries, $T_2[\psi(L,\tau) - \epsilon C_p(t)/C_p(0)]$, but also the solute which penetrates from the blood vessel lumen, $p\psi(L,\tau)$. Of course, this holds true only if $R_2 = 1$. Nevertheless, one would expect $T_L$ to be of greater magnitude than $T_2$. This is especially evident when one considers the disparity in the magnitudes of the pertinent driving forces, $\psi(L,\tau)$ for $T_L$ and $[\psi(L,\tau) - \epsilon C_p(t)/C_p(0)]$ for $T_2$. Since the latter driving force is much larger, in order to compensate, $T_L$ must be much greater than $T_2$. Hence, $T_L >> 0.1$ is the appropriate magnitude for $T_L$ in the theoretical analysis.

If the equilibrium condition is used for the $\eta = 1$ specification (6.25d), the equilibrium constant, $\kappa$, is injected into the solution $\psi(\eta,\tau)$. Inspection of the boundary condition (6.25c), reveals that the equilibrium condition is a special case of (6.25c)

$$
(R_2 p - T_L - T_2) \psi(1,\tau) - \frac{\partial \psi(1,\tau)}{\partial \eta} = - T_2 \epsilon \frac{C_p(t)}{C_p(0)}
$$

(6.25c)
For \( R_2 = 0, T_2 \approx 0.1, \ \frac{\partial \Psi}{\partial \eta} = 0, \) and \( T_L \gg 0.1, \) \( (6.25c) \)

reduces to

\[
\Psi(1, \tau) = \frac{T_2}{T_L \varepsilon} \frac{C_p(t)}{C_p(0)}
\]

(6.32)

Thus, the apparent equilibrium constant is approximately equal to \( T_2 \varepsilon/T_L. \)

For \( T_2 = 0.1, T_c = 10, \) and \( \varepsilon = 0.42, \) this expression is evaluated as

\( -0.0042. \)

Of course, if \( T_2 = T_L, \) then the apparent equilibrium constant will be closer to 0.4. From the previous discussion on the relative magnitude of \( T_2 \) and \( T_L, \) one can conclude that the apparent equilibrium constant is probably best taken closer to 0.0042 than to 0.42 since physical arguments suggest \( T_L \) must be considerably greater than \( T_2. \)

This provides the order of magnitude for \( K \) useful in evaluating the theoretical modules.

What is also useful from this order of magnitude analysis is an appreciation for the effect the ratio \( T_2/T_L \) will play in the solution to the problem. For a prescribed ratio, \( T_2/T_L, \) equation (6.32) can be used to determine the approximate value \( \Psi(1, \tau) \) might attain at equilibrium.

This is evident when one observes that (6.32) was developed assuming \( \frac{\partial \Psi(1, \tau)}{\partial \eta} = 0. \) That is to say, for a given \( T_2/T_L, \) e.g., 0.02, the local concentration gradient at \( \eta = 1 \) will be zero only if \( \Psi(1, \tau) = (0.02)\varepsilon \)

\[
\frac{C_p(t)}{C_p(0)}.
\]

Taking \( \varepsilon = 0.42, \) \( \Psi(1, \tau) \) becomes 0.0084 \( \frac{C_p(t)}{C_p(0)}. \) In a sense, \( 0.0084 \frac{C_p(t)}{C_p(0)} \) is therefore, the inflection concentration. For \( \Psi(1, \tau) < \)

0.0084 \( \frac{C_p(t)}{C_p(0)}, \) \( \frac{\partial \Psi}{\partial \eta} (1, \tau) \) will tend to be greater than zero. Conversely,

for \( \Psi(1, \tau) > 0.0084 \frac{C_p(t)}{C_p(0)}, \) \( \frac{\partial \Psi(1, \tau)}{\partial \eta} \) must be less than zero for (6.25c) to
hold. This analysis applies only for $T_L > 1$, or in other words, for large lymphatic removal rates relative to the diffusion of solute in the media. This will become more evident as the solutions $\psi(\eta, \tau)$ are discussed.

2. Effect of $T_1$

Using the foregoing order of magnitude estimates for the pertinent parameters in the problem, one can define the range of these parameters over which to test the behavior of the model. Consider first the equilibrium boundary condition case (6.25d). The variation in predicted profile with time and as a function of $T_1$ is presented on Figs. 56-60. For this case $\kappa$ was arbitrarily chosen as 0.0040. The plasma decay function, $C_p(t)/C_p(0)$, was taken from the experimental LDL data. The curves presented also correspond to the same time intervals used experimentally. For a vessel wall 240μ thick, $D_{eff}$ can be calculated from the relationship $\tau = D_{eff}t/L^2$. For this example $D_{eff} = 3.33 \times 10^{-8}$ cm²/sec. Note at the earliest time shown (Fig. 56), the sensitivity of the distribution to the value of $T_1$. For $T_1 = 0.1$ or 0.05, the predicted curves closely resemble the actual LDL data for 0.167 hours. Thus, the order of magnitude estimate of $T_1 = 0.155$ was fairly realistic. At $T_1 = 0.5$, the gradient is much steeper indicating that diffusion in the media is a more limiting transport process than for $T_1 = 0.1$.

At 0.5 hours (Fig. 57), the model predicts higher dimensionless concentrations throughout. The values for $T_1 \geq 0.1$ are especially high in comparison with the experimental observations. Yet the curvature of the distributions remain concave upward for all $T_1$ shown. At 4 hours (Fig. 58), such is not the case. All distributions are concave downward. Also note that the total accumulation, which is given by the area under each
Figure 56: Effect of $T_1$ - Equilibrium Boundary Condition

$\tau = 0.035$

$t = 0.167$ hours

$\varepsilon = 0.42$

$P = 1.0$

$\kappa = 0.0040$

$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.41t}$

Relative Concentration, $\psi = C/C_p(0)$

Relative Position, $\eta = x/L$
Figure 57: Effect of $T_1$-Equilibrium Boundary Condition

Relative Concentration, $\Psi = \frac{C}{C_p(0)}$

$t = 0.018$
$t = 0.5$ hours
$\epsilon = 0.42$
$P = 1.0$
$\kappa = 0.0040$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$

$C_p(0) = 0.33e^{-(0.775\tau)} + 0.67e^{-(0.041\tau)}$

Relative Position, $\eta = \frac{x}{L}$
Figure 58: Effect of $T_1$-Equilibrium Boundary Condition

$\tau = 0.83$  \hspace{1em} $\kappa = 0.0040$
$\varepsilon = 0.42$  \hspace{1em} $P = 1.0$
$C_p(t) / C_p(0) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$

Relative Concentration, $\psi = C/C_p(0)$

Relative Position, $\eta = x/L$
Figure 59: Effect of $T_1$-Equilibrium Boundary Condition

- $\tau = 5.0$
- $t = 24$ hours
- $\varepsilon = 0.42$
- $P = 1.0$
- $\kappa = 0.0040$

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

Relative Concentration, $\psi = \frac{C}{C_p(0)}$

Relative Position, $\eta = \frac{x}{L}$
Figure 60: Effect of $T_1$-Equilibrium Boundary Condition

\[
\psi = \frac{C(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

\[
\tau = 13.94 \\
t = 67 \text{ hours} \\
\varepsilon = 0.42 \\
P = 1.0 \\
\kappa = 0.0040
\]
each of the distribution curves, is greater at four hours than at 0.5 hours. At 24 hours (Fig. 59), the net accumulation for each particular T is less than at four hours. Thus, just as with the experimental data, this model predicts that solute accumulation should go through a maximum between four and 24 hours. At 67 hours (Fig. 60), the levels are still lower, reflecting the continuing efflux of labeled solute from the vessel wall as the plasma isotope concentration tends toward zero.

While the 0.167 hour and 0.5 hour predictions are similar in magnitude and in shape with the experimental observations, results at the three longer times are not. The shapes of the four hour distributions are not surprising, however, in light of the influence of convection in the transport process. In the case just discussed, P was taken as unity. Thus, convective and diffusive processes were equally important. Had P been assumed to be far less than unity, the profiles at four hours and longer would represent the classic diffusive "steady state" (if a true steady state were possible in this problem). That is, the distributions would be nearly straight lines.

3. Effect of Peclet Number, P

The effect of P on the solute distribution curves is presented on Figs. 61 to 65. At short times such as 0.167 hours (Fig. 61), a 500 fold difference in P affects the solute concentrations very little. However, at 0.5 and 4 hours (Figs. 62 and 63), the effect of P is more significant. The larger the value of P the lower the solute concentration predicted by this model. This at first may seem strange in that one might intuitively associate higher convective flows, or large P, with higher solute concentrations. However, upon reflection one can appreciate the fact that higher P results in lower concentrations by realizing that
Figure 61: Effect of P-Equilibrium Boundary Condition

\[ C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

- \( t = 0.167 \text{ hours} \)
- \( \tau = 0.03476 \)
- \( \varepsilon = 0.42 \)
- \( T_1 = 0.05 \)
- \( \kappa = 0.0040 \)

Relative Concentration, \( \psi = C/C_p(0) \)

Relative Position, \( \eta = x/L \)
Figure 62: Effect of P-Equilibrium Boundary Condition

\[ \frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

- \( t = 0.5 \) hours
- \( \tau = 0.104 \)
- \( \epsilon = 0.42 \)
- \( T_l = 0.05 \)
- \( \kappa = 0.0040 \)

Relative Concentration, \( \psi = \frac{C}{C_p(0)} \)

Relative Position, \( \eta = \frac{x}{L} \)
Figure 63: Effect of P-Equilibrium Boundary Condition

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

\[t = 4.0 \text{ hours} \quad \tau = 0.833 \quad \varepsilon = 0.42 \quad T_1 = 0.05 \quad \kappa = 0.0040\]
Figure 64: Effect of P-Equilibrium Boundary Condition

\[ t = 24 \text{ hours} \]
\[ \tau = 5.0 \]
\[ \varepsilon = 0.42 \]
\[ T_1 = 0.05 \]
\[ \kappa = 0.0040 \]
\[ C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

Relative Concentration, \( \Psi = \frac{C}{C_p(0)} \)

Relative Position, \( \eta = \frac{x}{L} \)
Figure 65: Effect of P-Equilibrium Boundary Condition

$t = 67$ hours
$	au = 13.94$
$\varepsilon = 0.42$
$T_1 = 0.05$
$\kappa = 0.0040$

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]
the amount of solute crossing the endothelium is controlled by the vesicular transport rate of the endothelium. Higher convective flux simply increases the rate at which solute is carried from the subendothelial space across the interior of the wall. Since the endothelial throughput is independent of P, the elevated clearance of solute from the subendothelial space serves to depress the solute concentration throughout the tissue. Note that at 4 hours, the profile for P = 0.1 is virtually a straight line. This low value of P corresponds to the case where transport is diffusively controlled. The downward concavity at larger P is, therefore, the result of appreciable convection superimposed on diffusion. Profiles at 24 and 67 hours also reflect the effect of the convective flux, but to a lesser degree. The distributions are less concave and the concentration is lower than at four hours, indicating efflux of solute from the wall at long times. The experimental LDL profiles, on the other hand, showed similar distributions of labeled solute, both in shape and in concentration, at four and 24 hours. Hence, while the shape of the 0.167 and 0.5 hour model profiles is similar to the experimental data, the qualitative agreement at the longer times with this model is poor.

4. Effect of Effective Diffusivity, $D_{\text{eff}}$

Both of the foregoing comparisons with varying $T_{1}$ and P were calculated with $D_{\text{eff}}$ estimated to be $3.33 \times 10^{-8}$ cm$^{2}$/sec. Using a different estimate of $D_{\text{eff}}$ changes the value of $T$ at each of the time intervals of interest. Were $T$ at four hours to be <<1, one might anticipate that the profile shape would still be in the initial "transient," or nonequilibrated, phase. That is to say, a smaller dimensionless time, $T$,
at four hours should result in the desired concave curvature observed in the previous examples at 0.167 and 0.5 hours. The effect of $D_{eff}$ on the profiles at the five intervals of interest is presented on Figs. 66 to 70. As would be expected, the extent of the penetration varies with the dimensionless time. At four hours (Fig. 68), a $D_{eff}$ of $5.6 \times 10^{-9}$ cm$^2$/sec corresponds to a dimensionless time of 0.140. Note that the concave upward shape of the profile is preserved. However, at 24 hours, for the same $D_{eff}$, $\tau = 0.84$ and the profile takes on its pseudo steady state shape; that is, it is almost linear. For this model to predict profiles with concave upward shapes at 24 hours, $D_{eff} < 10^{-9}$ cm$^2$/sec must be specified in order to ensure that $\tau << 1$. Unfortunately, selection of such a small $D_{eff}$ would be inconsistent with the observed experimental solute penetration at 0.167 hours. This fact can be appreciated on Fig. 66 where for $D_{eff} = 5.6 \times 10^{-9}$ cm$^2$/sec ($\tau = 0.0058$) solute has failed to penetrate beyond the initial 20% of the wall. This calculation was for $T_1 = 0.05$. A larger value for $T_1$ would lengthen the penetration depth slightly but result in gradients much steeper than the observed experimental 0.167 hour LDL data. Therefore, the selection of a smaller $D_{eff}$ improves the agreement at long times but at the sacrifice of the fit at the early times.

5. Effect of Equilibrium Constant, $\kappa$

The only other possible variation of this model which might account for the experimental observations both at early and long times would be a change in the $\eta = 1$ boundary condition. However, simply altering the estimate of $\kappa$ will not produce the desired effect. If $\kappa$ were set equal to 0.0080, for example, the four and 24 hour profiles with inter-
Figure 66: Effect of $D_{eff}$—Equilibrium Boundary Condition

$t = 0.167$ hours $L = 0.024$ cm
$p = 1.0$
$T_1 = 0.05$
$\varepsilon = 0.42$
$\kappa = 0.0040$

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}.
\]

Relative Concentration, $\Psi = \frac{C}{C_p(0)}$

- $\tau = 0.0696$ $D_{eff} = 6.67 \times 10^{-3}$ cm$^2$/sec
- $\tau = 0.0348$ $D_{eff} = 3.33 \times 10^{-8}$ cm$^2$/sec
- $\tau = 0.0058$ $D_{eff} = 5.6 \times 10^{-9}$ cm$^2$/sec

Relative Position, $\eta = x/L$
Figure 67: Effect of $D_{\text{eff}}$ - Equilibrium Boundary Condition

Variable Values:
\[ t = 0.5 \text{ hours} \]
\[ P = 1.0 \]
\[ T_1 = 0.05 \]
\[ \varepsilon = 0.42 \]
\[ \kappa = 0.0040 \]

Relative Concentration, $\psi = \frac{C}{C_p(0)}$:

\[ \frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

Length, $L = 0.024 \text{ cm}$

Curves:
- $\tau = 0.208 \quad D_{\text{eff}} = 6.67 \times 10^{-3} \text{ cm}^2/\text{sec}$
- $\tau = 0.104 \quad D_{\text{eff}} = 3.33 \times 10^{-8} \text{ cm}^2/\text{sec}$
- $\tau = 0.018 \quad D_{\text{eff}} = 5.6 \times 10^{-9} \text{ cm}^2/\text{sec}$

Relative Position, $\eta = \frac{x}{L}$
Figure 68: Effect of $D_{\text{eff}}$ - Equilibrium Boundary Condition

$t = 4 \text{ hours}$

$P = 1.0$

$T_l = 0.05$

$\varepsilon = 0.42$

$k = 0.0040$

$$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$$

$L = 0.624 \text{ cm}$

$D_{\text{eff}} = 5.67 \times 10^{-8} \text{ cm}^2/\text{sec}$

$D_{\text{eff}} = 3.33 \times 10^{-5} \text{ cm}^2/\text{sec}$

$D_{\text{eff}} = 5.6 \times 10^{-9} \text{ cm}^2/\text{sec}$
Figure 69: Effect of $D_{\text{eff}}$—Equilibrium Boundary Condition

$t = 24$ hours
$P = 1.0$
$T_1 = 0.05$
$\varepsilon = 0.42$
$\kappa = 0.0040$

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

$L = 0.024$ cm

$\tau = 5.0, 10.0$  
$D_{\text{eff}} = 6.67 \times 10^{-8}$ cm$^2$/sec, $3.33 \times 10^{-8}$ cm$^2$/sec

$\tau = 0.84$  
$D_{\text{eff}} = 5.6 \times 10^{-9}$ cm$^2$/sec

Relative Position, $\eta = x/L$
Figure 70: Effect of $D_{eff}$ - Equilibrium Boundary Condition

\[ t = 67 \text{ hours} \]
\[ P = 1.0 \]
\[ T_l = 0.05 \]
\[ \varepsilon = 0.42 \]
\[ \kappa = 0.0040 \]
\[ C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]
\[ \frac{C_p(t)}{C_p(0)} \]
\[ L = 0.024 \text{ cm} \]
mediate $D_{eff}$ still would not preserve the upward concavity of the earlier times. Moreover, the solute concentrations near $\eta = 1$ would be too high at the early times. Therefore, one can conclude that the simple model of convection with diffusion across the arterial wall with equilibrium conditions applying at the media-adventitia interface is inconsistent with the observed experimental results.

Likewise, for all other boundary conditions used at $\eta = 1$, agreement with all the experimental results is not achievable. First, for the case of no diffusional transport at $\eta = 1$ ($\frac{\partial y}{\partial \eta} = 0$), the calculated profiles at four, 24, and 67 hours are at considerable variance in shape from the LDL experimental results. This is illustrated in Figs. 71 to 75 for variation in $T_1$ and Figs. 76 to 80 for variation in $P$. By specifying only the slope of the profile at the boundary, the absolute concentration for $\tau \geq 1$ (four hours and longer) is set by the magnitude of $P$ and $T_1$. Moreover, the pseudo steady state profiles observed are horizontal showing concavity at four hours (Fig. 78) only for very small $P$ and showing no concavity whatsoever at 24 hours (Fig. 79). Hence, formulation of the problem with this boundary condition at $\eta = 1$ is totally unrepresentative of the experimental observations.

6. **Effect of $\eta = 1$ Boundary Condition**

The other possible formulation of the far side boundary condition (6.25c) does not result in predictions which agree with experimental observations either. The solution behavior is described by Figs. 81 to 85. The calculations plotted are for $T_2 = 0.5$, $T_1 = 0.05$, $P = 1.0$, $R_2 = 0$, and $\varepsilon = 0.42$, with $T_L$ ranging from 5 to 500. The 0.167 and 0.5 hour data indicate the effect of increasing values of $T_L$. The larger the
Figure 71: Effect of $T_1$ - No Diffusion at $\eta = 1$

$$t = 0.167 \text{ hours}$$

$$\tau = 0.035$$

$$P = 1.0$$

$$\varepsilon = 0.42$$

$$\frac{\partial \psi}{\partial \eta} = 0$$

$$\eta = 1$$

$$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$$

Relative Concentration, $\psi = C/C_p(0)$

Relative Position, $\eta = x/L$
Figure 72: Effect of $T_1$ - No Diffusion at $\eta = 1$

- $t = 0.5$ hours
- $\tau = 0.104$
- $P = 1.0$
- $\varepsilon = 0.42$
- $\frac{\partial \Psi}{\partial \eta} = 0$
- $C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
- $C_p(0)$

Relative Concentration, $\Psi = \frac{c}{C_p(0)}$

Relative Position, $\eta = x/L$
Figure 73: Effect of $T_1$ - No Diffusion at $\eta = 1$

$t = 4$ hours
$
\tau = 0.83
$
$P = 1.0$
$\varepsilon = 0.42$

$\frac{\partial \Psi}{\partial \eta} = 0$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$

Relative Concentration, $\Psi = C/C_p(0)$

Relative Position, $\eta = x/L$
Figure 74: Effect of $T_1$ - No Diffusion at $\eta = 1$

$t = 24$ hours \[ \frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

$\tau = 5.0$, $P = 1.0$, $\varepsilon = 0.42$ \[ \frac{\partial \psi}{\partial \eta}_{\eta = 1} = 0 \]
Figure 75: Effect of $T_1$ - No Diffusion at $\eta = 1$

$t = 67$ hours
$\tau = 13.94$
$p = 1.0$
$\epsilon = 0.42$

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

$\frac{\partial \psi}{\partial \eta} |_{\eta=1} = 0$

Relative Concentration, $\psi = \frac{c}{C_p(0)}$
Figure 76: Effect of $P$ - No Diffusion at $\eta = 1$

$$t = 0.167 \text{ hours}$$
$$\tau = 0.0348$$
$$T_1 = 0.05$$
$$\epsilon = 0.42$$
$$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$$
$$\frac{\partial \psi}{\partial \eta} = 0$$

Relative Position, $\eta = x/L$

Relative Concentration, $\psi = C/C_p(0)$
Figure 77: Effect of $P$ - No Diffusion at $\eta = 1$

$t = 0.5$ hours \hspace{1cm} \frac{\partial \psi}{\partial \eta} = 0, \eta = 1$

$\tau = 0.104$

$T_l = 0.05$

$\epsilon = 0.42$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$

Relative Concentration, $\Psi = \frac{C}{C_p(0)}$

Relative Position, $\eta = x/L$
Figure 78: Effect of P - No Diffusion at \( \eta = 1 \)

- \( P = 0.01 \)
- \( P = 0.1 \)
- \( P = 1.0 \)
- \( P = 5.0 \)

The figure illustrates the relative concentration, \( \psi = \frac{C}{C_P(0)} \), as a function of the relative position, \( \eta = \frac{x}{L} \), with different values of \( P \). The graph shows the concentration distribution for non-diffusion conditions at \( \eta = 1 \).

The equation for the concentration distribution is:

\[
C_P(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

At \( t = 4 \) hours:

- \( \eta = 1 \)
- \( \frac{\partial \psi}{\partial \eta} = 0 \)

Additional parameters:
- \( \tau = 0.83 \)
- \( T_1 = 0.05 \)
- \( \varepsilon = 0.42 \)
Figure 79: Effect of \( P \) - No Diffusion at \( \eta = 1 \)

\[
\psi = \frac{C(t)}{C_p(0)}
\]

\[ t = 24 \text{ hours} \]
\[ \tau = 5.0 \]
\[ T_1 = 0.05 \]
\[ \varepsilon = 0.42 \]
\[ C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]
\[ \frac{\partial \psi}{\partial \eta} = 0 \]
\[ \frac{\partial \psi}{\partial \eta} \eta = 1 \]

Relative Position, \( \eta = x/L \)
Figure 80: Effect of $P$ - No Diffusion at $\eta = 1$

- $t = 67$ hours
- $\zeta = 0$
- $\tau = 13.94$
- $T_1 = 0.05$
- $\epsilon = 0.42$

$$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$$
Figure 8.1: Effect of $T_L$ - Complex Boundary Condition

$$t = 0.167 \text{ hours} \quad P = 1.0$$

$$\tau = 0.03476 \quad R_2 = 0$$

$$T_2 = 0.5$$

$$\varepsilon = 0.42$$

$$T_1 = 0.05$$

$$\left[ p \frac{\partial \psi}{\partial \eta} = T_2 \left( \Psi - \varepsilon \frac{C_p(t)}{C_p(0)} + T_L \frac{\partial \psi}{\partial \eta}(1-R_2) \right) \right]_{\eta=1}$$

$$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$$

$$\frac{C_p(\eta)}{C_p(0)} = 0.33e^{-0.775\eta} + 0.67e^{-0.041\eta}$$
Figure 82: Effect of $T_L$ - Complex Boundary Condition

$t = 0.5$ hours  \hspace{1cm}  T_1 = 0.05  \hspace{1cm}  R_2 = 0
\tau = 0.104  \hspace{1cm}  T_2 = 0.5  \hspace{1cm}  P = 1.0
\varepsilon = 0.42

\[
\left[ p\psi - \frac{3\psi}{\partial \eta} = T_2 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \psi + P\psi(1-R_2) \right]_{\eta=1}
\]

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]
Figure 83: Effect of $T_L$ - Complex Boundary Condition

\[ t = 4 \text{ hours} \quad P = 1.0 \]
\[ \tau = 0.83 \quad R_2 = 0 \]
\[ T_2 = 0.5 \]
\[ \varepsilon = 0.42 \]
\[ T_1 = 0.05 \]
\[ \left[ \psi^\prime - \frac{\partial \psi}{\partial \eta} = T_2 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \psi + P \psi (1 - R_2) \right]_{\eta = 1} \]
\[ \frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

\[ \psi = \frac{C}{C_p(0)} \]

$t = 4$ hours \quad $P = 1.0$

$\tau = 0.83$ \quad $R_2 = 0$

$T_2 = 0.5$

$\varepsilon = 0.42$

$T_1 = 0.05$

$\left[ \psi^\prime - \frac{\partial \psi}{\partial \eta} = T_2 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \psi + P \psi (1 - R_2) \right]_{\eta = 1}$

$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
Figure 84: Effect of \( T_L \) - Complex Boundary Condition

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

- \( t = 24 \) hours
- \( \tau = 5.0 \)
- \( T_2 = 0.5 \)
- \( \varepsilon = 0.42 \)

\[
\frac{\partial \Psi}{\partial \eta} - T_2 \left( \frac{\partial \varepsilon}{C_p(t)} + T_L \frac{\partial \Psi}{\partial \eta} + P\eta (1-R_2) \right)_{\eta=1}
\]

- \( T_1 = 0.05 \)
- \( R_2 = 0 \)
- \( P = 1.0 \)
Figure 85: Effect of $T_L$ - Complex Boundary Condition

$t = 67$ hours  \quad T_L = 0.05$

$\tau = 13.94$  \quad P = 1.0$

$T_2 = 0.5$  \quad R_2 = 0$

$\varepsilon = 0.42$

\[
\left[ P\Psi - \frac{\partial \Psi}{\partial n} = T_2 \left( \Psi - \varepsilon \frac{C_p(t)}{C_p(0)} + T_L \Psi + P\Psi(1-R_2) \right) \right] \quad n=1
\]

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]
lymphatic removal rate constant, the lower the concentration, \( \psi(1, \tau) \).

This is as predicted previously. For \( T_L = 50 \), \( T_2/T_L = 0.01 \). Using (6.32) one would expect \( \psi(1, \tau) = 0.0042 \). Inspection of Fig. 81 reveals \( \psi(1, \tau) = 0.0038 \). Similarly for \( T_L = 150 \), the \( T_2/T_L = 0.003 \) would predict \( \psi(1, \tau) = 0.0014 \). The calculated value is 0.0013. Hence, the curves for \( T_L = 50 \) are approximately equivalent to \( k = 0.0042 \) in the equilibrium boundary specification. Note that \( T_L = 50 \) implies that

\[
\frac{K_{L \Lambda}}{A_m} = 6.9 \times 10^{-5} \text{ cm/sec} \left( \frac{K_{L \Lambda}}{A_m} = \frac{T_L D_{\text{eff}}}{L} \right).
\]

This is not an unreasonable value for a mass transfer coefficient.

Simply prescribing the ratio \( T_2/T_L \) with \( T_L > 1 \) does not always guarantee that the boundary condition will behave as a pseudo equilibrium condition. This will be true only for intermediate to large values of \( T_2 \) (i.e., \( T_2 > 0.1 \)). This is demonstrated on Fig. 86 where profiles for constant \( T_2/T_L (=1/70) \) are presented for \( T_L = 0.07 \) and \( T_2 = 0.56 \) at two different dimensionless times. Note that (6.32) suggests that for \( T_2/T_L = 1/70 \), one should expect \( \psi(1, \tau) \) to equal 0.0060. When \( T_L = 0.07 \), \( \psi(1, \tau) \) is only 0.0021 while for \( T_2 = 0.56 \), \( \psi(1, \tau) = 0.0050 \). Thus, the response time of \( \psi(1, \tau) \) is dependent on the magnitude of \( T_2 \). The larger the magnitude of \( T_2 \) the faster \( \psi(1, \tau) \) tends to approach its "equilibrium" limit or inflection value. Physically this means that the greater the vesicular flux across the adventitial capillary endothelium relative to the transmedial diffusive flux, the faster the boundary concentration will attain its equilibrium value. Therefore for \( T_2 > 1 \) one would expect that the transcapillary flux would be sufficiently faster than transmedial diffusion to guarantee that \( \psi(1, \tau) \) will instantaneously reach the value suggested by (6.32).
Figure 86: Effect of $T_L$ - Complex Boundary Condition

$T_1 = 0.07$  \hspace{1cm} A: $T_2 = 0.07$  \hspace{1cm} $T_L = 4.9$

$\varepsilon = 0.42$  \hspace{1cm} B: $T_2 = 0.56$  \hspace{1cm} $T_L = 39.2$

$P = 7.0$

$R_1 = 1.0$

$R_2 = 0.0$

$T_2/T_L = 1/70$

$$\left[ p\psi - \frac{\partial \psi}{\partial \eta} = T_2 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \psi + \frac{\psi}{1-R_2} \eta \right]_{\eta = 1}$$

$$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$$
To appreciate the fact that $T_L = 50$ and $T_2 = 0.5$ result in predicted profiles similar to the equilibrium boundary condition, compare the predicted four hour distributions (Figs. 63 and 83). They both possess concave downward curvature. Similar curvature inconsistent with experimental results is noted at 24 hours (Fig. 84). Moreover, the dimensionless concentrations are higher at four hours than at 24 hours, also contrary to the experimentally observed results.

Thus, variation of $T_2$ and $T_L$ in the more complex boundary condition (6.25c) does not result in theoretical profiles which are markedly more consistent with experimental results than was attainable with the equilibrium condition. The only improvement is that for small values of $T_2$ ($< 0.05$) the dimensionless concentration near $\eta = 1$ does respond more slowly to the instantaneous step change in $C_p$ at $t = 0$ than the case where the equilibrium condition is used. This rise in concentration with time near $\eta = 1$ is consistent with the LDL experimental results at 0.167 and 0.5 hours.

7. Effect of Solute Rejection at $\eta = 1$

The other parametric variation possible with (6.25c) as the boundary condition is for variable $R_2$, where $R_2$ is the lymphatic rejection coefficient. A rejection of unity implies that all solute is removed from the extravascular space into the lymphatic terminals by only diffusion, and not convection. The effect of $R_2$ on the solute profile is shown on Figs. 87 to 89 for three different dimensionless times with $P = 1$. Also shown are the profiles for $R_2 = 0.75$ when $P = 2$ and $P = 5$. At short times (Fig. 87), increased $R_2$ has only a minor impact on the distribution
Figure 87: Effect of $P$ and $R_2$ - Complex Boundary Condition

$t = 0.5 \text{ hours} \quad T_2 = 0.05$

$\tau = 0.104 \quad T_L = 2.5$

$T_1 = 0.05$

$\varepsilon = 0.42$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$

$\frac{C_p(t)}{C_p(0)}$

$P = 5, R_2 = 0.75$

$P = 2, R_2 = 0.75$

$P = 1, R_2 = 0.75$

$P = 1, R_2 = 0$
Figure 88: Effect of P and $R_2$ - Complex Boundary Condition

\[
\begin{align*}
\text{t} &= 4 \text{ hours} \\
\tau &= 0.833 \\
T_1 &= 0.05 \\
\varepsilon &= 0.42 \\
R_1 &= 1.0 \\
T_2 &= 0.05 \\
T_L &= 2.5 \\
\frac{C_p(t)}{C_p(0)} &= 0.33e^{-0.75t} + 0.67e^{-0.041t} \\
\end{align*}
\]
Figure 89: Effect of $P$ and $R_2$ - Complex Boundary Condition

$$t = 24 \text{ hours} \left[ p\psi - \frac{\partial \psi}{\partial \eta} = p\psi(1-R_2) + T_L \psi + T_2 \left( \psi - \frac{C_p(t)}{C_p(0)} \right) \right]_{\eta=1}$$

$\tau = 5.0$

$T_1 = 0.05 \quad R_1 = 1.0$

$\varepsilon = 0.42$

$$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$$

$T_2 = 0.05$

$T_L = 2.5$
with the only difference being slight elevation in $\Psi(\eta,t)$ near $\eta = 1$.

As noted previously, the elevation of $P$ tends to depress $\Psi$ near $\eta = 0$ at all times. At intermediate $\tau(=0.833)$, the effect of $R_2$ is more pronounced. For $P = 1$ (Fig. 88) an $R_2 = 0.75$ causes more of a buildup in solute concentration near $\eta = 1$ than for $R_2 = 0$. Note that for $P = 5$ and $R_2 = 0.75$ the profile has a marked upswing over $0.6 < \eta < 1$ at $\tau = 0.833$ while for $P = 2$ the gradient is less steep. These results are intuitively logical when one considers that $T_L = 2.5$ in this case.

What this demonstrates is that high rejections ($R_2 \approx 0.75$) result in marked solute polarization near $\eta = 1$ only for $P > T_L$. In physical terms, solute polarization occurs in the media only when the rate at which solute is convected across the media-adventitia junction exceeds the solute lymphatic clearance rate. A low clearance rate relative to the convection rate gives rise to solute accumulation near the media-adventitia interface. Thus, under physiological conditions where lymphatic clearance is sufficiently compromised, one would anticipate a positive solute concentration gradient near the media-adventitia interface. Note that even at long times, $\tau = 5.0$ (Fig. 89), the gradient for $P = 5$ and $R_2 = 0.75$ persists, albeit less steep than for $\tau = 0.833$.

While Figs. 87 to 89 do not represent an exhaustive parametric variation in $R_2$ for different $T_1$, $T_2$, $T_L$, and $P$, they do allow one to reach the conclusion that the likelihood that any combination of variables could result in theoretical profiles consistent with experimental findings is small. The main effect of rejection is, under the appropriate conditions, to provide a physical mechanism for solute accumulation near $\eta = 1$. Rejection has little effect on the profile shape for $0 < \eta < 0.5$. Thus, no
matter what \( T_1, T_2, T_L \), or \( \phi \) are chosen, the predicted profile shapes at 4 hours and later cannot be made to agree with the experimental data. Only at 0.167 and 0.5 hours can this theory be made to agree qualitatively with experiment. Therefore, convection with diffusion across a continuum with no internal reaction is not an appropriate model for the transport of LDL across the aortic wall.

C. Convection and Diffusion with Reaction

Given the inadequacy of the simple diffusion and convection model presented in the previous section, one may speculate that the problem cast in that manner was oversimplified. The next level of complexity would be to introduce the possibility of solute interaction with immobile constituents within the wall. The equation to be solved has been given previously as

\[
\frac{\partial}{\partial x} \left[ D_{\text{eff}} \frac{\partial C(x,t)}{\partial x} - \frac{J}{\varepsilon} C(x,t) \right] - R(C,x,t) = \frac{\partial C(x,t)}{\partial t} \quad (6.33)
\]

where \( R(C,x,t) \) is the rate at which solute is bound to the immobile phase.

1. Binding Model

The form of \( R(C,x,t) \) is dependent on the binding mechanism envisioned. Others have hypothesized that LDL binds with both elastin \((72, 73)\) and acid mucopolysaccharides (AMPS) \((77, 78)\) which exist within the arterial wall. Unfortunately, neither the kinetics nor the mechanism of these processes are known. However, some qualitative observations have been made concerning these binding processes. In the case of elastin, the binding is thought to be irreversible \((73)\). Treatment with hot alkalai could not remove the bound LDL from elastin. On the other hand,
the LDL-AMPS interaction is reversible under conditions of high ionic strength in vitro (79). Nothing has been reported on the relative binding affinities of elastin and AMPS. Hence, one can only speculate as to the degree to which any binding in the arterial wall may be reversible.

Other studies have shown that the amount of LDL which can be complexed with AMPS is dependent on the quantity of AMPS available. That is, in in vitro systems AMPS exhibits a binding saturation limit. Regardless of the amount of AMPS used, the ratio of the mg of AMPS to the mg of β-lipoprotein for maximum binding was found to be constant at 0.06 (82). In other words, for a given quantity of mucopolysaccharide, a limit exists as to the amount of β-lipoprotein which can be complexed under in vitro conditions. If such a limit exists within the arterial wall it is not known.

Inasmuch as so little is known about the potential binding mechanisms, one is forced to speculate as to the proper form of the binding kinetic expression. The usual approach taken for solute adsorption during flow through porous media is to assume that the solute (a) interacts reversibly with an immobile phase (b) in the manner \( a + b \xrightarrow{k_2} c \) such that the kinetic expression may be assumed to be of the form:

\[
\frac{\partial C}{\partial t} = k_1 C_a C_b - k_2 C_c = \text{rate of disappearance of a}
\]

(6.34)

where \( C_a, C_b \), and \( C_c \) denote the effective concentration of the species involved and \( k_1 \) and \( k_2 \) are the forward and reverse reaction rate constants.
Applying (6.34) to the case of LDL interaction with elastin and/or AMPS, we can assume that the concentration of the immobile phase, $C_b$, is in excess relative to $C_a$. Combining $k_1$ and $C_b$ we have

$$\frac{3C}{3t} = k_1 C_b (C_a - \frac{k_2 C}{k_1 C_b}) = k'_1 (C_a - \frac{k_2 C}{k_1})$$  \hspace{1cm} (6.35a)

Since

$$\frac{3C}{3t} = \frac{3C}{3t}$$

Thus, (6.33) becomes after dropping the subscript, $a$, for LDL,

$$\frac{3}{3x} \left[ D_{eff} \frac{3C(x,t)}{3x} \frac{j_f}{\varepsilon} C(x,t) \right] = \frac{3C(x,t)}{3t} + \frac{3C}{3t} (C(x,t))$$  \hspace{1cm} (6.36a)

$$\frac{3C}{3t} = k'_1 (C - \frac{k_2 C}{k_1})$$  \hspace{1cm} (6.36b)

The binding rate expression given by (6.36b) applies to nonequilibrium conditions. Presumably, any pre-existing LDL-AMPS interaction is already at equilibrium when the tracer dose of labeled LDL is administered. However, a perturbation analysis can be performed to show that (6.36b) still applies even for the case where a tracer dose is given. Denoting the pre-existing equilibrium concentrations by the subscript, eq, and the incremental tracer concentration by $\Delta C_a$, (6.34) becomes

$$\frac{3\Delta C}{3t} = k_1 (C_{a eq} + \Delta C_a) (C_{b eq} + \Delta C_b) - k_2 (C_{c eq} + \Delta C_c)$$  \hspace{1cm} (6.37)
For \( \Delta C_b = 0 \), and \( (k_1 C_{acq} C_{beq} - k_2 C_{c_{eq}}) = 0 \),

\[
\frac{\partial \Delta C}{\partial t} = k_1 C_{beq} \Delta C_a - k_2 \Delta C_c
\]  

(6.38)

Since \( C_a = C_a + C_{ae} \) and \( C_c \) can be written as \( C_{ca} + C_{ce} \), where \( u \) and \( e \) denote unlabeled and labeled solute, respectively,

\[
\frac{\partial \Delta C}{\partial t} = k_1 C_{beq} C_{au} \left( 1 + \frac{C_{ca}}{C_{au}} \right) - k_2 C_{cu} \left( 1 + \frac{C_{ce}}{C_{cu}} \right)
\]  

(6.39)

For \( C_a \ll C_{au} \) and \( C_c \ll C_{cu} \)

\[
\frac{\partial \Delta C}{\partial t} = \left( \frac{k_1 C_{beq}}{C_{ca}} C_{au} \right) C_{ac} - \left( \frac{k_2 C_{cu}}{C_{ce}} \right) C_{ca}
\]  

(6.40)

The terms within the parentheses are apparent lumped constants. Thus, the binding of the labeled solute is a measure of the total amount of solute bound. In other words, (6.36b) is the appropriate binding rate expression for the tracer dose experimental conditions.

Implicit in the use of (6.36b) are certain assumptions, not the least of which is that the kinetic expression, (6.34), indeed applies to LDL interactions within the arterial wall. Also assumed is that the immobile phase is not saturated with LDL. That is, the addition of incremental amounts of LDL as reflected in an increase in the amount of complex formed. If the binding substrate were saturated, this would not be possible. Moreover, it is assumed that saturation is never attained during the binding process. The amount of bound solute is only limited
by the ratio of the forward to reverse reaction rates and by the local concentration of unbound solute. This is another way of saying that the binding substrate concentration, \( C_b \), is everywhere in large excess relative to the concentration of unbound solute. Without experimental justification, this binding model with its implicit assumptions must be regarded as highly speculative. However, it may provide insight into how LDL interactions within the arterial wall may affect the spatial distributions over time.

One last specification is required before the solution to (6.36) can be obtained. One requires an initial condition on \( C_c \). As mentioned previously, \( C_c \) is actually at \( C_{c-eq} \) when the experiment starts. However, since only the labeled \( C_c \) is monitored,

\[
C_c (x,0) = 0
\]  

(6.41)

2. Solution

Thus, the problem is completely specified and the solution can be obtained in exactly the same manner as for the case where binding interactions were neglected. In dimensionless form, the problem can be stated as:

\[
\frac{\partial}{\partial \eta} \left( \frac{\partial \psi}{\partial \eta} - \psi \right) = \frac{\partial \psi}{\partial \tau} + \frac{\partial N}{\partial \tau} \tag{6.42a}
\]

\[
\frac{\partial N}{\partial \tau} = \Theta (\psi - m N) \tag{6.42b}
\]

at \( \eta = 0: \psi = \frac{\partial \psi}{\partial \eta} = T_1 \left( \epsilon \frac{C_p(t)}{C_p(0)} - \psi \right) + \frac{\epsilon_p C_p(t)}{C_p(0)} (1 - R_1) \tag{6.43a}
at \( \eta = 1 \):

\[
p\psi - \frac{\partial \psi}{\partial \eta} = T_2 \left[ \psi - \frac{C_p(t)}{C_p(0)} \right] + T_L \psi + p\psi (1 - R_2)
\]  
(6.43b)

or

\[
\psi = \kappa \frac{C_p(t)}{C_p(0)}
\]  
(6.43c)

at \( \tau = 0 \):

\[N = \psi = 0 \quad \text{for all } \eta \]  
(6.43d)

where \( \Theta \equiv k_1 C_b L^2_{\text{eff}} \) and \( m \equiv k_2 / k_1 C_b \) are the only new dimensionless groups introduced. \( \Theta \) is a dimensionless quantity, known as the Thiele modulus, indicative of the relative rates of solute adsorption and diffusion. For \( \Theta >> 1 \), the process would be dominated by reaction effects. For \( \Theta << 1 \), reaction rates are slow relative to diffusion. \( m \) represents the ratio of reverse to forward reaction rates. For \( m << 1 \), reverse reaction rate is relatively slow, and conversely, for \( m >> 1 \), solute "desorbs" relatively quickly.

The final form of the solution is quite similar to the case where the binding effect was excluded. It is given by:

\[
\psi(\eta, \tau) = A \left( B - 2 \sum_{\ell=1}^{\infty} \frac{Q_\ell \exp(S_{\ell} \tau)}{S_{\ell} Z_{\ell}} \right) + AB \left( \sum_{k=1}^{2} \alpha_k \frac{-\beta_k \tau}{k} \right) - \\
- 2A \sum_{\ell=1}^{\infty} \sum_{k=1}^{\infty} \frac{\alpha_k \beta_k t Q_\ell [\exp(-\beta_k \tau) - \exp(S_{\ell} \tau)]}{S_{\ell} Z_{\ell} \left[ \beta_k t + S_{\ell} \tau \right]} 
\]  
(6.44)

\[
N(\eta, \tau) = \Theta \left\{ A \left( B [1 - \exp(-m\Theta \tau)] - 2 \sum_{\ell=1}^{\infty} \frac{Q_\ell \left[ \exp(S_{\ell} \tau) - \exp(-m\Theta \tau) \right]}{S_{\ell} + \Theta m} \right) + \\
+ AB \left( \sum_{k=1}^{2} \frac{\alpha_k t [\exp(-\beta_k \tau) - \exp(-m\Theta \tau)]}{(m\Theta \tau - \beta_k t)} - \frac{\left[ 1 - \exp(-m\Theta \tau) \right]}{m\Theta} \right) \right\} 
\]
\[ -2A \sum_{k=1}^{\infty} \sum_{\ell=1}^{2} \frac{\alpha_\ell \beta_\ell \tau Q_\ell}{S_Z (\beta_\ell + S_\ell \tau)} \left[ \frac{\exp(-\beta_\ell \tau) - \exp(-m\Theta \tau)}{(\Theta \tau - \beta_\ell \tau)} \right] - \]

\[ \frac{[\exp(S_\ell \tau) - \exp(-m\Theta \tau)]}{(S_\ell + m\Theta \tau)} \right] \]  

(6.45)

where \( A \) is defined by (6.31a), \( B \) by (6.31c), \( Q_\ell \) by (6.31d) and \( \lambda_\ell \) by (6.31e). The other quantities appearing in the solution are:

\[ S_\ell = \frac{-1}{2} \left\{ m\Theta + \Theta + \frac{\Theta^2}{4} + \lambda_\ell^2 + \sqrt{(m\Theta + \Theta + \frac{\Theta^2}{4} + \lambda_\ell^2)^2 - 4m\Theta (\frac{\Theta^2}{4} + \lambda_\ell^2)} \right\} \]  

(6.46)

\[ Z_\ell = 1 + \frac{m\Theta^2}{(m\Theta + S_\ell)^2} \]  

(6.47)

Note that for \( \Theta = 0 \), (6.42b) vanishes and (6.42a) results in the solution presented for the case of no binding, equation (6.31). Thus, \( \psi(\eta, \tau) \) tends toward zero at long times as does \( N(\eta, \tau) \). As is shown in APPENDIX E, the solutions for constant, nondecaying plasma concentration are given by the first term of (6.44) and the first term of (6.45).

Defining \( \xi(\eta, \tau) \) as the total labeled solute, bound and mobile, in the wall at any instant, \( \tau \), we have for the case of steady plasma concentration

\[ \xi(\eta, \tau) = \psi(\eta, \tau) + N(\eta, \tau) = AB \left( 1 + \frac{1}{m} \right) \]  

(6.48)
Hence, the effect of binding is to increase the steady state dimensionless wall concentration by the factor \((1 + \frac{1}{m})\). This concept has an important bearing on the interpretation of the profiles calculated using (6.44) and (6.45), because it suggests that the effect of the binding model formulated here is simply to amplify the value which would be predicted without the binding reaction.

3. **Effect of Binding**

The effect of \(m\) is demonstrated on Figs. 90 to 94 where plotted are profiles calculated using the equilibrium boundary condition (6.43c). Note that at 0.167 and 0.5 hours (Figs. 90 and 91) a dimensionless binding constant of \(\theta = 0.3\) results in a family of curves similar in shape to the limiting no binding case. The largest deviations from the \(\theta = 0\) curve are seen for the smallest values of \(m\). That is, the net of the binding reaction is greatest for the slowest reverse reaction rate (i.e., the smallest \(m\)). At four hours (Fig. 92), the effect of \(m\) at \(\theta = 0.3\) persists. However, the effect at higher \(\theta\) is more pronounced. Note that, regardless of \(\theta\) or \(m\), all curves are similar in shape to the no binding case. Similarly, at 24 (Fig. 93) and 67 (Fig. 94) hours, symmetry with respect to the \(\theta = 0\) curve is evident. Therefore, including the reaction term in the convection-diffusion model with \(\theta \approx 1\) does not alter the shape of the profiles but rather the magnitude of the dimensionless concentrations.

Although larger values of \(\theta\) were not studied, one can argue that had they been tested, the same observation would hold, at least at four hours and later. The reasoning goes as follows: for \(\theta >> 1\) the forward reaction rate is fast relative to diffusion. Thus, the distribution of bound and
Figure 90: Effect of m

\begin{align*}
t &= 0.167 \text{ hours} \\
\tau &= 0.0347 \\
T &= 0.05 \\
\varepsilon &= 0.42 \\
P &= 1.0 \\
\left[\psi = 0.0040 \frac{C_p(t)}{C_p(0)} \right]_{\eta = 1} \\
\Theta &= 0.3 \end{align*}

\[ \psi = \frac{C_p(t)}{C_p(0)} \]
Figure 91: Effect of $m$

$t = 0.5$ hours
$\tau = 0.104$
$T_i = 0.05$
$\epsilon = 0.42$

$P = 1.0$
$\psi = 0.0040 \frac{C_p(t)}{C_p(0)} \eta = 1$

$\theta = 0.3$
Figure 92: Effect of $\Theta$ and $m$

\[ \psi = \frac{c}{c_p(0)} \]

- $t = 4$ hours
- $\tau = 0.83$
- $T_1 = 0.05$
- $\varepsilon = 0.42$
- $P = 1.0$
- $\psi = 0.0040 \frac{c_p(t)}{c_p(0)}$ for $\eta = 1$

Legend:
- $\Theta = 1.5$
- $\Theta = 0.7$
- $\Theta = 0.3$
- $\Theta = 0$ (no binding)

$\eta = x/L$
Figure 93: Effect of $\Theta$ and $m$

$t = 24$ hours
$\tau = 5.0$
$T_1 = 0.05$

$[\psi = 0.0040 \frac{C_p(t)}{C_p(0)}]^{\eta-1}$

$P = 1.0$
$\epsilon = 0.42$
Figure 94: Effect of $\Theta$ and $m$

\[
t = 67 \text{ hours} \\
t = 13.94 \\
T_1 = 0.05 \\
\tau = 0.42 \\
P = 1.0 \\
\psi = 0.0040 \frac{C_p(t)}{C_p(0)} \\
\eta = 0.0040 \frac{C_p(t)}{C_p(0)} \\
\Theta = 0.3 \\
\]

\[
\eta = x/L 
\]

Graph showing the effect of $\Theta$ and $m$ on $\eta = x/L$. The graph includes lines for different values of $m$ and $\Theta$. The graph shows how the concentration changes with $\eta$. 

- $\Theta = 0.2, 0.4, 0.6, 0.8$ for different $m$ values.
- $\Theta = 0$ (no binding) is also shown.
unbound solute will approach its equilibrium value much faster than in the cases presented on Figs. 90 to 94. Since this equilibrium distribution is everywhere the same as given by \( AB(1 + \frac{1}{m}) \), the total solute (bound plus unbound) distribution will everywhere equal the unbound concentration times \( (1 + \frac{1}{m}) \) at equilibrium. Only at short dimensionless times, when binding has not yet reached equilibrium, is it possible for reaction, as formulated here, to dramatically affect the shape of the profiles. At longer times (\( \tau > 1 \)) no matter what \( \Theta \) is used, the total solute distribution will simply represent an amplification of the unbound solute distribution. Therefore, simply increasing \( \Theta \) is not likely to preserve the 0.167 and 0.5 hour transient profile shapes to as long as four hours, since \( \tau \) must be greater than 1 at four hours. For \( \tau < 1 \) at four hours (i.e., for smaller \( D_{\text{eff}} \)), the solute penetration distance at 0.167 hours is much smaller than experimentally observed. Therefore, \( \tau \) must be greater than 1 and, hence, a \( \Theta \) greater than 1 will dictate that the four hour binding profile be simply an amplification of the non-binding case. In short, the binding model as formulated here, cannot result in predicted profiles which are any more consistent with the experimental data than can the nonbinding model.

4. Nonuniform Binding

One of the assumptions implicit in the foregoing binding model is that the concentration of binding sites, \( C_b \), is large and everywhere the same across the arterial wall. Recent topological studies have indicated, at least for bovine aorta, that acid mucopolysaccharides may not be uniformly distributed. Serial radial sections of bovine aorta
were analyzed for AMPS with the pertinent results shown on Fig. 95 (89). There existed a marked gradient in chondroitin sulfate composition across the wall, with the highest levels near the intima. Since chondroitin sulfate has an affinity for LDL (78), its distribution may have broad implications on how LDL distributes within the arterial wall. Its distribution across the bovine aorta would suggest that, if binding were important, LDL-AMPS interaction would be most prevalent near the intima. Note on Fig. 95 that the distribution for other AMPS are also given. They presumably also bind the LDL. However, spatially nonuniform distribution was not noted for these in bovine aorta.

To incorporate into the model the possibility of nonuniformly distributed binding sites within the rabbit aortic wall, one must first postulate mathematically, the form of the binding distribution. Since no experimental data are available which apply to rabbit aorta AMPS distribution, the solution was formulated so that profiles could be calculated for any prescribed distribution, g(η). This necessitated a numerical solution as described in APPENDIX E. The only difference in the problem formulation from that described by (6.42) and (6.43) involves the binding rate expression. For any g(η)

\[ \theta^* = \theta g(\eta) \left( \psi - \frac{m}{g(\eta)} N \right) \]  

(6.49)

where g(η) describes the distribution of binding sites, C_b. Thus, the numerical solution technique provides a means for studying the effect of various g(η) on the theoretical solute distributions over time.

The goal in this phase of the theoretical analysis was to find a binding distribution, g(η), which would bring the theoretical model into
Figure 95: Distribution of Acid Mucopolysaccharides Across Bovine Aorta (89)
better qualitative agreement with the observed experimental results. To be sure, the results of this approach do not validate the \( g(\eta) \) so chosen, but rather they provide insight into how sensitive the model is to \( g(\eta) \).

A number of different binding distributions were tested. First, however, a comparison of the profiles predicted by the numerical solution \([g(\eta) = 1]\) and the analytical solution is presented (Figs. 96 and 97). Note that the numerical solution results were lower than those calculated with the analytical solution at every time interval. However, differences were generally small, amounting to no more than 0.0006 at longer times and 0.0002 at the early times. Also note that the numerical solution was found to be in excellent agreement with the analytical solution when \( \theta = 0 \). The reasons for the larger error when \( \theta \neq 0 \) involve the details of the numerical solution method. These are discussed in APPENDIX E. Because the errors with the numerical method were small, the solution so obtained is deemed a reasonable approximation of the true solution.

The profiles predicted for five different binding distributions are presented on Figs. 98 to 102. For convenience the various \( g(\eta) \) are shown as well. In the cases shown, the equilibrium boundary condition was used at \( \eta = 1 \), and a moderate value of \( \theta = 0.7 \) was chosen. At 0.167 and 0.5 hours, all binding distributions resulted in virtually the same profiles (Figs. 98 and 99). Of course, had a larger value of \( \theta \) been used, an effect of \( g(\eta) \) would have been observed at these early times. At four hours \((\tau = 1.98, \text{ Fig. 100})\), \( g(\eta) \) can be seen to be important in determining the intramural distribution. Note that each \( g(\eta) \) used results in a profile which is higher in level than for the case of no binding. The
Figure 96: Comparison of Numerical Solution With Analytical Solution

\[ C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

\[ \tau = 0.0040 \]

\[ \eta = 0.42 \]

\[ t = 24 \text{ hours} \quad (t = 3.0) \]

\[ \eta = x/L \]

Analytical

Numerical
Figure 97: Comparison of Numerical and Analytical Solutions Without Binding

\[
\begin{align*}
0 &= 0 \\
\tau_1 &= 0.05 \\
\tau_2 &= 0.05 \\
\varepsilon &= 0.42 \\
\left\{ \left( P - (T_2 + T_L) + P(1-R_2) \right) - P(1-R_2) \right\} \frac{\partial \psi}{\partial \eta} &= -T_2 \varepsilon^2 \frac{C_\varepsilon(t)}{C_\varepsilon(0)} \\
\frac{C_\varepsilon(t)}{C_\varepsilon(0)} &= 0.33e^{-0.775t} + 0.67e^{-0.041t} \\
R_1 &= 1.0 \\
R_2 &= 0.75 \\
T_2 &= 0.05 \\
T_L &= 2.5 \\
\gamma &= \frac{\partial C}{\partial x} \\
\eta &= \frac{x}{L} \\
\tau &= 0.83 \\
\tau &= 0.104 \\
\tau &= 0.035
\end{align*}
\]
Figure 98: Effect of Binding Distribution, $g(\eta)$

- $t = 0.167$ hours
- $\tau = 0.083$
- $p = 1.0$
- $T_1 = 0.0288$
- $\epsilon = 0.42$
- $Q = 0.7$
- $m = 0.6$

$$\Psi (t, \tau) = 0.0025 \frac{C_p(t)}{C_p(0)}$$

$\bullet$ LDL experimental data
Figure 99: Effect of Reversible Binding Distribution

- $t = 0.5$ hours
- $\tau = 0.25$
- $P = 1.0$
- $T_1 = 0.0288$
- $\epsilon = 0.42$
- $\Theta = 0.7$
- $m = 0.6$
- $\Psi(1,\tau) = 0.0025C_p(t)/C_p(0)$

Relative Position, $\eta = x/L$
Figure 100: Effect of Reversible Binding Distribution

$t = 4$ hours $P = 1.0$

$\tau = 1.98$ $T = 0.0288$

$\varphi = 0.42$ $\Theta = 0.7$

$m = 0.6$

$\Psi(l, \tau) = 0.0025 \frac{C_p(t)}{C_p(0)}$

Relative Position, $\eta = x/L$

LDL Experimental Data
Figure 10.1: Effect of Reversible Binding Distribution

- $t = 24$ hours
- $\Theta = 0.7$
- $\tau = 11.9$
- $m = 0.6$
- $P = 1.0$
- $\psi(1, \tau) = 0.0025 C_p(t)/C_p(0)$
- $T_1 = 0.0288$

Relative Position, $\eta = x/L$
Figure 102: Effect of Binding Distribution

\[ t = 64 \text{ hours} \]
\[ \tau = 31.75 \]
\[ P = 1.0 \]
\[ \tau_1 = 0.0288 \]
\[ \varepsilon = 0.42 \]
\[ \theta = 0.7 \]
\[ m = 0.6 \]
\[ \Psi(1, \tau) = 0.0025 C_p(t)/C_p(0) \]
deviation from the no binding case at a particular \( \eta \) is directly proportional to the value of \( g(\eta) \) at that position. Hence, the linear distributions (3 and 5) result in profiles with the same concave downward curvature as for the no binding case. The exponential distributions (2 and 4) yield curves which are practically linear. Only the parabolic distribution (1) results in a profile which possesses curvature similar to the experimental data. Similar observations can be made based on the predicted profiles at 24 and 64 hours (Figs. 101 and 102). Certainly, as the values of \( P \) and \( T \) are varied, the nonbound solute distribution will change. This will, therefore, change the shapes and levels of the profiles for each of the \( g(\eta) \) used here. However, at four hours \( (\tau = 2) \) the nonbinding profile will always be either linear or concave downward depending upon the relative magnitudes of \( P \) and \( T \) so long as the equilibrium boundary condition is used at \( \eta = 1 \). Thus it follows that, if the equilibrium condition is appropriate, the only way to bring the theoretical predictions into qualitative accord with the experimental data is to require that the binding distribution, \( g(\eta) \), (1) either be identical in shape with the four hour LDL experimental data (i.e., concave upward) and specify a combination of \( P \) and \( T \) such that the non-binding profile is at some uniform level, independent of \( \eta \), below \( \psi = 0.0030 \), or (2) to require that the binding distribution go through a minimum somewhere between \( \eta = 0 \) and \( \eta = 1 \). If the equilibrium condition is not an accurate representation of the physical phenomena occurring at \( \eta = 1 \), then previous statement does not necessarily apply.

Assuming for the moment that the equilibrium condition does apply, one can successfully argue that under moderate binding rates, \( \theta \), both
strategies (1) and (2) result in profiles qualitatively consistent with the experimental data at four hours. Considering strategy (1), Fig. 58 and Fig. 63 demonstrate how making $T_1/P$ smaller affects the nonbinding profile at four hours. The smaller the ratio the flatter the profile. Taking for example, $P = 5.0$ (Fig. 63), and $T_1 = 0.05$, and superimposing a binding distribution similar in shape to the four hour LDL data, one can develop a theoretical profile which coincides very nicely with the experimental data since the total solute concentration is the sum of the bound and unbound solute contributions. Unfortunately, a $T_1/P = 0.01$ results in profiles at 0.167 hours and 0.5 hours which are much flatter' than the experimental data (Figs. 56 and 57 or Figs. 61 and 62). Therefore, while strategy (1) may be successful in bringing the four hour predictions into accord with the experimental data, it does so at the expense of the fit at earlier times. Of course, this holds true only for $\Theta \approx 1$ in which case binding at early times is insignificant. One cannot rule out the possibility that much larger values of $\Theta$ would eliminate this inconsistency at the earlier times. Thus, with the possible exception of large $\Theta$, one can deduce that strategy (1) will not bring theory and experiment into agreement at all time intervals.

The effect of a binding distribution which goes through a minimum [strategy (2)] is illustrated by the parabolic distribution ($\theta 1$) on Fig. 100. The theoretical profile is in reasonable agreement with the experimental data at four hours as well as at 24 hours (Fig. 101). Since this distribution was arbitrarily chosen, undoubtedly other $g(\eta)$ which also to through a minimum might fit the data equally well or better. The important point to be made, however, is that the distribution of binding sites
can have a marked effect on the distribution of LDL in the arterial wall. Thus, accurately knowing the distribution is critical to the optimization of any theoretical model.

Notice that Figs. 98 to 102 were calculated using the equilibrium boundary conditions at \( n = 1 \). Similar results are obtained when the more complex boundary condition (6.43b) is used with \( T_L = 120T_2 \). This is illustrated on Figs. 103 and 104 where the effect of the \( n = 1 \) boundary condition is assessed. The parabolic binding distribution was employed with \( T_1 = 0.0288, P = 1.0, \Theta = 0.9, \) and \( m = 0.6 \). At the two early times (0.5 and 0.167 hours), the effect of the boundary condition is only felt in the outer 50% of the wall. At four hours and longer, the \( n = 1 \) boundary condition affects the dimensionless concentrations throughout the wall. Had \( T_L/T_1 \) been chosen greater than 120, e.g., 160, the (6.43b) boundary condition would have given profiles even more similar to the equilibrium condition. But because the complex condition predicts curves that do not have a downturn near \( n = 1 \), one is tempted to speculate that this is the more physically realistic condition.

5. Spatially Nonuniform \( \text{D}_{\text{eff}} \) and Binding

Returning to the question of how the binding site distribution might affect the distribution of LDL across the aortic wall, one must also consider the role AMPS are alleged to play in restricting the diffusion of LDL. Thus far, we have tacitly assumed that the arterial wall is a continuum, and as such, is isotropic as far as its transport properties are concerned. This idealization may not be justifiable based on (1) the layered structure of the aortic wall, and the idea that (2) the diffusion of LDL may be restricted by AMPS which is nonuniformly distributed.
Figure 103: Effect of Boundary Condition - Nonuniform Binding

A: Equilibrium Condition

\[ T_1 = 0.0288 \quad T_2 = 0.1 \quad \psi(1, \tau) = 0.0025 \frac{C_p(t)}{C_p(0)} \]

B: Complex Condition

\[ \frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

\[ g(\eta) = 3.8 (0.5 - \eta)^2 + 0.05 \]
Figure 104: Effect of Boundary Condition - Nonuniform Binding

A: Equilibrium Condition

\[ \psi(1, \tau) = 0.0025 \frac{C_p(t)}{C_p(0)} \]

B: Complex Condition

\[ \frac{\partial \psi}{\partial \tau} = T_2 (\psi - \frac{C_p(t)}{C_p(0)}) + T_L \psi + P \psi (1 - R_2) \]

\[ C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

\[ g(\eta) = 3.8 (0.5 - \eta)^2 + 0.05 \]
The implications of the first possibility are obvious. If the individual layers, as defined by the intervening space between two adjacent elastic laminae, possess different transport properties, treatment of the arterial wall as a continuum cannot be justified. Perhaps a convection-diffusion analysis performed over 29 layers in series would be more appropriate. However, the experimental data do not justify such a complex approach at this stage.

The second possibility -- AMPS restricting the diffusion of LDL -- is also not without some physiochemical justification. Experiments have been performed which demonstrate that the LDL sedimentation rate is retarded by the presence minute quantities of hyaluronic acid in the analytical ultracentrifuge (78). This retardation is thought due to the formation of a molecular chain network which acts as a "filter" to LDL. The author does not present any viscosity data so one is forced to assume, on faith, that the hyaluronic acid concentrations used did not increase viscosity, thereby causing the observed reduction in the sedimentation velocity. No direct experimental evidence exists, however, on the extent to which AMPS in the arterial wall affect LDL diffusion. If one assumes that diffusion is restricted in regions of high AMPS concentration, and furthermore, if one takes the AMPS to be distributed as shown on Fig. 95 for chondroitin sulfate in bovine aorta, then one may speculate that LDL diffusion coefficients monotonically increase in value from the intima toward the media-adventitia junction.

To account for this possibility, the theoretical model was formulated to allow for any diffusion coefficient distribution, \( f(\eta) \), one might wish
to specify. The details of the solution are presented in APPENDIX E.

As one might \textit{a priori} predict, an \( f(\eta) \), such as the one shown on Fig. 105, results in steeper concentration gradients near \( \eta = 0 \) at early times. This is demonstrated on Fig. 106 where a comparison between the effect of uniform and nonuniform LDL diffusion coefficients is plotted. The complex boundary condition at \( \eta = 1 \) was used. At longer times, the effect of nonuniform diffusivity is less (Fig. 107). Thus, inclusion of variable \( D_{\text{eff}} \) in the theoretical model is seen to steepen gradients near \( \eta = 0 \), bringing the model in closer agreement with the experimental results. Only at 67 hours is there any marked difference. This discrepancy may be attributable either to inadequacy of the model or to the wide range in the three experimental results at 67 hours. Regardless of the reason(s) for the discrepancy, the improvement of the fit effected by allowing \( D_{\text{eff}} \) to vary with position must be appreciated. This is not to prove, however, that the LDL \( D_{\text{eff}} \) does indeed show spatial dependence. Only appropriately designed experiments can attest to this possibility. Rather, it only suggests that the experimental results are consistent with the hypothesis that wall constituents restrict the transport of LDL both by binding and by limiting the LDL diffusion rate.

\textbf{a. Effect of } \( T_1 \)

To demonstrate how sensitive the model is to variation in the endothelial conductance (i.e., the vesicular transport coefficient), \( T_1 \), parametric curves for all time intervals of interest are presented on Figs. 108 to 112. Except for the variation in \( T_1 \), the conditions used are identical to the variable diffusivity case presented on Figs. 106 and 107.
Figure 105:

Assumed Variation in Effective LDL Diffusion Coefficient and AMPS Binding Sites

\[ D_{\text{eff}}^*(n) = D_{\text{eff}} f(n) \]
\[ \Theta^*(n) = \Theta g(n) \]
\[ f(n) = 1.002236 - 0.902236e^{-6n} \]
\[ g(n) = 1.0114e^{-6n} + 1.009e^{-9(1-n)} - 0.0116 \]
Figure 106 - Effect of Variable Diffusivity With Variable Binding

\[ \frac{\partial^2 \phi}{\partial \eta^2} = \frac{T_0}{\lambda} \frac{T}{T_0} \frac{\partial \phi}{\partial \eta} + \frac{T}{T_0} \frac{\partial^2 \phi}{\partial \eta^2} + \frac{T}{T_0} \frac{\partial \phi}{\partial \eta} \]

\[ T_0 = 0.0288 \]
\[ P^0 = 0.6 \]
\[ \varepsilon = 0.42 \]
\[ \gamma_1 = 1.0 \]

\[ \begin{align*}
T_0 & = 0.0288 \\
P^0 & = 0.6 \\
\varepsilon & = 0.42 \\
\gamma_1 & = 1.0 \\
\end{align*} \]

\[ \phi \left( \frac{\partial}{\partial \eta} - \varepsilon \frac{C_p(\varepsilon)}{C_p(0)} \frac{\partial}{\partial \eta} \right) + \frac{\partial^2 \phi}{\partial \eta^2} + \phi - T \]

\[ \eta = 1 \]

\[ T_2 = 0.1 \]
\[ \Theta = 0.7 \]
\[ T_L = 12.0 \]
\[ m = 0.6 \]
\[ \gamma_2 = 0.0 \]

\[ C_{p}(\varepsilon) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

\[ C_{p}(0) \]

\[ g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157 \]

\[ f(\eta) = 1.002236 - 0.902236e^{-6\eta} \]

--- = Variable $D_{eff}$
--- = Constant $D_{eff}$

\[ \eta = \frac{x}{L} \]
Figure 107 - Effect of Variable Diffusivity With Variable Binding

\[ \frac{\partial \psi}{\partial t} = T_1 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} + T_2 \psi + P \psi(1 - R_2) \right) \]

- \( T_1 = 0.0288 \)
- \( P = 0.6 \)
- \( \varepsilon = 0.42 \)
- \( R_1 = 1.0 \)

\( \psi \) and \( C_p(0) \) are constants.

\( \psi \) is a variable.

- \( T_2 = 0.1 \)
- \( \theta = 0.7 \)
- \( R_2 = 0.0 \)

\( C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \)

\( C_p(0) = 0.33e^{-0.775} + 0.67e^{-0.041} \)

\( \eta = x/L \)

- \( \tau = 13.97 \) (\( t = 24 \) hr)
- \( \tau = 37.24 \) (\( t = 64 \) hr)

--- Variable \( D_{\text{eff}} \)

--- Constant \( D_{\text{eff}} \)
Figure 10: Effect of $T_1$ - $t = 0.167$, $\tau = 0.0972$

\[
P = 0.6, \quad \varepsilon = 0.42, \quad R_1 = 1.0
\]

\[
\left[\frac{\partial \Psi}{\partial \eta} = T_2 (\Psi - \varepsilon C_p(t)) + T_L \Psi + \Psi(1 - R_2) \right] \eta = 1
\]

\[
\eta = 0.1 \quad \theta = 0.7
\]

\[
T_L = 12.0, \quad m = 0.6
\]

\[
R_2 = 0.0
\]

\[
C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

\[
C_p(0)
\]

\[
g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157
\]

\[
r(\eta) = 1.002236 - 0.902236e^{-6\eta}
\]

\[
\eta = \frac{x}{L}
\]
Figure 109: Effect $T_1 - t = 0.5, \tau = 0.29$

$P = 0.6$
$\varepsilon = 0.42$
$R_1 = 1.0$

$$\left[ \frac{\partial W}{\partial \eta} = T_2 \left( \psi - \varepsilon \frac{C_p(\tau)}{C_p(0)} \right) + T_L \psi + P\psi(1-R_2) \right]_{T=1}$$

$T_2 = 0.1 \quad \theta = 0.7$
$T_L = 12.0 \quad m = 0.6$
$R_2 = 0.0$

$C_p(\tau) = 0.33e^{-0.775\tau} + 0.67e^{-0.041\tau}$
$C_p(0)$

$\psi (\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157$
$f (\eta) = 1.002236 - 0.902236e^{-6\eta}$

$T_1 = 0.0488$
$T_1 = 0.0288$
$T_1 = 0.0188$
Figure 110: Effect of $T_1$ - $t = 4$ hr, $\tau = 2.33$

$P = 0.6$
$\epsilon = 0.42$
$R_1 = 1.0$

\[
\frac{\partial \Psi}{\partial \eta} = T_2 (\Psi - \epsilon \frac{C_p(t)}{C_p(0)}) + T_L \Psi + \frac{P \Psi (1-R_2)}{t=1}
\]

$T_2 = 0.1$  $\theta = 0.7$
$T_L = 12.0$  $m = 0.6$
$R_2 = 0.0$

$T_1 = 0.0488$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.042t}$

$C_p(0)$

$g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157$

$f(\eta) = 1.002236 - 0.902236e^{-6\eta}$

$\eta = x/L$
Figure 11: Effect of $T_1$ - $t = 24$ hrs, $\tau = 13.97$

$P = 0.6$
$\epsilon = 0.42$
$R_1 = 1.0$

\[
\frac{p\psi}{\partial \eta} = T_2 (\psi - \varepsilon \frac{C_p(t)}{C_p(0)}) + T_L \psi + \frac{p\psi(1-R_2)}{P}
\]

$T_2 = 0.1$, $\theta = 0.7$
$T_L = 12.0$, $m = 0.6$
$R_2 = 0.0$

$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$

$g(\eta) = 1.011e^{-6\eta} + 1.009e^{-g(1-\eta)} - 0.01157$

$f(\eta) = 1.002236 - 0.902236e^{-6\eta}$
Figure 112: Effect of $T_1$  - $t = 64$ hrs, $\tau = 37.2$

\[
P = 0.6 \\
\varepsilon = 0.42 \\
R_1 = 1.0 \\
\left[ \frac{\partial \psi}{\partial \eta} = T_2 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \psi + P \psi(1 - R_2) \right]_{\eta = 1}
\]

$T_2 = 0.1$  $\Theta = 0.7$
$T_L = 12.0$  $m = 0.6$
$R_2 = 0.0$
$C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
$C_p(0)$
$\beta(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157$
$f(\eta) = 1.002236 - 0.902236e^{-6\eta}$

$\eta = x/L$
Notice that a 2.6-fold increase in $T_1$ (from 0.0188 to 0.0488) results in a proportionate change in $\psi(0,0.097)$ (Fig. 108). At longer times the effect of elevated $T_1$ is felt in the form of a generally higher dimensionless concentration over all $\eta$. The experimental data, for the most part, suggest that $T_1 = 0.03$ is the most reasonable estimate at the given conditions. However, the agreement between experiment and theory is sufficiently poor to prevent the accurate estimation of $T_1$.

b. **Effect of Peclet Number, $P$**

The effect on the profiles induced by variations in $P$, the dimensionless convective flux, is shown on Figs. 113 to 117. As was the case when no binding was assumed, a higher $P$ depresses the dimensionless concentrations at times four hours and later. At early times, the effect of $P > 1$ is to decrease $\psi$ at $\eta = 0$ and to broaden the concentration gradient over the inner 50% of the wall. At four hours, $P = 1$ fits the data best. At 24 hours, however, a $P$ closer to 0.06 is a better approximation. There is no particular value at 64 hours which is consistent with the experimental data. Hence, one cannot conclude with any certainty based on this model what the true value of $P$ is for arterial tissue. Clearly, small changes in $P$ about $P = 1$ make a large difference in the predicted solute distributions.

c. **Effect of $D_{eff}$**

The other critical parameter in the model is $D_{eff}$. Since all of the figures presented thus far were in terms of dimensionless parameters, one has difficulty in appreciating how variation in $D_{eff}$ might affect the profiles assuming all other variables are held constant. Presented on Figs. 118 to 122 are theoretical distributions for $D_{eff}$ between
Figure 113: Effect of P - Nonuniform Diffusivity and Nonuniform Binding - $t = 0.167, \tau = 0.097$

\[ T_1 = 0.0288 \]
\[ \varepsilon = 0.42 \]
\[ \Theta = 0.7 \]
\[ m = 0.6 \]
\[ R_1 = 1.0 \]

\[ \left[ \frac{P\Psi}{2} - \frac{3\Psi}{2\eta} \right] = T_2 \left( \Psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_1 \Psi + \sum_{n=1}^{\infty} P^{n+1}(1-R_2^n) \]

\[ g(\eta) = 1.011e^{-6\eta} + 1.003e^{-9(1-\eta)} \]

\[ f(\eta) = 1.002236 - 0.902236e^{-6\eta} \]

\[ T_2 = 0.1 \]
\[ T_L = 12.0 \]
\[ R_2 = 0.0 \]

\[ \frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]
Figure 114: Effect of $P$ - Nonuniform Diffusivity and Nonuniform Binding - $t = 0.5$ hr, $\tau = 0.29$

\[
T_1 = 0.0288, \quad e = 0.42, \quad \Theta = 0.7, \quad m = 0.6, \quad R_1 = 1.0
\]

\[
\left[ \frac{\partial \psi}{\partial \eta} = T_2 \left( \psi - \epsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \psi + \psi(1-R_2) \right]_{p=1}
\]

\[
\begin{align*}
\psi (\eta) &= 1.011 e^{-6\eta} + 1.009 e^{-9(1-\eta)} - 0.01157 \\
\psi (\eta) &= 1.002236 - 0.902236 e^{-6\eta}
\end{align*}
\]

\[
T_2 = 0.1, \quad T_L = 12.0, \quad R_2 = 0.0
\]

\[
\frac{C_p(t)}{C_p(0)} = 0.33 e^{-0.775t} + 0.67 e^{-0.041t}
\]
Figure 115: Effect of P - Nonuniform Diffusivity and Nonuniform Binding - t = 4 hr, τ = 2.33

\[ T_1 = 0.0288 \quad C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]
\[ \varepsilon = 0.42 \quad \frac{C_p(t)}{C_p(0)} \]
\[ \Theta = 0.7 \]
\[ \eta = 0.6 \]
\[ R_2 = 1.0 \]

\[ \left[ p \psi - \frac{\partial \psi}{\partial \eta} = T_2 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + \frac{T_L \psi + p \psi (1-R_2)}{\eta - 1} \right] \]

\[ g(\eta) = 1.001e^{-6\eta} + 1.000e^{-9(1-\eta)} - 0.01157 \]
\[ f(\eta) = 1.002236 - 0.902236e^{-6\eta} \]
\[ T_2 = 0.1 \]
\[ T_L = 12.0 \]
\[ R_2 = 0.0 \]
Figure 116 Effect of $P$ - Nonuniform Diffusivity and Nonuniform Binding - $t = 24$ hr, $\tau = 13.97$

\[
\begin{align*}
T_1 &= 0.0288 \\
\epsilon &= 0.42 \\
\Theta &= 0.7 \\
n &= 0.6 \\
R_1 &= 1.0 \\
\end{align*}
\]

\[
\left[ \frac{\partial \psi}{\partial \eta} = T_2 (\psi - \epsilon \frac{C_p(t)}{C_p(0)}) + T_2 \psi + P \psi (1 - R_2) \right]_{\eta=1}
\]

\[
\begin{align*}
\psi_0 (\eta) &= 1.011 e^{-6\eta} + 1.005 e^{-9(1-\eta)} - 0.01157 \\
\epsilon (\eta) &= 1.002236 - 0.902236 e^{-6\eta} \\
T_2 &= 0.1 \\
T_2 &= 0.0 \\
R_2 &= 0.0 \\
\end{align*}
\]

\[
\frac{C_p(t)}{C_p(0)} = 0.33 e^{-0.775t} + 0.67 e^{-0.041t}
\]
Figure 117: Effect of P - Nonuniform Diffusivity and Nonuniform Binding - t = 64 hrs, \( t = 37.2 \)

\[
\frac{\partial \Psi}{\partial t} = T_2 \left( \Psi - \varepsilon \frac{C_p(t)}{C_p(\theta)} \right) + T_L \Psi + \frac{P \Psi (1-R_2)}{R_2}
\]

\[ g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.0157 \]

\[ f(\eta) = 1.002236 - 0.902236e^{-6\eta} \]

\[ T_2 = 0.1 \]
\[ T_L = 12.0 \]
\[ R_2 = 0.0 \]

\[ \frac{C_p(t)}{C_p(\theta)} = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]
Figure 118: Effect of $D_{\text{eff}}$ - Nonuniform Diffusivity and Nonuniform Binding - $t = 0.167$ hr

$K_1 = 1.12 \times 10^{-7}$ cm/sec

$J_f = 0.0084$ ml/cm$^2$-hr

$\epsilon = 0.42$

$R_1 = 1.0$

$[p_\psi - \frac{\partial \psi}{\partial \eta} = T_2 (\psi - \epsilon \frac{c_p(t)}{c_p(0)} + T_L \psi + PV(1-R_2))]$

$K_2 = 3.8 \times 10^{-7}$ cm/sec

$K_L = 4.6 \times 10^{-5}$ cm/sec

$R_2 = 0.0$

$k_1 = 1.13 \times 10^{-4}$ sec$^{-1}$

$f (\eta) = 1.002236 - 0.902236e^{-6\eta}$

$g (\eta) = 1.011e^{6\eta} + 1.009e^{-9(1-\eta)} - 0.01157$

$\frac{c_p(t)}{c_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
Figure 119 Effect of $D_{\text{eff}}$ - Nonuniform Diffusivity and Nonuniform Binding - $t = 0.5 \text{ hr}$

- $K_1 = 1.12 \times 10^{-7} \text{ cm/sec}$
- $J_f = 0.0084 \text{ ml/cm}^2\text{-hr}$
- $\varepsilon = 0.42$
- $R_1 = 1.0$
- $P = \frac{1}{1-R_2}

\[
\Psi = T \left( \Psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \Psi + P \Psi (1-R_2)
\]

- $K_2 = 3.8 \times 10^{-7} \text{ cm/sec}$
- $K_L = 4.6 \times 10^{-5} \text{ cm/sec}$
- $R_2 = 0.0$
- $k_1 = 1.13 \times 10^{-4} \text{ sec}$
- $f(\eta) = 1.002236 - 0.902236e^{-6\eta}$
- $g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157$
- $C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
- $C_p(0)$

$D_{\text{eff}} = 2.79 \times 10^{-8} \text{ cm}^2/\text{sec}$

$D_{\text{eff}} = 5.59 \times 10^{-8}$

$D_{\text{eff}} = 9.31 \times 10^{-8}$
Figure 120: Effect of $D_{\text{eff}}$ - Nonuniform Diffusivity and Nonuniform Binding - $t = 4$ hr

\[
K_1 = 1.12 \times 10^{-7} \text{ cm/sec} \quad K_2 = 3.8 \times 10^{-7} \text{ cm/sec} \\
J_f = 0.0084 \text{ ml/cm}^2\text{-hr} \quad K_L = 4.6 \times 10^{-5} \text{ cm/sec} \\
\varepsilon = 0.42 \quad R_2 = 0 \\
R_L = 1.0 \quad k_1 = 1.13 \times 10^{-4} \text{ sec}^{-1} \\

\left[ \frac{\psi}{\partial \eta} \right]_{\eta=1} = T_2 \left( \psi - \frac{C_p(t)}{C_p(0)} \right) + T_L \psi + P\left[1 - R_2 \right] \\
\text{f} (\eta) = 1.002236 - 9.02236e^{-6\eta} \\
\text{g} (\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157 \\
C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \\
C_p(0) \\

D_{\text{eff}} = 2.79 \times 10^{-8} \text{ cm}^2/\text{sec} \\
D_{\text{eff}} = 5.59 \times 10^{-8} \text{ cm}^2/\text{sec} \\
D_{\text{eff}} = 9.31 \times 10^{-8} \text{ cm}^2/\text{sec} \]
Figure 121: Effect of $D_{\text{eff}}$ - Nonuniform Diffusivity and Nonuniform Binding - $t = 24$ hr

$K_1 = 1.12 \times 10^{-7}$ cm/sec
$J_f = 0.0084$ ml/cm$^2$-hr
$\epsilon = 0.42$
$R_1 = 1.0$

\[
\frac{\partial \Psi}{\partial \eta} = \frac{K_2}{\eta} (\Psi - \epsilon \frac{C_p(t)}{C_p(0)}) + T_L \Psi + P^\Psi (1-R_2)
\]

$K_2 = 3.8 \times 10^{-7}$ cm/sec
$K_1 = 4.6 \times 10^{-5}$ cm/sec
$R_2 = 0.0$

$k_1 = 1.13 \times 10^{-4}$ sec$^{-1}$
$f(\eta) = 1.002236 - 0.902236e^{-6\eta}$
$g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157$

$\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
Figure 122. Effect of Def - Nonuniform Diffusivity and Nonuniform Binding - t = 64 hr

\[ D_{\text{eff}} = 2.79 \times 10^{-8} \text{ cm}^2/\text{sec} \]

\[ D_{\text{eff}} = 5.39 \times 10^{-8} \text{ cm}^2/\text{sec} \]

\[ D_{\text{eff}} = 9.31 \times 10^{-8} \text{ cm}^2/\text{sec} \]
2.79 x 10^{-8} \text{ cm}^2/\text{sec} and 9.31 x 10^{-8} \text{ cm}^2/\text{sec}. Since the model allows D_{eff} to vary in a manner defined by f(\eta), for the f(\eta) used the diffusivity at \eta = 0 is an order of magnitude lower than at \eta = 1 where the diffusivities quoted above apply. At 0.167 and 0.5 hours the lowest diffusivity results in steeper concentration gradients and a lower net accumulation (Figs. 118 and 119). At four hours and longer, the period during which efflux of labeled solute from the wall occurs, a lower diffusivity is reflected in a decreased clearance rate. Thus, the dimensionless concentrations are higher over all \eta. As far as agreement with the data is concerned, only at four hours is the choice of the best D_{eff} easily made. At the other time intervals, while variation in D_{eff} does affect the theoretical profiles, the experimental data are too scattered to permit saying more than that D_{eff} for LDL is in the range of 2-9 x 10^{-8} \text{ cm}^2/\text{sec}.

Finally, presented on Figs. 123 to 127 is the effect of different binding rates, \theta, and different binding equilibrium ratio, m. As before, the complex boundary condition is used at \theta = 1 with the g(\eta) and f(\eta) functionalities as shown on Fig. 105. \theta was varied in the range 0.07 to 17. Due to computational difficulties, higher values of \theta were not studied. The effect of both \theta and m is similar to the case where D_{eff} was taken to be uniform. Only for large values of \theta (>1), does binding become detectable at 0.167 hours. As one would predict, \theta = 17 steepens the concentration gradient at this time (Fig. 123) as well as at later times. Undoubtedly, this change in the gradient is also attributable to the particular binding distribution g(\eta) used. At four hours and longer, small values of m (relatively slow reverse reaction rate)
Figure 123: Effect of Binding Parameters - Nonuniform Diffusivity ($t = 0.167 \text{ hr}$, $\tau = 0.097$)

$m = 0.4$, $p = 1.0$

$T_1 = 0.0288$, $C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t}$

$\epsilon = 0.42$, $C_p(0)$

$R_1 = 1.0$

$$\left[ p \psi - \frac{\partial \psi}{\partial \eta} = T_2 \left( \psi - \epsilon C_p(t) \right) + T_L \psi + p \psi (1-R_2) \right]_{\eta=1}$$

$T_2 = 0.1$

$T_L = 12.0$

$R_2 = 0.0$

$g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157$

$f(\eta) = 1.002236 - 0.902236e^{-6\eta}$

--- $\theta = 17.0$

--- $\theta = 7.0$

--- $\theta = 0.7$
Figure 124: Effect of Binding Parameters - Nonuniform Diffusivity \( t = 0.5 \text{ hr}, \tau = 0.29 \)

\[
\begin{align*}
T_1 &= 0.0288 \quad T_2 = 0.1 \\
P &= 1.0 \quad T_L = 12.0 \\
\varepsilon &= 0.42 \quad R_2 = 0.0 \\
R_1 &= 1.0 \quad \mu = 0.4 \\
\left[ F_p - \frac{3F_p}{3\eta} = T_2 \left( \Psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + T_L \Psi + F_p(1-R_2) \right]_{\eta=1} \\
C(t) &= 0.33e^{-0.775t} + 0.67e^{-0.041t} \\
\frac{C_p(t)}{C_p(0)} &= 1.002236 - 0.902236e^{-6\eta} \\
g(\eta) &= 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157 \\
\end{align*}
\]
Figure 1.25: Effect of Binding Parameters - Nonuniform Diffusivity ($t=24$ hr, $T = 13.97$)

$$T_1 = 0.0288 \quad T_2 = 0.1$$
$$P = 1.0 \quad \tau_L = 12.0$$
$$\varepsilon = 0.42 \quad R_2 = 0.9$$
$$R_1 = 1.0$$

$$\psi' = \frac{\partial \psi}{\partial n} = T_2 \left( \psi - \varepsilon \frac{C_p(t)}{C_p(0)} \right) + \tau_L \psi + \Omega \psi (1 - R_2) \quad \eta = 1$$

$$f(\eta) = 1.002236 - 0.902236 e^{-5\eta}$$
$$g(\eta) = 1.014 e^{-5\eta} + 1.009 e^{-9(1-\eta)} - 0.01157$$

$$C_p(t) = 0.33 e^{-0.775t} + 0.67 e^{-0.041t}$$

$\eta = x/L$
Figure 126: Effect of Binding Parameters - Nonuniform Diffusivity $t = 4$ hr $\tau = 2.33$

\[ T_1 = 0.0288 \quad T_2 = 0.1 \quad C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

\[ T_L = 12.0 \quad C_p(0) \]

\[ \varepsilon = 0.42 \quad R_2 = 0.0 \]

\[ R_1 = 1.0 \]

\[ \frac{\partial \psi}{\partial \eta} = T_2 (\psi - \varepsilon \frac{C_p(t)}{C_p(0)}) + T_L \psi + \psi (1-R_2) \]

\[ f(\eta) = 1.002236 - 0.902236e^{-6\eta} \]

\[ g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.01157 \]

\[ N = 0.4 \quad \theta = 7.0 \]

\[ N = 0.4 \quad \theta = 0.7 \]

\[ N = 0.4 \quad \theta = 0.07 \]

\[ \eta = x/L \]
Figure 127: Effect of Binding Parameters - Nonuniform Diffusivity
$t = 64 \text{ hr} \quad \tau = 37.2$

$T_1 = 0.0288 \quad T_2 = 0.1$
$P = 1.0 \quad T_L = 12.0$
$\varepsilon = 0.42 \quad R_2 = 0.0$
$R_1 = 1.0$

\[ \frac{P \psi}{\eta} - \frac{3 \psi}{3 \eta} = T_2 (\psi - \varepsilon \frac{C_p(t)}{C_p(0)}) + T_L \psi + P \psi (1 - R_2) \]

$f(\eta) = 1.002236 - 0.902236 e^{-6 \eta}$
$g(\eta) = 1.011e^{-6 \eta} + 1.009e^{-9(1-\eta)} - 0.01157$

$\theta = 0.7 \quad \frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}$
result in solute distributions which preserve the past history of the process (e.g., \( m \approx 0.1 \)). Like all of the other parametric variations presented, there does not appear to be any one combination of \( \Theta \) and \( m \) which are perfectly representative of the experimental data.

To further demonstrate the importance of the assumed binding distribution and the magnitude of \( m \), the mobile solute dimensionless concentration, \( \Psi \), is compared with the total, \( N + \Psi \), at \( t = 4 \) hours (Fig. 128) and at \( t = 24 \) hours (Fig. 129). At both times, the mobile solute concentration is virtually linear. The binding distribution, \( g(\eta) \), provides the curvature observed in the \( N + \Psi \) profiles. The ratio of mobile to total concentrations, \( \Psi/(N + \Psi) \) varies with position and depends on \( m \). At \( \eta = 1 \), \( t = 24 \) hours, and \( m = 0.1 \), \( N/(N + \Psi) \approx 0.1 \) (Fig. 28). Similarly, for \( m = 0.4 \), \( N/(N + \Psi) \approx 0.25 \). These ratios are consistent with equation (6.48) which shows that the effect of binding is to increase \( \Psi \) by the factor \((1 + \frac{1}{m})\) for steady state conditions.

The lower the value of \( m \), the greater the tendency of the profile to mimic the assumed binding distribution, \( g(\eta) \) at long times. Thus, for the \( g(\eta) \) used here, the greatest proportions of bound solute are found near the intima and media-adventitia interface.

The net conclusion to be reached based on the figures presented above is that the experimental data are in accord with the hypothesis that some wall constituent, such as AMPS, or wall property, such as the layered structure, results in a spatially dependent \( D_{\text{eff}} \) for LDL. However, the experimental results for LDL are best used not to confirm or refute the possibility of varying \( D_{\text{eff}} \), but rather to determine the approximate magnitude of the transport parameters involved in the problem.
Figure 128: Distribution of Bound and Mobile Solute - $t=4$ Hours

\begin{align*}
\tau &= 2.33 \\
T_1 &= 0.0288 \\
P &= 1.0 \\
\epsilon &= 0.42 \\
\theta &= 0.7 \\
K_1 &= 1.0 \\
K_2 &= 0.0 \\
T_1^r &= 12.0 \\
T_2^r &= 0.1 \\
\end{align*}

\[
\frac{C_p(t)}{C_p(0)} = 0.33e^{-0.775t} + 0.67e^{-0.041t}
\]

\[
g(\eta) = 1.011e^{-6\eta} + 1.009e^{-9(1-\eta)} - 0.016
\]

\[
f(\eta) = 1.002 - 0.902e^{-6\eta}
\]
Figure 129: Distribution of Bound and Mobile Solute - t=24 Hours

\[ C_p(t) = 0.33e^{-0.775t} + 0.67e^{-0.041t} \]

\[ f(\eta) = 1.002 - 0.902e^{-6\eta} \]

\[ g(\eta) = 1.011e^{6\eta} + 1.009e^{-9(1-\eta)} - 0.0116 \]
d. **Estimated Value for Transendothelial Vesicular Transport**

First, as is seen on Figs. 106 to 113, $T_1 = 0.0288$ fits the data fairly well. Physically, since this $T_1 \ll 1$, the endothelium must play a controlling role in the regulation of LDL transport into the arterial wall. The vesicular transport rate using this $T_1$ and $D_{eff} = 9.31 \times 10^{-8}$ cm$^2$/sec is calculated to be $4.7 \times 10^{-8}$ cm/sec ($=K_1$). Earlier in this section the estimate of $K_1 = 1.02 \times 10^{-7}$ cm/sec was made based on other data in the literature. The degree of agreement between the two numbers is amazing considering the speculative nature of the model proposed here.

e. **Estimated Value for Hydraulic Flux**

The best value of $P$ is found to be of order unity. For the $P = 0.6$, which was used for the curves on Figs. 106 and 107, one calculates the hydraulic flux to be $0.0035$ ml/cm$^2$-hr ($=J_f$). Again this value is in excellent agreement with literature values which are approximately $0.01$ ml/cm$^2$-hr in *in vitro* systems. The other ramification of the magnitude of $P$ is that it suggests that both convection and diffusion are important transport mechanisms for LDL movement across the aortic wall. If the model is truly realistic, then the hydraulic flux is critical in clearing solute from the blood vessel wall, since at true steady state, the diffusive LDL concentration gradient is probably small and clearance most likely is effected mainly by the hydraulic flux.

f. **Estimated Value for Effective LDL Diffusivity**

The other parameters used for the curves shown on Figs. 106 and 107 are also reasonable based on available knowledge. For example, $D_{eff}$ is taken to vary between $9.31 \times 10^{-9}$ cm$^2$/sec near the intima and $9.21 \times 10^{-8}$ cm$^2$/sec near the adventitia. This is quite a wide range, but a
realistic range if one anticipates the intramural LDL diffusivity to be five-to-ten fold lower than in water as is the case for glucose and norepinephrine (see III. INTRODUCTION). The aqueous diffusion coefficient for LDL has been reported to be $2.25 \times 10^{-7}$ cm$^2$/sec (235). Thus, the range used in the calculations on Figs. 106 and 107 is well within expectations. What perhaps is surprising is that the diffusion coefficient is estimated to be this high. The gross appearance of an aorta in cross would tend to convince most that the elastic laminae and general fibrous nature of the tissue would seriously restrict diffusion. However, aortic tissue is 70% water. So, viewed in this context, perhaps it is not so surprising, after all, that the estimated LDL diffusivity range is as high as it is.

g. Lymphatic Removal Rate Constant and Capillary Transport Estimates

The deduced values for the capillary vesicular transport rate ($K_A c / A_m$) and the lymphatic clearance constant ($K_L A_m$) are calculated to be on the order of $1.6 \times 10^{-7}$ cm/sec and $2 \times 10^{-5}$ cm/sec, respectively. The value for $K_A c / A_m$ is consistent with the effective capillaries in the adventitia having approximately 300% greater total surface area ($A_c / A_m = 3.0$) than the luminal endothelium. Earlier it was shown that $A_c / A_m$ could be as much as 12. Although no parametric curves with $T_2$ were presented for the variable binding and diffusivity model, one must acknowledge that $T_2$ can be varied over a wide range with no great effect on the predicted profiles so long as the ratio, $T_2 / T_L$, is of order 0.01. Thus, the model would suggest that the lymphatic clearance rate relative to the capillary input rate is a critical determinant of the
solute concentration in the blood vessel wall. Since no data are available on the lymphatic solute removal rate from aortic tissue, no comparisons of the number estimated here \( \frac{K_{LA}}{A_m} \) with independent estimates are possible. However, one observes that the value, \( 2 \times 10^{-5} \) cm/sec, is two orders of magnitude greater than the capillary vesicular transport coefficient. This suggests that the mechanism for solute transfer into lymphatic terminals may be much faster and more complex than simple vesicular transport.

h. Reaction Rate Estimates

The other parameters, which affect the predicted profiles and which had to be estimated, are the binding constants. For \( \theta = 0.7 \), the forward reaction rate constant \( \left( \frac{C_b}{k_1} \right) \) turns out to be \( 1.1 \times 10^{-4} \) sec\(^{-1} \), for \( D_{eff} = 9.31 \times 10^{-8} \) cm\(^2\)/sec and \( L = 0.024 \) cm. This suggests that the reaction kinetics are quite slow. Intuitively, one might guess that LDL would react faster than it would diffuse if AMPS indeed have a high affinity for LDL. So, perhaps, much larger values of \( \theta \) should have been investigated. However, until one knows how any alleged binding sites are distributed and what experimentally is the order of both \( \theta \) and \( m \), parametric variations are likely to be inconclusive.

6. Analysis of the Experimental Albumin Profiles

The albumin experimental results were only preliminarily compared with theory. However, certain conclusions can be tentatively reached based simply on the theoretical analyses of the LDL studies. First of all, the U-shaped albumin profiles at all time intervals studied suggest that albumin may also interact with wall constituents. And secondly, the higher solute uptake at 10 minutes indicates that the
transendothelial transport rate may be greater for albumin than for LDL. This could be due to an albumin rejection, \( R_l \), which is less than unity (e.g., \( = 0.999 \)), at the endothelial surface. Just as in the case of LDL, a more detailed knowledge of the potential for albumin to bind within the aortic wall is required to permit a thorough quantitative analysis of the experimental results.

D. Summary

A number of theoretical models were developed to aid in the interpretation of the experimental LDL data. The simplest model — solute movement by both convection and diffusion without internal reaction — did not adequately describe the experimental data at all times. As complexities were added to the model, such as the inclusion of spatially dependent binding sites and solute diffusivity coefficient, the theoretical predictions were brought in closer accord with experimental observations. However, the final model involved too many unknown parameters, all of which affected the calculation in some way, to truly optimize the fit without a more complicated multivariate regression analysis. Given the almost total lack of knowledge concerning the appropriate binding and diffusivity distributions, any detailed optimization of the fit cannot be justified at this time. The important ideas to retain from this modelling work are (1) that the experimental data are consistent with the concept of the endothelium regulating the transport of large protein molecules into the blood vessel wall; (2) the transport process is more complex than simple convection and diffusion across an isotropic material; (3) spatially variable reaction and/or solute diffusivity may be important; and (4) convection and diffusion are of relatively equal importance in the overall transport process. Clearly, as more experimental data become available
on AMPS distribution, binding kinetics and mechanism, and the role of lymph clearance, refinements can be made to improve this preliminary model.
The experiments reported here demonstrate that both labeled LDL and albumin enter and accumulate within the aortic wall of rabbits. Both solutes penetrate the entire wall thickness in less than 10 minutes. The intramural distributions suggest that both solutes gain entry through the intima and across the media-adventitia interface. However, the rate of entry and accumulation is different for the two solutes. Labeled LDL accumulation goes through a maximum between one-half and 24 hours. Albumin net accumulation is higher than for LDL at all time intervals but does not go through a maximum. Theoretical analysis shows that the LDL influx and accumulation rates are consistent with the concept that the solute is carried across the endothelium solely by vesicular transport. However, the change in the solute concentration profiles over time cannot be explained by a simple convection and diffusion analysis applied to an isotropic medium. Only if spatially nonuniform binding of LDL to wall constituents is assumed can one bring theory and experiment into reasonable accord.

In the sections that follow, the results of these experiments will be discussed in light of the work of others. Suggestions for future work will be offered where appropriate.

A. Entry of Labeled Albumin and LDL into Arterial Tissue

The results reported here showing the accumulation of both labeled LDL and albumin within the aortic wall are consistent with the observations of others. Scott and Hurley (21) have demonstrated the
entry of radio-iodinated albumin and LDL into the aortas of humans. Duncan, et al. have shown the uptake of labeled albumin by rabbit aortas (231) and by the aortas of dogs (123, 125, 232). They also demonstrated the accumulation of protein labeled LDL in dog aortas (126). More recently, still others have confirmed that rabbit and hog aortas are permeable to albumin (128, 133) and in the rabbit studies, to protein labeled β- and γ-globulins (128). In this latter study, a mixed globulin fraction was labeled. Thus, the results summarized here are the first evidence that labeled β-lipoprotein by itself penetrates the arterial wall of the rabbit. In the light of the previous research, one is not surprised with this finding.

What at first may seem unusual is how rapidly both labeled LDL and labeled albumin distributed throughout the entire thickness of the aorta. Isotope concentrations in the center of the media were significantly above background levels after only 10 minutes. A similar observation was made by Somer and Schwartz (132) who studied the aortic distribution of cholesterol labeled plasma. They, too, found labeled solute in the aorta of hogs only 10 minutes after the administration of the label. However, most of the intra-aortic label was in the form of free cholesterol, which means that the label did not necessarily trace the transport of LDL since LDL free cholesterol could have exchanged with cholesterol in the arterial endothelium. Thus, based on their work, one cannot, without qualification, conclude that LDL does actually penetrate the wall in 10 minutes. On the other hand, Duncan, et al. (231) found significant labeled
albumin uptake by rabbit aorta in as little as two minutes. They assumed that, since the uptake did not change between two and 10 minutes, this radioactivity must represent endothelial surface contamination and not intramural accumulation. Based on the results reported here, this assumption is probably no longer justifiable. While both of the previous studies just mentioned suggest that labeled macrosolute penetration into the arterial wall is very rapid, one may additionally argue on physical grounds alone that the 10 minute profiles observed in this study are reasonable. In the previous chapter theoretical profiles were calculated which indicated that solute with an effective diffusion coefficient of $3.3 \times 10^{-8} \text{cm}^2/\text{sec}$ would penetrate to the center of the aortic media in 10 minutes or less. Certainly, this value is in the realm of the possible for both LDL and albumin. Keller (233) has reported an aqueous albumin diffusivity on the order of $10^{-7} \text{cm}^2/\text{sec}$. Others (234) have suggested slightly higher values. Aqueous LDL diffusion coefficients were found to be $2.25 \times 10^{-7} \text{cm}^2/\text{sec}$ (235). Therefore, even if tortuosity effects increased the effective diffusion path length across the aortic wall such that $D_{\text{eff}}$ was a factor of 10 lower than in aqueous solutions, both the albumin and LDL $D_{\text{eff}}$ would be consistent with the observed 10 minute concentration profiles. Hence, the rapid penetration of the labeled solutes is not inexplicable.

Still, one might argue that the uptake of label does not necessarily mean that labeled solute has penetrated the arterial wall. In all tracer studies, one is tempted to assume that the presence of the label in the tissue is tantamount to proving the presence of the
labeled solute. Strictly speaking, until one has demonstrated that the label remains with the original solute carrier throughout the experiment, no such conclusion is truly justified. Thus far, no investigators have provided proof that the labeled solute which enters the blood vessel wall is the same labeled albumin or LDL which was given to the animal. This study is no exception. Nevertheless, a fairly good case, based mainly on circumstantial evidence, can be made to support the contention that the labeled material which accumulated in the aorta was, at worst, LDL apoprotein and albumin, and, at best, intact LDL.

First of all, the purity of the injected LDL was checked by a number of different methods. Neither immunoelectrophoresis, immunodiffusion, paper electrophoresis, nor gel permeation chromatography indicated the presence of any contaminating material. Secondly, the labeled LDL preparations were resuspended in saline and separated a second time by ultracentrifugation prior to injection into the animals. Any denatured material with an aqueous density greater than 1.050 gm/ml would have been removed by this procedure. Thirdly, the injectates were dialyzed to remove labeled small polypeptides and free radioactive iodine. The injectate radioactivity was, on average, better than 95% precipitable in 10% trichloracetic acid, in the case of LDL, and better than 99% precipitable for albumin preparations. While this small fraction of nonprecipitable radioactivity could possibly account for the uptake of label by the arterial wall, it is highly unlikely. Ultracentrifugal analysis of the labeled rabbit plasma indicated that better than 98% of the precipitable radioactivity
floated in the LDL density range. Moreover, all tissue samples were washed at least twice in TCA before counting. This procedure removed virtually all nonprecipitable radioactivity as evidenced by the fact that the third TCA wash contained, on average, less than 1% of the total nonprecipitable radioactivity removed from the tissue slices. Thus, any nonprotein-bound radioactivity which accumulated in the aorta was most likely removed before the tissue slices were counted. And, since there is no reason to presume preferential uptake of the small (2%) non-LDL radioactive fraction, one may reasonably infer that the labeled solute in the aortic wall was LDL protein.

Unfortunately, the presence of TCA-precipitable label in the tissue slices is not sufficient evidence to prove that intact LDL entered the arterial wall. The LDL could possibly have shed part or all of its lipid constituency in the uptake process. Only double label experiments in which the lipid and protein moieties are separately labeled can resolve this uncertainty. Klimov, et al. (58) recently reported one such experiment. However, the authors were sufficiently vague about their techniques that the reliability of the results may be open to question. They found that the ratio of protein label to lipid label in the rabbit aorta was identical to that in the circulating plasma. However, their paper left unanswered questions concerning the purity of the labeled preparations and whether blood contamination of the aorta was a factor.

In the experiments performed here, attempts were made to extract the labeled solute from the vessel wall and to identify it using immunodiffusion techniques. The low levels of radioactivity in the
tissue and less than optimum isolation procedures probably in part explain why no immunologically reactive LDL could be detected. Smith and Slater (8) have recently devised an immunoelectrophoretic technique for extracting and identifying LDL apoprotein in arterial tissue. While they have not proved that the extractable, immunoreactive material is intact LDL, they have shown that it does contain lipid stainable substances. Thus, the inference can be made that the LDL does not lose all, if any, of its lipid while being transported from the blood plasma into the arterial wall. If their techniques were applied, one might be able to show that the uptake of label is the same as proving the uptake of LDL in these animal studies.

Another possible way of interpreting the uptake of labeled material is in terms of aggregated material. Despite the fact that the LDL injected into the rabbits was purified with a second ultracentrifugation step, aggregated material remained. This aggregated material, which presumably consisted of denatured protein, was removed by filtration through a 0.22μ filter prior to the injection. The results of the in vivo screening experiment suggest that this procedure was sufficient because both screened and unscreened solute accumulated equally fast. Thus, it is highly unlikely that the labeled solute accumulation represented the preferential uptake of aggregated material. In addition, these results tend to refute the possibility that uptake was fast because the labeled solute, in the case of human LDL, was a foreign protein. Moreover, when rabbit LDL was used, the total accumulation and distribution was very similar to the results from the human LDL experiments. Hence, neither species
difference nor aggregation of the labeled material significantly influenced the results.

One factor which may have accounted for the rapid influx of the label was the conscious state of the experimental animals. For the experiments of four hour duration or less, the animals were restrained in a box. Undoubtedly, fright or anxiety may have been inadvertently induced by the procedure. In fact, an occasional surge in blood pressure was qualitatively observed when blood samples were taken. If blood pressure levels were elevated during the experiment, one might anticipate unusually rapid uptake of labeled solute. In the one study performed on an unconscious rabbit, the resulting intramural accumulation was no different than from the average of all conscious rabbit experiments. Although this one comparison is not adequate to prove that fright or anxiety were not factors in the experimental results, it does suggest that the effect of the conscious state, if any, was small.

Still another possible explanation exists for the detection of labeled solute in the center media after only ten minutes. This explanation concerns experimental technique. The argument could be made that the endothelial surface of the tissue was contaminated with highly radioactive plasma, and that during the mounting procedures part of this plasma was displaced into the media. This possibility is unlikely for several reasons. First, the aorta was rinsed twice in a saline jet immediately after it was excised from the animal. Secondly, the endothelial surface was blotted dry just prior to mounting the tissue for sectioning. Thus, the residual amount of
radioactive plasma on the endothelial surface was minimal. Thirdly, if contamination were significant, one would expect the average label concentration in the slice nearest the endothelium to decrease with increasing experimental time, just as the plasma isotope concentrations decay with time. The opposite result was observed. The average dimensionless tissue concentration nearest the intima was higher at each ensuing time interval up to four hours in the LDL studies. And finally, the experiment performed with radioactively labeled red blood cells gave no indication that blood contamination near the intima was a problem. Thus, surface contamination, while it cannot be excluded as a possibility, most likely did not significantly influence the uptake results.

This part of the discussion of the experimental results may be summarized by stating that labeled solute penetrates the aorta of rabbits within ten minutes. In all likelihood, the presence of the label in the wall is representative of the transport of labeled LDL-protein and albumin from the blood plasma. This rapid uptake suggests that changes in plasma protein, and perhaps lipid, concentrations will affect the concentration of these solutes within the arterial wall.

B. Distribution of Labeled LDL and Labeled Albumin

1. Behavior of Concentration Profile Near $\eta = 0$

The concentration gradients near $\eta = 0$ (the intima) at ten minutes for both labeled solutes suggest that solute entered the arterial wall through the intima. These observations are consistent with the experimental results of others reported in the literature.
In the studies of Duncan, et al. (126) on the uptake of protein labeled LDL by canine aorta, the innermost aortic layer contained the highest concentration of isotope of any of the layers at six hours, the earliest time interval monitored. Similarly, Scott and Hurley (21) observed significant amounts of label in the inner aorta with their studies on the uptake of protein labeled LDL and albumin by human aortas. In experiments where the lipid moiety of the lipoprotein was labeled, Adams, et al. (130) in rabbits, and Somer and Schwartz (132), in swine, found a decrease in label concentration away from the intima at the earliest time intervals studied (two hours in the work of Adams et al., and ten minutes in the experiments of Somer and Schwartz). In other work on swine, Bell, et al. (133, 134) noted a steep concentration gradient near $\eta = 0$ for both labeled albumin and labeled fibrinogen at two hours. The only studies possibly inconsistent with the concept of a rapid influx of label across the intima are those of Adams, et al. (128) in which labeled albumin and a protein labeled mixture of $\beta$- and $\gamma$-globulins were used with rabbits. In those experiments the gradient was relatively flat, in the case of the $\beta$-$\gamma$-globulin, or sloping upward toward the adventitia, in the case of albumin. However, these profiles were determined one day after the injection of the labeled material. Thus, they do not necessarily exclude the possibility that a considerable amount of labeled material originally entered through the intima. Assigning the heaviest weight to only those observations made ten minutes after the injection of label, one may safely conclude that labeled solute gains entry into the arterial wall, in part, by
passing from the plasma through the intima.

The rate at which the intimal solute concentration approaches its equilibrium value would appear to be relatively slow. In the LDL experiments, the average dimensionless solute concentration rose from 0.0053 at ten minutes to 0.0111 at four hours. It remained at this level through 24 hours, falling to 0.0027 only after 67 hours. Similar behavior was observed in the albumin experiments up to 24 hours. Both Somer and Schwartz (132) and Adams (130) noted the same slow rise in intimal concentration over times up to 48 hours in their studies with lipid labeled plasma on swine and rabbits. Adams interprets this to mean that cholesterol turns over very slowly in the inner aortic wall. He cites as a supporting reference the work of Scott and Hurley on humans (21) as also showing that the intimal concentration of protein labeled LDL is not at equilibrium with the plasma even after 14 days. Actually, the experiments of Scott and Hurley do not demonstrate this at all. They presented data on the radioactivity found in the human inner aortic wall relative to the terminal plasma isotope concentration as a function of time. A summary of this data is given in Table X. Note that the tissue radioactivity relative to the terminal plasma activity does increase monotonically up to 14 days. However, this is a misleading way to present the data because this monotonic increase could be due not only to increasing tissue radioactivity, but also to decreasing plasma activity. When the decay in plasma activity is taken into account such that tissue activity is expressed relative to the initial plasma isotope concentration, the "relative" tissue radioactivity is
TABLE X

Uptake of Labeled LDL by Human Aorta (21)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>( \psi^* )</th>
<th>( \frac{C_p(t)}{C_p(0)} )</th>
<th>( \psi^{**} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.056</td>
<td>( \approx 1 )</td>
<td>( \approx 0.056 )</td>
</tr>
<tr>
<td>1.1</td>
<td>0.058</td>
<td>0.25 - 0.50</td>
<td>0.0145 - 0.029</td>
</tr>
<tr>
<td>1.7</td>
<td>0.256</td>
<td>0.16 - 0.39</td>
<td>0.041 - 0.100</td>
</tr>
<tr>
<td>1.9</td>
<td>0.08</td>
<td>0.15 - 0.38</td>
<td>0.012 - 0.030</td>
</tr>
<tr>
<td>4.3</td>
<td>0.119</td>
<td>0.047 - 0.17</td>
<td>0.006 - 0.020</td>
</tr>
<tr>
<td>6.9</td>
<td>0.129</td>
<td>0.018 - 0.09</td>
<td>0.002 - 0.012</td>
</tr>
<tr>
<td>9.2</td>
<td>0.199</td>
<td>0.006 - 0.05</td>
<td>0.001 - 0.01</td>
</tr>
<tr>
<td>13.5</td>
<td>0.171</td>
<td>0.0015 - 0.016</td>
<td>0.00 - 0.003</td>
</tr>
<tr>
<td>14.0</td>
<td>0.201</td>
<td>0.0 - 0.015</td>
<td>0.00 - 0.003</td>
</tr>
</tbody>
</table>

* \( (\text{cpm/gm})_{\text{tissue}} / (\text{cpm/ml})_{\text{terminal plasma}} \)

** \( (\text{cpm/gm})_{\text{tissue}} / (\text{cpm/ml})_{\text{initial plasma}} \)
seen to actually decline in time (Table X). Thus, Scott and Hurley's work does not support the contention that the intimal cholesterol, or LDL, concentration takes better than 14 days to equilibrate with the plasma. However, the results presented here and the results of Somer and Schwartz (132) do indicate that a response time does exist, but that it is more on the order of 30 minutes to four hours, rather than 14 days.

Physically speaking, the fact that the intimal concentration does not instantaneously attain an equilibrium value is indicative of the existence of a surface mass transfer resistance. This surface resistance could be either in the fluid phase or at the fluid-wall interface. Studies by Caro, Nerem and coworkers (178, 179) suggest that a fluid phase mass transfer boundary layer resistance is unlikely. However, they did note that labeled albumin uptake was dependent on the local wall shear stress imparted by the fluid phase. This suggests that the uptake mechanism is sensitive to local hemodynamic events. The theoretical analysis presented here in the preceding chapter also sheds new light on the nature of the uptake process. Assuming that transintimal LDL transport were accomplished solely by vesicular transport, one calculates intimal solute concentrations which do increase with time up to approximately four hours. Thus, the slow equilibration time is consistent with the concept that LDL enters the arterial wall mainly by vesicular transport across the endothelium. Exactly how local hemodynamic events, such as an increased shear stress, influence vesicular transport, if at all, is not clear at this time.
The mention of hemodynamically induced variation in the solute uptake rates relates to another aspect of this study, and that is the manner in which the data were reduced and presented. All profile data were grouped and averaged according to spatial position within the arterial wall without regard to whether the profile represented tissue from the arch region or the descending part of the thoracic aorta. Others besides Nerem, Caro, et al. have shown that labeled albumin and labeled LDL uptake varies with position along the aorta (125, 126) of dogs. Since the averaging technique tacitly implies that a true mean isotope concentration exists at each relative depth for a particular time interval, one might be justified in criticizing this method of data analysis in light of the known variation in uptake along the aorta. However, it should be pointed out that only descending thoracic aorta was analyzed in this study. Duncen, et al. found the greatest variation in uptake (4.5 fold) to occur between ascending thoracic aorta and the thoracic aorta nearest the diaphragm. While variation also was found between the most proximal and distal segments of descending thoracic aorta, it was considerably less (1.7 fold) than the difference between the ascending aorta and the distal segment of the descending thoracic aorta (4.5 fold). Moreover, each grand average profile in this study was based on many profiles. With equal likelihood of having the same proportion of upper and lower descending thoracic aortic contributions, the grand average result at each time interval was most likely influenced to the same degree by spatial variation in uptake. Thus, there is justification for the comparison in profile level and shape at the various time intervals.
Unfortunately, the exact location along the aorta from which each profile originated was not always recorded. This prevented a comparison of the profiles from the upper and descending regions. In all future studies this comparison should be made.

Along this same line, Somer and Schwartz (131, 132) and Bell, et al. (133, 134) have found that focal differences exist in labeled solute uptake. Along with others, they have shown that regions where the aortic endothelium is permeable to the azo dye, Evans Blue, are also more permeable to labeled plasma cholesterol, labeled albumin, and labeled fibrinogen. In swine, the aortic arch is the most permeable to Evans Blue, in accord with the observations by Duncan, et al. (125) on the uptake of labeled albumin by dog aorta. However, there also exist isolated regions of higher permeability near the origins of the intercostal arteries along the descending part of the thoracic aorta. For the most part, though, the descending aorta is impermeable to the dye, suggesting that local variation in permeability is important only in the immediate vicinity of the intercostal arteries. In the preparation of the tissues for sectioning in this study, the tissue closest to the intercostal arteries was discarded. So, even if similarly oriented focal regions of high permeability exist along the rabbit aorta as in the hog aorta, their effect on the results of this study would be minimal.

2. Behavior of the Concentration Profiles Near $\eta = 1$

Both the albumin and LDL ten minute concentration profiles are consistent with the hypothesis that solute enters the rabbit aortic wall from the adventitia. The concentration gradients are
positive, indicating that solute diffusion occurs toward the center of the media. Adams (130) observed similar gradients at early times (two hours through 48 hours) in the concentration profiles for lipid labeled plasma also in rabbit aorta. On the other hand, no such gradient exists at early times in the concentration profiles across swine aorta (132) for either lipid labeled plasma or for protein labeled albumin and fibrinogen (133, 134). The latter authors suggest that this discrepancy might be due either to the species difference or to the incomplete removal of the adventitial vasa vasorum in the studies of Adams (133). There are major differences between the anatomy of the rabbit and hog aortas. The rabbit aorta has no vasa vasorum in the media while the hog aorta does. Thus, if Adams failed to completely remove the adventitia from the rabbit aortas before they were sectioned, the steep gradient he found is explicable. As an example of what the profiles look like when adventitial slices are not excluded from the analysis, data from a four hour LDL study (Exp. 145) are presented in Fig.130. Note the abrupt change in dimensionless concentration near the media-adventitia interface in each case. Many of the adventitial slices were noticeably contaminated with blood. Had Adams, et al. not thoroughly removed the adventitia before slicing, considerable error would result. While the gradients in the media are not nearly as steep as those Adams, et al. reported, the results reported here agree with Adams' contention that solute does enter the rabbit aorta from the adventitia. Since in this study tissue slices with discernible blood contamination were excluded from the analysis, it would seem that species differences, rather than
Figure 130  Labeled LDL Distribution Near Media-Adventitia Interface (Exp. 145)

Relative Tissue Concentration, \( y = \frac{C}{C_0(0)} \)

Position, \( x \), Relative to Media-Adventitia Interface (\( \mu \))
differences in technique, explain why the concentration profiles in swine aorta are different from those in the rabbit aorta near the media-adventitia interface. In this respect, it is interesting to note that using swine as the experimental animal represents a better simulation of the human situation because the media of the aorta, like the human aorta, is penetrated by capillaries. But the point to be emphasized here, at any rate, is that the adventitia definitely contributes to the solute accumulation within the rabbit aortic media.

Another interesting aspect of the behavior of the solute concentration near $\eta = 0$ is the change which occurs over time. In the LDL experiments, the level increased monotonically from ten minutes up to four hours. Thereafter, it remained constant. In the albumin experiments, the concentration also increased but it attained a much higher average level than was observed in the LDL studies (0.0125 vs. 0.0046 at 24 hours). Several explanations are possible for this difference in accumulation between albumin and LDL.

First of all, one must consider what mechanisms dictate the concentration of solute in this region. Presumably, a number of factors are at play. For solute to diffuse from the adventitia into the media, it must first pass from the capillaries in the adventitia into the adventitial extravascular space. Studies on muscle capillaries (53) indicate that they are partially permeable to the smaller molecular weight solutes ($<70,000$), and relatively impermeable to the high molecular weight solutes ($>100,000$). In the case of albumin and LDL, one would expect the former to pass from the adventitial capillaries much faster than the latter. Thus, depending on the rate
of clearance, albumin concentrations in the extravascular space of
the adventitia could be much higher than those for LDL. Although very
little is known about how lymphatic vessels are distributed in
relation to the aortic media-adventitia interface, it is probably
safe to assume their existence in this region, and furthermore, to
assume that the majority of the protein which passes from the adventi-
tial capillaries is returned to the general circulation via these
lymphatic channels. Thus, the rate of albumin and LDL clearance from
the extravascular adventitial space is most likely proportional to
the lymphatic removal rate. If the lymphatic removal mechanism is
postulated to be dependent on the movement of bulk fluid as well as
some concentration driving force across the lymphatic wall, then it
is conceivable that the concentrations of labeled albumin and LDL in
the adventitial extravascular space might be considerably different.
If the albumin concentration is higher, the driving force for
diffusion from the adventitia into the aortic media would be greater.
This would explain why on average, isotopic albumin concentrations
near the media-adventitia interface tend to be higher than those for
LDL. More importantly, if the media-adventitia interface presents
very little resistance to mass transfer, one would expect the media
concentrations to equilibrate rapidly with the solute concentrations
in the adventitial extravascular space. This would explain why the
LDL concentration near $\eta = 1$ reached its maximum level in just 30
minutes.

The foregoing reasoning, unfortunately, does not explain why
the LDL average dimensionless concentration near $\eta = 1$ remained close
to 0.0040 from 30 minutes through 67 hours with very little change. Since the plasma level in these isotope experiments was decaying in time, one would expect the tissue isotope concentration near \( n = 1 \) to fall as well if it equilibrated rapidly with the plasma. Why it did not is not at all clear. Perhaps once inside the media, labeled LDL was immobilized by interactions with elastin or acid mucopolysaccharides, for example, so that it was prevented from diffusing or being convected back out at the same rapid rate at which it entered. Alternatively, one might possibly argue that although both the plasma and lymph isotope concentrations were rapidly decaying in time, the lymph concentration decay lagged behind the plasma decay. Such an effect has been noted for labeled albumin in dogs (91). The net effect of this time lag may have been to somehow stabilize the local concentration of labeled solute in the media over the time interval studied. However, because the role of the lymphatic removal mechanism is so poorly understood, any explanations involving the lymphatics are necessarily quite speculative.

Another observation concerning the shape of the LDL profiles near the outer edge of the media worthy of discussion is the fact that the gradients for the average profiles were quite shallow for most every time interval. In contrast, the albumin gradients were steep over the outer 20% of the media. In this respect the LDL data are in conflict with the profiles which Adams found for lipid-labeled plasma penetration into rabbit aorta (130). On the other hand, the albumin data are in qualitative agreement with his albumin experiments. The flat LDL gradients observed here suggest that, if
the profile represents the distribution of diffusible labeled solute, then there is less labeled LDL diffusion from the adventitia than albumin diffusion. Perhaps this is due to higher labeled albumin concentrations in the adventitia. In any event, more albumin label seems to be entering across the media-adventitia interface than LDL. Thus, molecular size may be an important determinant in solute uptake and accumulation at the outer edge of the media.

3. **Concentration Profiles between η = 0 and η = 1**

The shapes of the average LDL and albumin profiles at early times yield qualitative information on the relative importance of transport across the intima and solute entry from the adventitial side of the aorta. At ten minutes for both solutes the label concentration is higher near the intima than near the outer edge of the media. Although in the case of LDL, the difference is small, it would still appear that labeled solute entered the wall more rapidly from the intimal side of the media. Perhaps this is due to the pressure gradient across the aortic wall. Near the intima diffusion, and pressure driven convection, both transport solute toward the adventitia. At the other extreme edge of the media, these two transport mechanisms are opposing each other. Of course, the assumption is made here that both transport mechanisms are operative with neither one dominating the other. An alternate explanation for why more solute might enter through the intima is that the intimal endothelium is initially exposed to a much higher labeled solute concentration than is the opposite side of the media. Thus, the labeled solute driving force would be higher, thereby accounting for the greater
labeled solute uptake. Whatever the explanation, the net effect is the same - the total solute accumulation over the inner one-half of the aorta is higher than over the outer one-half at ten minutes.

At 30 minutes the same observation applies. The shapes of both the average labeled LDL profile and the average labeled albumin profile suggest slightly greater influx through the intima. Note that for both solutes the minimum in dimensionless concentration is found between $\eta = 0.4$ and $\eta = 0.7$. The fact that the range in which this minimum falls is skewed toward the adventitial side perhaps can also be construed as evidence that transintimal entry is the more dominant route for the rabbit aorta.

The profiles at four hours and longer are less informative about the route of influx and more useful in terms of saying something about how the labeled solutes accumulate and are removed from the aortic wall. The LDL four hour profile still maintains the gradient over the inner one-third of the media. The theoretical analysis of the preceding chapter showed that if convection and diffusion were the assumed transport mechanisms, and the aorta were isotropic, then one would expect the distribution at four hours to be close to linear from the intima toward the adventitia. That is, in order to account for the total wall solute penetration at ten minutes, the solute diffusion coefficient must be on the order of $5 \times 10^{-8} \text{cm}^2/\text{sec}$. However, at four hours with this diffusion coefficient, "pseudo" steady state is theoretically attained. (If the plasma isotope concentration were not falling, true steady state would be approximated.) Hence, the profile should be essentially a straight line.
Several reasons can be offered as to why the four hour LDL profile was not linear as the most simplified theory predicts. As was suggested in the theory chapter, the arterial wall may be far from isotropic with respect to its solute transport properties. Secondly, local interactions with elastin, acid mucopolysaccharides, or some other wall component might give rise to the steep gradient in labeled solute concentration near the intima at four hours. Still a third possibility is that the experimental data for $\eta < 0.30$ are unreliable due to either undetectable contamination on the surface of the endothelium or due to errors introduced when correcting data for tissue slices which were of some fractional area of the original. Note that ignoring data in this region does result in a flat, linear solute distribution. Were it not for the fact that the tissues were well rinsed and blotted before slicing, the fact that the differences between the 30 minute profile and four hour profile were statistically significant ($p < 0.05$) in this region, and the fact that the $^{3}\text{H}_2\text{O}$, $^{125}\text{I}$, and 67 hour profiles were flat as one would expect, the third explanation might be the most likely one. However, since it is reasonable to postulate that the arterial wall is an isotropic and that any number of interactions between the labeled solute and constituents in the wall are possible, one is justified in rejecting the argument involving alleged artifacts introduced by experimental technique. Moreover, when an arbitrary binding mechanism is assumed, the experimental data are found to be consistent with theory. Thus, the four hour LDL data suggest that the arterial wall may possess properties which tend to retain high concentrations of LDL near the
intima.

Although no theoretical analysis of the albumin data was presented, the same reasoning applied to the LDL data can be used on the average albumin profile. It is not theoretically possible to account for the observed experimental data without making some assumptions about the solute interaction with wall components and/or the anisotropic nature of the tissue itself. Further experimentation is required to better define these important wall properties.

The change in average LDL profiles from four hours through 67 hours gives an indication as to how labeled solute leaves the arterial wall. So often in this type of study, major emphasis is placed on the origin of the accumulated material with little attention paid to how it is removed. That efflux is occurring is evident from the lower dimensionless concentrations observed at 67 hours compared with four hours over the inner 40% of the media. The average concentrations over the outer 60% did not change during the same interval. This suggests that just as solute enters through the intima more rapidly than from the adventitia, so it leaves the aorta by this route as well. Moreover, if one assumes that a significant portion of the labeled solute in the wall has been immobilized due to interaction with wall constituents, then the failure of the dimensionless concentration to fall in the outer 60% of the wall can be explained. It should be pointed out that of the three 67 hour LDL experiments, one resulted in a flat average profile near $\Psi = 0.0010$, another near $\Psi = 0.0030$, and the third was also quite flat near $\Psi = 0.0050$. Thus, in the one study almost all of the
solute was cleared. Certainly, the binding properties of rabbit aorta may vary from animal to animal. This may be why variation between the three experiments was as wide as it was, but the fact remains that labeled LDL was cleared from the wall.

No decline was noted in the albumin studies, however. One reason might be that the albumin plasma isotope concentration decayed slower than in the LDL experiments. The isotope concentration driving force was, therefore, not as favorable for net efflux. Had the albumin studies been extended to 67 hours, net clearance of labeled solute from the wall might have been observed.

To summarize this discussion on the qualitative information provided by the shapes of the labeled solute profiles, the short time results indicate a rapid influx of solute into the aortic wall, with slightly more coming through the intima than from the adventitia. The four hour profiles are inconsistent with a simple diffusion and convection theoretical analysis, thereby suggesting anisotropy or reaction effects are important. At long times, labeled solute is carried out of the wall. In short, the uptake, retention and efflux of labeled solute by rabbit aorta is a complex, dynamic process requiring more detailed knowledge of the properties of the arterial wall before it can be well understood.

C. Comparison of LDL and Albumin Total Solute Accumulation

Thus far the discussion has been restricted to how labeled LDL and albumin distribute across the aortic wall with increasing time. Very little has been said about the relative amount of the two labeled solutes which accumulated.
The comparison of the average LDL and average albumin profiles at 10 minutes, 30 minutes and four hours indicates that more albumin entered the aorta than LDL relative to their respective initial plasma isotope concentrations. Duncan, et al. made a similar observation when they compared the uptake of lipid labeled plasma and labeled albumin in dogs (124). They found albumin to enter two to four times as fast as the cholesterol labeled lipoproteins, which in the dog, are presumably the α-lipoproteins. Another study where albumin and labeled lipoproteins were studied at the same time was that of Adams, et al. (128). Unfortunately, they did not present their results in a fashion which permits any conclusions about the relative uptake rates of the two solutes. The only other studies, to this writer's knowledge, which compare the transport rates of various solutes across vascular tissue involve capillary permeability studies. Thus, they shed light on endothelial permeability but not on arterial wall transport.

These capillary studies may be of some interest here if one assumes that capillary endothelium and arterial endothelium possess similar transport properties. Since the capillary endothelium is more permeable to solutes with lower molecular weights (53), this might explain why albumin uptake is greater than LDL uptake in the rabbit aorta. That is, the endothelium probably controls the rate of intimal solute uptake. In the case of albumin, some of the solute may diffuse and/or be convected through the intercellular junctions. LDL, on the other hand, most likely gains entry exclusively by vesicular transport, since its molecular size prevents its access to
the intercellular junctions. Thus, any theoretical analysis of albumin uptake, in the fashion described in the preceding chapter, should take into account the possibility of this additional transport mode through the endothelium. More importantly, this probably explains why the albumin penetration into the rabbit thoracic aorta observed in these studies was higher than that of LDL.

D. Experimental Techniques

Throughout the discussion to this point, comparisons have been made between the results of this study and the only other reported work on the detailed distribution of albumin and LDL across aortic wall. Interspersed have been various arguments vouching the reliability of the techniques used in this study. Since poor technique can invalidate the results of any experiment more than any other single factor, a few words are in order about the differences in the techniques employed in the various studies.

First of all, the most important requirement of the experimental techniques used to determine the solute distribution is that they truly represent the distribution which exists under physiological, in vivo conditions. Otherwise, the results are meaningless in terms of assessing how solute enters and accumulates within the blood vessel wall. Short of making measurements inside the living animal, which is obviously impossible in this type of study, the best one can do is to somehow preserve the solute concentration profile as it exists at the time the animal is sacrificed. Emphasis is to be placed on the word preserve because, in dealing with diffusible substances, failure to somehow fix the profile immediately would allow diffusion to
continue even though the aorta is no longer in the animal. The technique used in this study was to freeze the aorta as quickly as possible after its removal from the animal. The assumption was made that no solute could diffuse under these frozen conditions, and hence, the labeled solute concentration profile could be preserved indefinitely. In the studies of Adams, et al. (128 - 130), Somer and Schwartz (132), and Bell, et al. (133, 134) no special precautions were taken to prevent post mortem diffusion of the labeled solute before the tissue was sectioned. This failure to fix the profile casts serious doubts on the reliability of their results in so far as representing actual physiological conditions is concerned. Any gradients for diffusible solutes were most likely lost by the time the tissue was sectioned. Close inspection of their data (see Figs. 6 - 8) reveals that the distributions are fairly uniform except near the borders of the media, regardless of the solute studied. This observation is consistent with the possibility that significant solute diffusion occurred during the preparation of the tissue for sectioning, thereby invalidating their results.

In criticism of the freezing technique of this study, the argument could be made that local surface tension gradients were introduced within the tissue causing displacement of solute as the tissue was being frozen. Such concerns have often been expressed over techniques used to prepare tissues for scanning electron microscopy. However, since aorta is 70% water, and the water is uniformly distributed, there is no good reason to believe that solute would be preferentially concentrated in any particular
region of the aorta during ice crystal formation. The only other conceivable harmful effect of the freezing procedure would be that the labeled protein could possibly lose its label upon subsequent thawing. This is an important possibility because, before counting, all non-protein-bound label was washed from each tissue section using 10% trichloracetic acid. If the freezing and subsequent thawing after the tissue was sliced caused isotopic iodine to be released from the carrier protein, then the measured tissue isotope concentrations would be artifactually low. Presumably, the shape of the profile would not be affected were this possibility real since the release of previously bound isotope should be independent of the position of the slice within the aorta. The assumption was made in this study that no label was released from the carrier protein on account of the freeze-thaw history. A freeze-thaw test performed on an aqueous solution of iodinated LDL did not result in any change in the amount of nonprotein-bound label in the sample. Thus, the freezing technique was thought not to introduce artifact into the experimental procedures.

Mention of the treatment of the samples with 10% TCA before counting brings up another important aspect of the procedures others have used in aortic solute distribution studies. In APPENDIX D results were presented which demonstrated that at 24 hours a significant fraction of the LDL radioactivity found in the tissue slices was not precipitable in 10% TCA. This was true despite the fact that the terminal plasma sample radioactivity was better than 95% TCA-precipitable. The chemical identity of this nonprecipitable labeled
material is not known. However, it definitely was not LDL protein. Otherwise, it would have precipitated. Failure to remove this contaminating labeled material would have certainly compromised the validity of the results. In the studies of Adams, et al. (128 - 130) and Bell, et al. (133, 134) where iodinated proteins were used, no evidence was offered that the radioactivity associated with each tissue sample actually was protein-bound. Adams, et al. (128) state that the "layers...were...counted in 10% formalin by means of a well scintillation counter." Whether this should be taken to mean that all nonprotein-bound radioactivity was removed is not at all clear. Bell, et al. (133) thoroughly rinsed each tissue segment in six changes of saline followed by two changes of 5% KI (w/v). This procedure is disturbing from two standpoints. First, there is no way of knowing that all nonprotein-bound labeled material was removed. Secondly, if one assumes that it was removed, what is to prevent a good deal of the intramural, diffusible labeled albumin from also being rinsed away? Even if only negligible amounts of labeled albumin were cleared, one still has to worry that the distribution within the wall was significantly altered. Therefore, until the above reservations can be dispelled, the solute distributions observed in those studies must be regarded as speculative.

Still a third criticism can be leveled at each of the preceding studies. This concerns the actual slicing technique itself. In both cases, the aortic tissue was mounted with the endothelial surface facing the upward direction. Adams, et al. (128) state that "by adjusting the chuck it was possible to arrange the knife
exactly parallel with the upper surface (inner intima) of the aortic sample." This assumes that the intima surface was perfectly planar, which is unlikely. Even more importantly, taking the length of the aorta across which the microtome knife passed as 1 cm (the authors do not state the size of the tissue which was sectioned), one calculates that a misalignment of $1^\circ$ would result in a tissue thickness of 20$\mu$ where the knife first contacts the tissue and thickness of 194$\mu$ where it completes its cut. To adjust the chuck so perfectly that the angle between the plane described by the intimal surface and the knife cutting plane is significantly less than $1^\circ$ would seem to be a humanly impossible feat. Since the total thickness of the rabbit aorta (not counting the adventitia) is approximately 240$\mu$, the slightest misalignment would result in the first slice having an average thickness considerably different from the subsequent slices. In addition, the labeled solute distribution would apt to be very misleading because the same slice could entail contributions both from the outer media and the inner media. The net distribution, therefore, may in no way reflect the real distribution of solute from the intima to the outer side of the media.

The studies of Somer and Schwartz (132) and Bell, et al. (133, 134) suffer from the same shortcoming. However, since the hog aorta in their studies was between 1100 and 1500$\mu$ thick, the relative error introduced by tissue-knife misalignment is smaller. This is not to say that it is negligible. If the misalignment angle is greater than $1^\circ$, clearly, the error could be as significant as in the rabbit aorta studies of Adams, et al. The important point to be made here is
that mounting the tissue with the intima surface upwards, as in the preceding studies, considerably enhances the possibility of introducing significant artifacts into the results.

In this study, the aorta was placed endothelial surface face-down onto a mounting plane which was exactly parallel to the knife cutting plane. The surface onto which the tissue was placed was "planed off" by the knife just prior to mounting the tissue. No adjustments of the chuck or knife were made in the interim. Therefore, perfect alignment was assured. The major shortcoming of this technique was the failure of the tissue to always adhere to the mounting surface as the slicing plane approached the intima. Sometimes, portions of tissue in excess of the nominal cutting thickness of 20μ would be taken. This required that corrections be made for those slices with either a surface area equal to some fraction of the original or a thickness greater than 20μ. As shown in APPENDIX B, these corrections were not thought to introduce gross errors. Certainly, these corrections might have been responsible for some of the scatter in the data for slices taken near the endothelium, but the grand average profiles were probably still a good representation of the actual solute distribution.

In summary of this section on the experimental techniques, the methods used in this study present a marked improvement over the techniques used in earlier experiments reported in the literature. While improvements in the slicing technique are still needed, the experimental methods developed here provide a reliable way to determine the distribution of labeled solute within aortic tissue.
E. Hydraulic Permeability of Rabbit Aorta

The hydraulic permeability measurements of this study are at variance with the results from other investigators. For normal aorta, the flux was found to be relatively pressure insensitive and to range only as high as \(0.0030\) ml/cm\(^2\)-hr. The studies performed by Yamartino (112), also on rabbit aorta, yielded values that did change with increasing pressure. The average flux was approximately \(0.0125\) ml/cm\(^2\)-hr at 100 mm Hg. This four fold difference in results is thought to be attributable to the different techniques used. In this study the aorta was clamped flat in the bottom of a filtration cell. In Yamartino's studies, the aorta was maintained in its native cylindrical configuration. The latter approach would seem to be better based on the results of this study for one basic reason: the tissue was compressed significantly in the filtration cell used in this study. This tissue compression undoubtedly decreased the tissue hydraulic permeability. Thus, aortic hydraulic permeabilities are best measured in systems where compressive forces are absent.

To explain further, significant fluid displacement was observed from the filtration cell chamber in which the tissue had been covered with an impermeable plastic sheet. Since the plastic sheet prevented fluid from passing through the tissue, any fluid which was displaced into the flow meter must have been the result of tissue compaction effects. The tissue was supported by a rigid, porous plastic frit. So the only way to account for the observed fluid flow (the system was leak-tight) is either to assume that the tissue was gradually
pressed into the porous frit, displacing the fluid originally in the frit, or to postulate that fluid was squeezed from the tissue by compactive forces. In all likelihood, both possibilities occurred. At the conclusion of the experiments, the aorta and porous frit were firmly meshed together. This suggests that the tissue was indeed pressed into openings on the surface of the frit. Also, if one compares the total fluid displaced from the tissue which was covered with the plastic sheet, with the total estimated water volume of the tissue, the fact that much of tissue water might have been squeezed out can be appreciated. For example, assume the thickness of the aorta was 0.025 cm. With the filtration surface area equal to $1.01 \text{ cm}^2$ and the weight fraction of tissue water equal to 0.7, the total water content of the tissue is calculated to be 0.0177 ml (assuming a density of 1.0 gm/ml for aortic tissue). As shown in Table XI, the average total displacement due to compaction was $0.0170 \pm 0.0051$ ml. Thus, if all of the fluid displacement were due simply to compaction, there would be no water left in the tissue. The situation would be analogous to wringing a sponge dry. Clearly, the same compactive forces were present about the tissue which was not covered by the plastic sheet. While fluid was able to pass through this tissue, it did so only very slowly due to these compressive effects, which most likely decreased the tissue hydraulic conductance. Therefore, the hydraulic fluxes determined in this study are probably lower than the true physiological values.

For reasons given in the III. INTRODUCTION, Yamartino's results are also likely to be in error. In his studies the endothelium was
TABLE XI
Total Volume of Fluid Displaced From Covered Tissue

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Duration (min)</th>
<th>Volume Displaced (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>230</td>
<td>0.0183</td>
</tr>
<tr>
<td>108a*</td>
<td>302</td>
<td>0.0193</td>
</tr>
<tr>
<td>108b*</td>
<td>302</td>
<td>0.0192</td>
</tr>
<tr>
<td>118+</td>
<td>352</td>
<td>0.0223</td>
</tr>
<tr>
<td>120+</td>
<td>120</td>
<td>0.0154</td>
</tr>
<tr>
<td>121</td>
<td>191</td>
<td>0.0149</td>
</tr>
<tr>
<td>123</td>
<td>457</td>
<td>0.0060</td>
</tr>
<tr>
<td>124</td>
<td>492</td>
<td>0.0208</td>
</tr>
</tbody>
</table>

Average = 0.0170 ± 0.0051

* Both chambers covered in this experiment
+ Atherosclerotic aorta
not intact. Thus, the hydraulic flux estimates are probably on the high side. Hence, one may speculate that the true hydraulic flux across rabbit aorta at physiological pressures lies somewhere between 0.0030 and 0.0125 ml/cm²-hr.

F. The Relative Importance of Diffusion and Convection

The overall purpose of this thesis was to ascertain the relative importance of diffusion and pressure driven convection in the transport of LDL across aortic tissue. The results do not permit any firm conclusions in this respect. Nonetheless, certain new insight into the factors which may affect intramural LDL transport has been provided.

First of all, the theoretical analysis suggests that the effective diffusivity of LDL in rabbit aorta is at least $3.3 \times 10^{-8}$ cm²/sec. This observation is based on the rapid penetration of the labeled solute after but ten minutes. In this analysis the assumption was made that all solute crossed the endothelium solely by vesicular transport. With $D_{\text{eff}}$ lower than the aforementioned value, solute would not reach the center of the media in ten minutes. It is difficult to assign an upper limit on $D_{\text{eff}}$ based solely on these studies. Intuitively, one would anticipate a value no larger than the LDL aqueous diffusivity of $2.25 \times 10^{-7}$ cm²/sec. Realistically, the true LDL $D_{\text{eff}}$ should be smaller than this due to tortuosity effects which increase the effective diffusion path length through the aorta. Others have reported aortic diffusivity values for nor-epinephrine and glucose which are lower than the aqueous values by a factor of five to ten (121, 122). There is no reason not to
believe that the same relative decrease applies to LDL diffusion in the arterial wall as well. This, of course, assumes that the effective diffusivity is the same throughout the wall. These studies do not allow one to exclude the possibility, for example, that $D_{\text{eff}}$ is quite small near the intima and relatively large at the outside edge of the media. Further experimentation is required to more precisely define $D_{\text{eff}}$ for LDL.

As far as the importance of hydraulic convection is concerned, the values measured in vitro are in order of magnitude consistent with the labeled solute profiles predicted by theory which assumes binding of solute to wall components and a nonuniform $D_{\text{eff}}$. The real importance of convection may not be in terms of moving across the intima into the wall, but rather in terms of clearing the solute toward the lymphatic system near the outer edge of the media. Assuming that no LDL crossed the endothelium by convection, then the ratio of the endothelial mass transfer coefficient, $K_1$, to the convective velocity, $J_\ell$, dictates the amount of labeled solute which will accumulate intramurally. A $J_\ell$ of zero would result in theoretical profiles totally at variance with the observed experimental distribution at four hours and longer. Solute accumulation would be much higher than observed experimentally. Thus, these studies strongly suggest the existence of a hydraulic flow across the rabbit aortic wall. The precise value may range as high as 0.0125 ml/cm²·hr, assuming that the in vitro studies of Yamartino (112) set the upper limit.

At first the concept that a higher hydraulic flux is beneficial (in terms of minimizing solute accumulation) may seem at odds with
the widely held belief that high blood pressure promotes the risk of atherosclerosis. It could very well be that hypertension affects the permeability of the endothelium in sufficient degree to offset the enhanced rate of clearance a higher hydraulic flux would otherwise afford. In vitro studies have shown that aortic uptake of albumin is enhanced by a mechanically stretching (236) of the aorta. Whether hypertension has the same effect in vivo has yet to be established. Assuming it were possible in vivo, then the junctions between endothelial cells might be expanded to allow the passage of LDL. This would then enhance the flux into the wall and partially explain the correlation between hypertension and the incidence of atherosclerosis. In short, the theoretical analysis presented here applies only to the case where solute uptake is affected by transendothelial vesicular transport. Circumstances which alter the endothelial permeability must be analyzed with the possibility of convective transport into the blood vessel wall considered.

One of the most interesting results of this study is that the ten minute and 30 minute LDL profiles are consistent with the hypothesis that LDL enters the normal arterial wall solely by vesicular transport. Moreover, if appropriate binding reactions are assumed, the data for all time intervals are in accord with this transendothelial transport mechanism as well. The best theoretical fit of the data is for the endothelial transfer coefficient in the neighborhood of $1.1 \times 10^{-7}$ cm/sec. This corresponds to a vesicular flux of approximately 4 vesicles/sec-$\mu^2$. Shea and Karnovsky (237) predicted vesicular fluxes of approximately 9 vesicles/sec-$\mu^2$ for
capillary endothelium. Certainly, this degree of agreement should
capillary endothelium. Certainly, this degree of agreement should
be considered quite good. More importantly, however, is the magni-
tude of this endothelial mass transfer coefficient. As the theoreti-
cal analysis demonstrated, the influx of solute is very sensitive to
this parameter. In other words, transendothelial transfer is the
rate limiting step for solute uptake by arterial tissue. Factors
which affect either the vesicular transport rate or the integrity
of the endothelium are likely to result in significantly altered
uptake rates.

There is always danger in making any definite conclusions on
what factors affect the distribution of solute across the wall.
Since the aortic wall is like an organ in the sense that it serves
a very specialized function and that it is composed of living cells,
one must avoid the temptation to idealize it simply as an inert,
homogenous medium through which solute diffuses and convects.
Chances are the distribution and composition of components such as
elastin and mucopolysaccharides greatly influence where LDL, for
example, is predisposed to accumulate. This distribution may have
an overriding influence compared with the effect of diffusion and
convection. Thus, to simply make judgments on the relative im-
portance of convection and diffusion certainly might not be the
single most relevant characterization of the aortic wall transport
properties. A detailed knowledge of the wall composition and how it
related to the accumulation of solute is equally important.

Also, to fully appreciate the transport process much more in-
formation about the solute clearance and removal mechanisms is
required. Naturally, certain solutes are catabolized by the living smooth muscle cells in the wall. This is one form of clearance in that the original solute is destroyed in the process. But, of course, the catabolic waste products themselves must be removed by some mechanism as well. So there must be some process wherein the waste products and unreacted solutes are collected and returned to the general circulation. The most logical route would be through the lymph system. Just as alterations to endothelial function could lead to higher influx of solute into the wall, so a decreased clearance rate would result in greater solute accumulations. If the accumulation of cholesterol, for example, is the propagating factor in the atherosclerotic process, the malfunction of the lymphatic system could be the direct cause. The work of Pinkas and Spaet (103) demonstrated abnormal accumulation of the enzyme, horseradish peroxidase, in the abdominal aorta of the rabbit when the adventitia was stripped away from the media. While a number of explanations are possible, the most likely one is that the lymphatic system was destroyed by the experimental manipulation. With no route of egress, the enzyme accumulated in the aortic wall. Hopefully, more studies of this variety will be performed to conclusively demonstrate the precise function of the lymphatic system in aortic transport processes.

Along these same lines, one conceivable explanation for the shape of the albumin profiles up through 24 hours is the existence of a solute removal system in the center of the rabbit aortic media. This would explain why all albumin distributions went through a
minimum in this region. Although the LDL profiles were not nearly so U-shaped as the albumin distributions, they, too, possessed minima near the center of the media. If the exact location of the lymphatic terminals in rabbit aorta were known, this mid-media clearance explanation might be supportable with anatomical evidence. Thus, the extent to which lymphatic terminals are found in the media of the rabbit aorta is another piece of knowledge which is required to properly interpret the results of these solute distribution studies.

Also required to facilitate interpretation of these results is the determination of the maximum possible concentration of labeled solute in the aorta at true equilibrium. The theoretical analysis assumed that the inulin space was totally available for LDL intramural accumulation. This is a reasonable assumption in that inulin is thought to distribute only in the extracellular space. Since most cholesterol accumulation in normal arterial wall is extracellular, one is justified in assuming that labeled LDL can accumulate only in this region as well. However, molecular size considerations may result in the "LDL space" being smaller than the inulin space. In vitro incubation experiments should be conducted to determine if this possibility is real. An alternate approach would be to maintain a constant plasma isotope level by a programmed constant infusion of labeled LDL.

In summary of this section on the importance of diffusion and convection, it must be concluded that, while diffusive mechanisms are most likely important in the intimal solute uptake process and
convective processes important in maintaining sufficient efflux of solute from the wall, equally important, but poorly understood, are the roles solute reaction with wall constituents and the lymphatic system play in dictating the distribution of aortic solutes.

G. Implications of Atherogenesis

In any study which uses experimental animals to model human disease processes the applicability of the results is limited by the degree to which the experimental conditions simulate the human analogue. Certainly, the rabbit aorta has characteristics sufficiently different from the human aorta to render any extrapolation of the results highly speculative. The relative thicknesses of the two vessels, the orientation of the vasa vasorum, the location of lymphatic terminals within the media and the fact that rabbit aorta do not develop spontaneous atherosclerotic lesions are differences which may make extrapolation of the results unwarranted. Nevertheless, there are enough similarities, such as structure and function, to suggest that the transport properties may not be all that different. Therefore, one would expect the endothelium of human aortas to regulate the passage of plasma macrosolutes and microsolutes into the blood vessel wall. Plasma cholesterol concentrations undoubtedly directly influence the concentration of cholesterol in the aorta by affecting the transendothelial transport driving force. Total cholesterol accumulation is probably not only a function of influx allowed by the endothelium but also the retention and clearance properties of the subendothelial and medial parts of the aorta proper. While the results of this study do not prove these speculations,
they do show that macrosolutes do gain entry into the vessel wall quite rapidly thereby lending support to the theory that excessive accumulation of cholesterol, for example, is a propagating factor in the disease atherosclerosis.
VIII. CONCLUSIONS AND RECOMMENDATIONS

A. Conclusions

1. Labeled LDL and labeled albumin enter and accumulate within the rabbit aortic wall.

2. Entry is gained both through the intima and across the media-adventitia interface.

3. The rate of influx is greater for labeled albumin than for labeled LDL, suggesting that the uptake mechanism may be, in part, dependent on molecular size.

4. The influx of labeled LDL is consistent with the concept of transendothelial passage solely by vesicular transport.

5. Solute accumulation and distribution cannot be explained in terms of convection and diffusion of solute across an isotropic medium.

6. The LDL distribution data are in accord with theoretical predictions which assume the existence of spatially non-uniform interaction of LDL with wall components.

7. All in vitro hydraulic flux determinations should be performed in a system which does not allow the tissue to be compacted during the experiment.

B. Recommendations

1. Experiments should be performed to test the validity of the theoretical model involving convection, diffusion and reaction of LDL with wall components. These experiments should include:
a. Studies on the mechanism and kinetics of interactions between LDL and wall components such as elastin and acid mucopolysaccharides.

b. Studies on the intramural distribution of these interacting components in rabbit and human aortas.

c. Studies on the intramural distribution of labeled LDL following mechanical and chemical alteration of endothelial function, such as by embolectomy or challenge with various vasoactive substances.

d. Studies on the intramural distribution of labeled LDL following perturbations in the function of the aortic lymphatic system.

e. **In vitro** experiments to determine the labeled LDL concentration in the aorta which is in equilibrium with the isotope concentration in the bathing medium.

f. **In vitro** studies on the effect of pressure on the rate of movement of labeled LDL across aortic tissue.

g. **In vitro** studies to determine the effective LDL diffusion coefficient in rabbit aortic media.

2. Experiments, using doubly labeled LDL, should be initiated to determine if LDL penetrates the arterial intima as intact LDL.

3. Anatomical studies should be undertaken to determine if lymphatic terminals exist within rabbit aortic media.
IX. APPENDIX

A. Theories of Atherogenesis Relating to the Transport Properties of Arterial Tissue

1. Injury Theories

One school of thought in atherogenesis holds that some kind of endothelial injury, either mechanical or chemical, triggers the development of lesions. These two types of injury will be discussed in the sections that follow.

a. Mechanical Injury

One possible sequence of events has recently been summarized (137). They postulate that local endothelial injury leads to enhanced plasma infiltration. Depending on the severity and nature of the injury, the endothelium is either repaired and normal function returned or outside forces (hypertension, hormonal imbalances, and/or the composition of plasma proteins) dictate incomplete endothelial repair accompanied by smooth muscle cell proliferation and lesion development. The hypothesis that endothelial injury leads to lesions is supported by studies of Bjorherud and Bondjers (138-142). They found that mechanically induced transverse (oriented normal to the direction of blood flow) injuries to the endothelium of rabbits led to intimal thickening and lipid accumulation. On the other hand, longitudinal injuries (coaxial with blood flow) were effectively repaired. Others have shown that traumatized endothelium results in abnormally large lesion development in hypercholesterolemic rabbits (143) and increased vascular permeability to albumin (88, 144).
Similar results have been observed with pigs (145). It is noteworthy that long periods of time, up to seven months (146), are required for endothelial regeneration. Even after repair, abnormalities are sometimes persistent (147). The extreme measures used in these types of studies, i.e., denudation with balloon catheters and microprobes, are obviously quite unphysiological. However, physiological mechanical trauma may be induced over long periods of time by either hypertensive or hemodynamic factors.

i. Hypertension induced injury

Hypertension is an excellent risk factor for atherosclerosis (148). A number of animal studies (125, 149-154) have shown that the accumulation of lipid, fibrinogen, and albumin in the arterial wall is enhanced by elevated blood pressures. Indirect studies on the capillary protein leakage rate in humans also suggest elevated protein flux due to increased pressures (155-157). What mechanism(s) is (are) involved? Some additional animal studies provide insight into this question. Four possible modes of action have been postulated: 1. that hypertension increases wall permeability by opening the endothelial cell junctions, 2. by increasing the vesicular transport flux, 3. by increasing the convective driving force for solute penetration, and/or 4. by altering the biochemistry of the arterial wall in such a way to favor lipid entrapment and accumulation. The supporting evidence for these concepts is provided below.

A few studies have shown that under hypertension endothelial junctions either expand or are destroyed. These studies
have involved electron microscopic evaluation of arterial endothelium (158-159) as well as capillary endothelium (26, 29). Solute flux across capillary endothelium with colloidal carbon (160) and dextran (95) is also enhanced in hypertension allegedly due to stretching of the junctions. In vivo work with dog aortas (117-118, 161-163) has shown that the short-term accumulation of radioactively labeled albumin is invariant with pressure but is highly sensitive to the degree of stretch. While by themselves these in vitro studies do not prove that the opening of endothelial junctions is involved, in combination with the aforementioned in vivo studies, a fairly strong case can be made that this is actually so.

Some other studies have been made, however, which are contradictory. Experiments with dog and pig aortas in vitro (80) did show that LDL uptake varied linearly with applied pressure. Given the long term nature of these experiments, however, it is highly likely that the endothelium was unphysiologically damaged and removed. Thus, the validity of the experimental results is open to question.

The evidence in support of increased vesicular transport induced solely by hypertension is minimal. The renal artery of hypertensive rats was studied (164) and an increase in vacuolization and degeneration of endothelial cells was noted. However, others have found that transport of ferritin, a protein probe which penetrates endothelium exclusively by pinocytosis, is not enhanced under hypertension (26, 164). Thus, more experimental work is required to resolve this particular question.
Some have suggested that hypertension increases the convective driving force thereby causing increased plasma infiltration. The endothelial electron microscopic studies using horseradish peroxidase as a marker could be interpreted in this fashion (165, 29, 26). In vitro studies have demonstrated an increased arterial filtration rate with increasing transmural pressure drops (107, 165). Heart perfusion studies have also indicated that the direction of transport of horseradish peroxidase across the ventricular endocardium is dependent on the direction of the pressure gradient (166). Thus, hypertension may increase the driving force as well as affect the permeability of vascular tissue.

Another possibility is that hypertension adversely affects the metabolic activity of the arterial wall resulting in an altered permeability. Experiments conducted with hypertensive rats have demonstrated an increase in the thickness of the aorta. This change is accompanied by elevated amounts of elastin and collagen (74, 167) which could bind and entrap greater quantities of lipid. Stretching itself has been found to increase the affinity of elastin for serum lipid in vitro (75). That elastin strain is increased during hypertension has been shown (168). Thus, hypertension may act in yet another manner to interfere with arterial homeostasis. Of the four proposed effects of hypertension, the evidence demonstrating increased stress altering endothelial permeability seems the most persuasive.

ii. Hemodynamic Injury

As with hypertension, hemodynamic forces may increase the permeability of the endothelium. Fry, et. al. (162, 169)
determined a yield shear stress of 350-400 dynes/cm$^2$ above which significant endothelial damage was observed in vivo and in vitro in the aorta of dogs. They hypothesized that such shear stress may be physiologically realizable at branch points such as the iliac bifurcation. If so, one would expect elevated vascular permeabilities in these regions and a higher frequency of lesion development. Attempts to determine the actual wall shear stresses attained in the aorta and at the iliac bifurcation have recently been reviewed by Gessner (170). The most pertinent studies (171-174) indicate maximim attainable shear stresses slightly below the yield stress estimates of Fry. However, endothelial fatigue may be a factor. It might be possible, for example, for a 280 dynes/cm$^2$ shear stress acting over a long period of time to produce the same effect that a 400 dynes/cm$^2$ shear stress does over a matter of hours. This contention is supported by findings of regions of increased permeability to cholesterol (131, 132), albumin (133, 175, 176), and fibrinogen (134) in the arteries of pigs and rabbits. As predicted, these regions were often found at branch points (177). That these high permeabilities might be attributable to shear stress effects has also been shown in recent studies (161, 162, 178, 179) performed in vitro and in vivo with dog arteries. The exact nature of the shear-stress induced damages has not been elucidated. However, the sensitivity of the endothelium to changes in shear stress has been demonstrated (180). They found that endothelial cells orient with their major axes parallel to the flow streamlines. Others have found, using electron microscopy, gaps in the endothelial lining at the junction of the iliac artery and the
aorta in swine (181-182). It has also been shown that aortic endothelial cell turnover rates are higher in regions of high permeability (183). Thus, abundant evidence exists to support the hypothesis that elevated shear stresses damage the endothelium, thereby increasing its permeability. Taken with the evidence that damaging the endothelium leads to lesion development, hemodynamic forces are probably a major causal factor in atherogenesis.

b. Chemical Injury

Just as local hemodynamic forces may injure the endothelium so may certain chemical challenges increase endothelial permeability. The sequellae of events leading to lesions is the same as for mechanical injury, i.e., plasma infiltration stimulating elastin and medial degeneration. Different types of chemical injury have been postulated and evidence in their behalf is presented below.

i. Lipid-induced Injury

As with hypertension, serum cholesterol levels are an excellent risk factor for assessing an individual's susceptibility to atherosclerosis (154). Since the early 1900's when Anitschkov (184) discovered that hypercholesterolemic rabbits developed fatty lesions similar to human fatty streaks, high serum cholesterol has been regarded by many as an initiating factor in human atherosclerosis. However, this today remains a very controversial issue. For example, in a recent review (63), high serum cholesterol is postulated to be the result of abnormally high lipase enzyme activity on the surface of endothelium. That is, high cholesterol may be an effect rather than a cause. However, recent electron and light microscopic studies
of endothelium have revealed a possible mechanism whereby serum cholesterol might initiate the vascular changes that result in atherosclerosis (185, 186). These studies suggest that cholesterol stimulates active contraction of endothelial cells and the opening of endothelial junctions to macromolecules such as LDL. These events might explain why the aorta of cholesterol-fed rabbits is more permeable than normal to albumin (197) and why the extent of lesion development in cockerels correlates with serum cholesterol values (188). Others have recently shown, moreover, that the effect of cholesterol acts synergistically with mechanical trauma to produce lesions more rapidly in pigs (145). These results could be interpreted, however, as evidence that cholesterol simply accelerates the atherosclerotic process rather than causing it. Perhaps serum cholesterol levels do not affect the endothelium at all. Given the inability of blood vessel cells to metabolize cholesterol efficiently, the small fraction of the high serum cholesterol that enters the normal wall may represent an overwhelming challenge. The blood vessel wall, in responding to this challenge through smooth muscle cell proliferation and intimal thickening, may become more susceptible to plasma infiltration. Hence, a runaway cycle may develop wherein the arterial wall becomes progressively more permeable (19). Studies on humans have been infrequent, but a reduced mortality due to coronary heart disease among patients on a low cholesterol diet has been noted (189). Hence, while the exact role, if any, which cholesterol plays in atherogenesis is debatable, lowering blood cholesterol levels by drug therapy and diet regulation are logical strategies in the treatment of the disease.
ii. Vasoactive-amine-induced Injury

Another form of chemical injury that may be of physiological significance involves vasoactive agents. Catecholamine compounds are known either to constrict or dilate capillaries (190). If they have similar effects in the larger vessel, it is conceivable that vascular permeability changes could be effected. For example, colloidal carbon (characteristic size 250 Å) uptake in the aorta of mice was significantly greater in the presence of histamine, serotonin and adrenalin (191). Giese observed the same phenomenon in the capillaries of rats which dilated in response to angiotensin II (160). Higher lymph flows, indicating elevated transcapillary hydraulic flux, were found due to histamine stimulation (91). Ionic (calcium and sodium) flux into rabbit and dog aorta is also enhanced by vasoactive amines (192, 193). Hence, under the artificially high catecholamine levels used in these experiments, permeability was enhanced.

More recent work suggests possible mechanisms for this permeability enhancement. In electron microscopy studies, histamine and serotonin challenge resulted in the formation of large 0.1-0.8μ wide gaps between rat muscle capillary endothelial cells (194). Other studies on both rats and rabbits confirm this observation (197-199). These investigators postulate that endothelial cell contraction accounts for the widening of the junction. Some studies were conducted with LDL (99, 199, 200). In all cases LDL transport into the artery was increased in the presence of either angiotensin or epinephrine. It is not clear that open junctions necessarily
account for all of the permeability changes noted. Robertson et. al. (198, 199) found serotonin and angiotensin II to cause a marked increase in the number of vesicles in both endothelial and smooth muscle cells. Hence, increased vesicular transport may be a factor as well. Also, since these vasoactive compounds affect blood pressure, increased LDL uptake due either to dilation of the vessel wall or an increased convective driving force may contribute.

A valid criticism of these studies is that the dose of vasoactive amine required to produce the effect may be unphysiological. Normal levels of angiotensin II in the blood are considerably below those used by Constanides and Robertson (6, 198, 199). However, they argue that local concentration near the endothelial surface may be drastically higher than blood levels due to the formation of platelet thrombi. Platelets contain large quantities of serotonin and inflammatory cationic proteins (201). If all of these substances were released at the endothelial surface, endothelial cell integrity could be affected. Platelet aggregation and deposition are thought to be continually occurring along the vascular wall (202, 203). Hence, high local concentrations of inflammatory compounds may indeed be physiologically realistic. It should be noted that elevated plasma LDL levels tend to render platelets more prone to aggregation in in vitro studies (205). Hence, platelet function and vascular permeability may be altered in hyperbetalipoproteinemia.

Other forms of chemical injury have been shown to affect vascular endothelium. Osmotic, pH, and temperature effects alter vascular permeability (205, 206) as well as enzymes, surfactants (207) and
denatured proteins (208). The physiological significance of these factors is extremely dubious, however.

iii. Role of Platelets in Injury

The role that platelets may play in relation to vascular injury merits further discussion. Over 100 years ago platelet thrombi formation on the endothelial surface was hypothesized to lead to atherosclerosis (209). Recent reviews summarize the evidence to support this hypothesis (210, 211). Local defects in the endothelial lining brought about by hemodynamic forces and/or the natural growth and development of the endothelium are thought to promote thrombus formation (203, 212-214). If conditions are favorable, the thrombus may become permanent, in which case it is thought that endothelial regeneration envelopes the thrombus. Hence, platelets, fibrin, and platelet debris may be incorporated directly into the intima. Given the high lipid and cationic protein content of platelets, a tissue inflammatory response may ensue which increases the permeability of the wall. Plasma infiltration may accentuate the inflammation and fibrous plaque formation may be the end result. Of course, variations in sequence of events are possible. However, the most important idea is that thrombus formation may lead to fibrous lesions. Mural thrombi have been found on the endothelium of normal (202) and atherosclerotic pigs (215). Other studies with pigs have demonstrated the presence of mural thrombi in regions of damaged endothelium and thickened intima (175, 216). One case of platelet deposition in a human atheromatous plaque has been reported (18). Smith and Slater also found fibrin in ostensibly normal intima
of humans. While evidence in support of this theory is the least substantial of all hypotheses, this does not detract from the importance of thrombogenic processes in the final occlusive stages of the disease.

In summary, considerable evidence is available to suggest that some form of injury, chemical and/or mechanical, alters the normal homeostatic function of the blood vessel wall. Changes in the endothelium which result in increased permeability to plasma constituents are a common theme among most of the injury-related hypotheses. Hence, a fundamental knowledge of the factors which determine vascular permeability in both normal and atherosclerotic vessels is a prerequisite to an overall understanding of the atherosclerotic process.

2. Theories Related to the Metabolism of the Blood Vessel Wall—Hypoxia

Thus far, the discussion of atherogenic theories has been restricted to events which may occur at the blood-wall interface. To ignore the possibility of inherent changes in the biochemistry of the blood vessel wall itself is to forget that the disease develops within a living tissue, and, hence, is to risk oversimplification of the disease process. The purpose of the discussion that follows is to place in proper perspective the role that metabolic processes may play in atherogenesis.

The arterial wall should be regarded as an individual organ which requires oxygen for its metabolic processes. Animals placed daily in a hypoxic environment for short periods of time develop
lesions in their arteries within two weeks (217). Abnormally low oxygen tensions in the arterial wall also gives rise to medial necrosis. Kirk (218) has shown that normal oxygen tensions barely provide an adequate diffusional driving force to meet arterial tissue demand. Thus, any circumstances which result in an increase in the diffusion path length (e.g., thrombus formation on the endothelial surface, intimal thickening, etc.) or a reduction in the oxygen diffusion coefficient (e.g., a highly concentrated protein layer on the endothelial surface) (219) may jeopardize the metabolic activity of the central medial cells. For example, anoxia results in a decreased ground substance synthesis rate in rabbit aorta (220). This is a direct reflection of decreased metabolic activity of arterial cells. With depletion of ground substance, the arterial wall becomes more permeable to plasma solutes. This has been demonstrated in capillaries (99, 221). Anoxia has also been shown to stimulate lipid uptake in tissue culture cells (61) and in ligated rabbit arteries (224, 223). It also damages arterial endothelium (205). Given that cells within arterial lesions consume more oxygen than normal (218), one can visualize a sequence of events, starting with an anoxic episode, which causes lesions to develop. Moreover, once the lesion starts to form, the oxygen demand is greater, and the process can, therefore, be self perpetuating. What would constitute an anoxic episode is not clear. However, one cannot discount the possibility that oxygen transport and utilization may be of significance in atherogenesis.
3. Summary of Theories of Atherogenesis

The exact mechanism that initiates the atherosclerotic process is not known. However, under certain artificially induced conditions atherosclerotic-like lesions can be induced in experimental animals. Thus, one can speculate that some form of injury, (chemical or mechanical) or metabolic insufficiency (anoxia) leads to plasma infiltration, local inflammation, and in certain cases, to fibrous plaque formation.
B. Sample Calculations

Sample calculations are given below for both the solute distribution and hydraulic permeability studies. A summary of the detailed experimental results can be found in APPENDIX D. Details of Experimental Results.

1. Solute Distribution Studies

a. Normalized Tissue Radioactivity

The radioactivity in each tissue slice was normalized with respect to the initial plasma isotope concentration as follows:

\[ \psi = \frac{C}{C_p(0)} = \frac{R/(A) (\Delta L_i) (f)}{C_p} \]

(B1)

where:
- \( R \) = net counting rate (counts/min)
- \( A \) = area of tissue slice (cm\(^2\))
- \( \Delta L_i \) = Thickness of tissue slice (cm)
- \( C_p \) = net counts per minute per ml plasma
- \( f \) = area correction factor

The net counts per minute (R) for each sample radioactivity determination was calculated by subtracting the background counting rate, \( R_B \), from the observed sample counting rate, \( R_S \):

\[ R = R_{S,i} - R_{B,i} \]  

(B2)

Each of the 100 tube holders on the gamma-well spectrometer had a slightly different background counting rate. Thus, the subscript \( i \) in Equation (B2) denotes the tube holder number. Both background and sample tubes were counted for 10 minutes. The standard deviation of counting was \( \sqrt{R/10} \).
The area \((A)\) in equation (B1) was determined by weighing the outline of the tissue perimeter traced onto transparent plastic sheets. Knowing the weight of the sheet per \(\text{cm}^2\), and the weight of the plastic tissue outline, the area of the tissue sample was calculated:

\[
A = D \times W
\]

where: 
\(D\) = plastic sheet conversion factor \((\text{cm}^2/\text{gm})\)
\(W\) = weight of plastic tissue outline \((\text{gm})\)

The conversion factor was constant to within 1\% for a given sheet of plastic. The method of outlining the tissue shape onto the plastic was estimated to have introduced a 5\% error.

The thickness \((AL)\) of the tissue section was taken from the setting on the refrigerated microtome. In all studies 20\(\mu\) was used. On average 12.18 slices were recovered between the media-adventitia junction and the endothelial surface. Assuming a 20\(\mu\) thickness per slice, the calculated average tissue thickness was 243.5\(\mu\). This agrees with direct thickness measurements made on arterial cross-sections viewed under a microscope where average tissue thickness was reported as 248 \(\mu\) (112). Thus, the error introduced by assuming a 20\(\mu\) thickness was 1.8\%. Actual variation from slice to slice was determined by counting successive cross sections of a frozen \(^3\text{H}_2\text{O}\) cylinder. The average standard deviation about the mean was 6.2\% The range of thicknesses was up to 19\% deviant from the mean.

These results are summarized later (see APPENDIX D. Details of Experimental Results).

Some slices represented some fraction, \(f\), of the original area. This fraction was estimated visually as the tissue was being sliced.
Estimates for $f$ were judged to be within $\pm 0.05$ of the true value.

Denoting the standard deviation in each determination by $\sigma$, combination of (B1) - (B3) yields:

$$
\psi = \frac{c_T}{c_{\text{init. plasma}}} = \left[ \frac{(R_{S,i} \pm \sigma_{S,i}) - (R_{B,i} \pm \sigma_{B,i})}{(D \pm \sigma_D)(W \pm \sigma_W)(\Delta L \pm \sigma_L)(f \pm \sigma_f)} \right]_{\text{init. plasma}}
$$

The initial plasma isotope concentration $\{c_p(0)\}$ was taken as the average of at least three separate determinations. Background corrections were made for each determination. Thus,

$$
c_{\text{init. plasma}} = \frac{1}{n} \sum_{i=1}^{n} \frac{(R_{S,i} - R_{B,i})}{V_i}
$$

where: $V_i$ is the volume in ml of the plasma sample counted. In most experiments the original plasma sample was first diluted by a factor of 101 before counting. Of this diluted solution, usually 0.1 ml was counted. Thus, the effective plasma dilution factor was $\frac{101}{0.1}$ or 1010. That is, only 1/1010 ml of the original plasma was counted. If two plasma aliquots were diluted 1 : 101 and three aliquots of each dilution were counted, the plasma radioactivity was determined by taking the average of all six determinations:

$$
c_{\text{init. plasma}} = \frac{1}{6} \sum_{i=1}^{6} \frac{(R_{S,i} - R_{0,i})}{V_i}
$$
An example of the use of equations (B4) and (B6) is given below.

Example 1: Calculation of Normalized Tissue Radioactivity (f=1)

Equations: (B4) and (B6)

Data: Experiment 165, profile # 49, slice I, date: 3/2/74

Fractional area, \( f = 1.0 \pm 0.05 \)

Tissue radioactivity, \( R_{S,53} = 157.7 \pm 4.0 \) counts/min

Background radioactivity, \( R_{B,53} = 59.3 \pm 2.4 \) counts/min

Plastic conversion factor, \( D = 65.58 \pm 0.66 \) cm\(^2\)/gm

Plastic tissue outline weight, \( W = 0.0078 \pm 0.0001 \) gm

Slice thickness, \( AL = 0.0020 \pm 0.001 \) cm

<table>
<thead>
<tr>
<th>( i )</th>
<th>Tube Holder No.</th>
<th>( K_{S,i} )</th>
<th>( R_{B,i} )</th>
<th>( V_i ) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>29,636</td>
<td>56.3</td>
<td>1/1010</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>25,595</td>
<td>55.8</td>
<td>1/1010</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>26,002</td>
<td>57.0</td>
<td>1/1010</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>26,896</td>
<td>59.9</td>
<td>1/1010</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>23,262</td>
<td>56.6</td>
<td>1/1010</td>
</tr>
<tr>
<td>6</td>
<td>67</td>
<td>26,564</td>
<td>54.6</td>
<td>1/1010</td>
</tr>
</tbody>
</table>

Calculations: (a) Plasma Isotope Concentration

\[
C_p \pm \sigma_C \quad \text{(Equation B6)}:
\]

\[
C_p \pm \sigma_C = \left( \frac{(R_{S,i} - R_{B,i})}{V_i} \right)_{\text{ave.}} \]

Substituting:

\[
\begin{array}{c|c|c|c|c}
\hline
i & (R_{S,i} - R_{B,i}) & \text{counts/min} \\
\hline
1 & 29,579.7 & " \\
2 & 25,539.2 & " \\
3 & 25,945.0 & " \\
4 & 26,836.1 & " \\
5 & 23,205.4 & " \\
6 & 26,509.4 & " \\
\hline
\text{Ave. \pm S.D.} & 26,268.3 \pm 2,067.0 \text{ counts/min} & \\
\text{\( \div V \) (= \frac{1}{1010} \text{ ml})} & 26,530,980 \pm 2,087,710 \text{ counts/min-ml} \\
\hline
\end{array}
\]
Substituting:
\[
\psi = \left( \frac{(157.7 - 4.0) - (59.3 - 2.4)}{(65.58 - 0.66) (0.0078 - 0.0001) (0.001 - 0.0001) (1 - 0.05)} \right)
\]

\[
\psi = \frac{98.4 \pm 4.7}{27,142.5 \pm 2905.3} = 0.0036 \pm 0.0004
\]

Thus, the normalized tissue concentration was calculated with a standard deviation of 11% in cases where the fractional area \((f)\) was 1. In situations where \(f < 1\), the error associated with \(\psi\) was greater. This is illustrated below.

Example 2: Calculation of Normalized Tissue Radioactivity \((f < 1)\)

Equation: \((B4)\)

Data: Experiment 165, profile #50, slice N; date: 3/3/74

Fractional area, \(f = 0.40 \pm 0.05\)

Tissue radioactivity, \(R_{S,13} = 293.1 \pm 5.4\) counts/min

Background radioactivity, \(R_{B13} = 55.4 \pm 2.4\) counts/min

Plastic Conversion factor, \(D = 65.58 \pm 0.66\) cm²/gm

Plastic tissue outline weight, \(W = 0.0098 \pm 0.0001\) gm

Slice thickness, \(\Delta L = 0.00200 \pm 0.00004\) cm

Initial plasma isotope concentration, \(C_p(0) = 26,530,980 \pm 2,087,710\) counts/min-ml
Calculation:

\[
\psi = \frac{C}{C_p(0)} = \frac{(293.1 \pm 5.4) - (55.4 \pm 2.4)}{(65.58 \pm 0.66)(0.0098 \pm 0.0001)(0.0020 \pm 0.0001)(0.4 \pm 0.05)}
\]

\[
(65.58 \pm 0.66)(0.0098 \pm 0.0001)(0.0020 \pm 0.0001)(0.4 \pm 0.05)
\]

\[
(26,530,980 \pm 2,087,710)
\]

\[
\psi = \frac{(237.7 \pm 5.9)}{(13,640.8 \pm 2,136.1)}
\]

\[
\psi = 0.0174 \pm 0.0028
\]

Hence, with area corrections involved, the relative error is higher, and in this case, equal to 16%.

Most area corrections concerned tissue slices at or near the endothelium. Thus, this partially explains the greater variability of the data in this region of the tissue profile.

The maximum possible error involved with any one slice analysis would occur when one takes into account the possibility that the slice thickness was as much as 19% greater or less than 0.0020 cm.

Using the data from the previous example, we have:

\[
\psi = \frac{C}{C_p(0)} = \frac{(293.1 \pm 5.4) - (55.4 \pm 2.4)}{(65.58 \pm 0.66)(0.0098 \pm 0.0001)(0.0020 \pm 0.0004)(0.4 \pm 0.05)}
\]

\[
(26,530,980 \pm 2,087,710)
\]

\[
\psi = \frac{(237.7 \pm 5.9)}{(13,640.8 \pm 3397.1)}
\]

\[
\psi = 0.0174 \pm 0.0044
\]

Thus, an occasional slice dimensionless concentration could be in error by as much as 25%.
b. **Double Isotope Sample Radioactivity Analysis**

In some experiments two labeled materials were given to the same animal. Either $^{131}$I and $^{125}$I were used together, or, in one experiment, $^{51}$Cr and $^{125}$I isotopes were injected simultaneously. In both cases the subsequent radioactivity analysis of both the tissue slices and the rabbit blood was performed on a dual channel gamma spectrometer. Since each isotope has a characteristic energy spectrum (see APPENDIX C: Calibration of Gamma Counter for Double Isotope Studies), the two channels on the gamma counter could be adjusted such that each isotope decay contributed primarily to only one of the two channels. Thus, the total counts registered in each of the two channels is given by the sum of the contributions of each isotope.

\[
R_1 = \varepsilon_{1,F} a_A + \varepsilon_{1,B} a_B
\]  
\[
R_2 = \varepsilon_{2,A} a_A + \varepsilon_{2,B} a_B
\]

where:  
$R_1$, $R_2$ = net counting rate in channel 1 and 2, respectively (counts/min)  
$a_A$, $a_B$ = total counting rate of isotope A and B, respectively (counts/min)  
$\varepsilon_{i,j}$ = fraction of the $j^{th}$ isotope decay recorded by the $i^{th}$ channel

Combination of (B7a) and (B7b) allows one to calculate the activity of each isotope ($a_A$, $a_B$) knowing all $\varepsilon_{i,j}$. 
As with all radioactivity determinations, the net counting rates, \( R_1 \) and \( R_2 \), were the observed counting rates corrected for background.

\[
R_1 = R_{1\text{-obs}} - R_{1\text{-backgd}}. \quad (B9a)
\]

\[
R_2 = R_{2\text{-obs}} - R_{2\text{-backgd}}. \quad (B9b)
\]

The fractions \((\varepsilon_{i,j})\) of the isotope decay recorded in each of the two channels was determined experimentally with samples containing only one of the two isotopes. Thus, \( a_A \) and \( a_B \) could be calculated using equations (B8) and (B9). Substitution into (B4) and (B6) results in the relative tissue isotope concentration.

Example 3: Dual Isotope Radioactivity Analysis

Equations: (B8), (B9) and (B4)

Data: Experiment 149: \(^{131}\text{I-LDL}, ^{125}\text{I-LDL}\), profile # 850, slice I
Observed counting rates:

channel 1, \( R_{1-obs} = 318.2 \pm 5.6 \) counts/min
channel 2, \( R_{2-obs} = 233.1 \pm 4.8 \) counts/min

Background counting rates:

channel 1, \( R_{1-backgd} = 144.3 \pm 3.8 \) counts/min
channel 2, \( R_{2-backgd} = 80.9 \pm 2.8 \) counts/min

Channel counting rate fractions:

\( ^{131}\text{I} \) in channel 1, \( \epsilon_{1,131} = 1.0 \)
\( ^{131}\text{I} \) in channel 2, \( \epsilon_{2,131} = 0.134 \pm 0.015 \)
\( ^{125}\text{I} \) in channel 1, \( \epsilon_{1,125} = 0.008 \pm 0.003 \)
\( ^{125}\text{I} \) in channel 2, \( \epsilon_{2,125} = 1.0 \)

Plastic conversion factor, \( D = 58.867 \pm 0.59 \) cm\(^2\)/gm

Plastic tissue outline weight, \( W = 0.0143 \pm 0.0001 \) gm

Slice thickness, \( \Delta L = 0.0020 \pm 0.0001 \) cm

Fractional area, \( f = 1.00 \pm 0.05 \)

Initial plasma \( ^{131}\text{I} \) concentration, \( C_{131} = 16,507,460 \pm 825,370 \) counts/min-ml

Initial plasma \( ^{125}\text{I} \) concentration, \( C_{125} = 21,355,940 \pm 1,067,800 \) counts/min-ml

Calculations:

\[ R_1 = (318.2 \pm 5.6) - (144.3 \pm 3.8) \]
\[ R_1 = 173.9 \pm 6.8 \text{ counts/min} \]
\[ R_2 = (233.1 \pm 4.8) - (80.9 \pm 2.8) \]
\[ R_2 = 152.2 \pm 5.6 \text{ counts/min} \]
Calculating the relative tissue isotope concentrations - (B4):

\[
\Psi_{131I} = \left( \frac{C_{131I}}{C_{131I}^{(0)}} \right) = \left( \frac{174.1 \pm 8.1}{(58.867 \pm 0.59)(0.0143 \pm 0.0001)(0.002 \pm 0.0001)(1.00 \pm 0.05)}{16,507,460 \pm 825,370} \right)
\]

\[
\Psi_{131I} = \frac{(172.8 \pm 6.8)}{(27,791.9 \pm 2430.7)} = 0.0062 \pm 0.0006
\]

\[
\Psi_{125I} = \left( \frac{C_{125I}}{C_{125I}^{(0)}} \right) = \left( \frac{129.0 \pm 8.8}{(58.867 \pm 0.59)(0.0143 \pm 0.0001)(0.002 \pm 0.0001)(1.00 \pm 0.05)}{21,355,940 \pm 1.067,800} \right)
\]

\[
\Psi_{125I} = \frac{(129.0 \pm 8.8)}{(35,954.8 \pm 3144.6)} = 0.0036 \pm 0.0004
\]
Hence, as in the single isotope experiments, the relative tissue concentrations were determined to within 11% precision for 
\[ f = 1.00 \pm 0.05 \] in the double isotope studies.

c. Calculating Aortic \( {^{125}}I \) Radioactivity Due to Blood Contamination

In one study \( {^{51}}Cr \)-labeled red blood cells were given with \( {^{125}}I \)-LDL in order to determine the fraction of \( {^{125}}I \) radioactivity in each slice attributable to the presence of blood. The calculation was based on the assumption that the ratio of \( {^{51}}Cr \) to \( {^{125}}I \) radioactivity in the terminal venous blood sample also applied to the blood in adventitial capillaries of the aorta. Thus, knowing the \( {^{51}}Cr \) red cell radioactivity associated with each tissue slice, one could calculate the attendant concentration of blood-borne \( {^{125}}I \) radioactivity. This result taken with the measured total \( {^{125}}I \) radioactivity allowed the calculation of the fraction attributable to blood contamination.

Some of the equations used in the calculation have already been covered. For example, the total \( {^{51}}Cr \) and \( {^{125}}I \) radioactivity was calculated using Equations (B8) and (B9). Since some of the tissue \( {^{51}}Cr \) radioactivity was thought to be the result of "free" plasma \( {^{51}}Cr \) (not bound to red cells) accumulating in the vessel wall, a correction was made to determine just the \( {^{51}}Cr \) associated with red blood cells.

\[
\left( a_{^{51}Cr-RBC} \right)_T = \left( a_{^{51}Cr-TOT} \right)_T - \left( a_{^{51}Cr-free} \right)_T \quad (B10)
\]

where: \( a_{^{51}Cr-RBC} = {^{51}}Cr \) radioactivity associated with red cells in each slice
\[ a^{51}\text{Cr-TOT} = \text{total slice }^{51}\text{Cr radioactivity} \]

\[ a^{51}\text{Cr-free} = \text{slice non-red cell }^{51}\text{Cr radioactivity} \]

The "free" \(^{51}\text{Cr}\) radioactivity was estimated by assuming that the equilibrium partition factor between the plasma and the aortic tissue was 0.5.

\[
\left( C_{51\text{Cr-free}}^{\text{TIS}} \right) = (0.5) \left( C_{51\text{Cr-free}}^{\text{plasma}} \right) \quad (B11)
\]

Multiplying by the tissue volume to convert isotope concentration to total slice radioactivity yields:

\[
\left( a^{51}\text{Cr-free} \right)_T = \left( C_{51\text{Cr-free}}^{\text{T}} \right) (A) (\Delta L) \quad (B12)
\]

Defining the ratio of \(^{125}\text{I}\) radioactivity to \(^{51}\text{Cr}\) red cell radioactivity in the terminal blood as \(p = \{ C_{125\text{I}} / C_{51\text{Cr}} \}^{\text{blood}} \), we have for each tissue slice:

\[
F = \frac{a^{51}\text{Cr-tot} - (0.5) (C_{51\text{Cr-free}}) (A) (\Delta L) (p)}{a^{125}\text{I-tot}} \quad (B13)
\]

where \(F\) is the fractional \(^{125}\text{I}\) radioactivity due to the presence of blood. In the example below \(F\) is calculated with and without allowance for the contribution of "free" \(^{51}\text{Cr}\) in each tissue slice.

Example 4: Calculation of Fractional \(^{125}\text{I}\) Radioactivity Attributable to Blood Contamination

Equations: (B8), (B9), and (B13)
Data: Experiment 137, Slice 741Q (x = 100), Date: 8/24/73

Sample Counting Rate, channel 1, \( R_{1-{\text{obs}}} = 150.0 \pm 3.9 \) counts/min
Sample Counting Rate, channel 2, \( R_{2-{\text{obs}}} = 439.1 \pm 6.6 \) counts/min
Background Counting Rate, channel 1, \( R_{1b} = 135.0 \pm 3.7 \) counts/min
Background Counting Rate, channel 2, \( R_{2b} = 101.2 \pm 3.2 \) counts/min

Tissue Plastic Outline Weight, \( W = 0.0145 \pm 0.0001 \) gm

Plastic weight to area conversion factor, \( D = 58.867 \pm 0.59 \, \text{cm}^2/\text{gm} \)

Ratio of \(^{125}\text{I} \) to \(^{51}\text{Cr} \) Radioactivity in terminal blood
\[ p = 0.841 \pm 0.024 \]

Tissue thickness, \( \Delta L_4 = 0.0020 \pm 0.0001 \) cm

Area correction factor, \( f = 1.0 \pm 0.05 \)

Isotope channel overlaps:
\[ \text{fraction} \, ^{51}\text{Cr} \text{ read in channel 2}, \varepsilon_{2-A} = 0.079 \pm 0.003 \]
\[ \text{fraction} \, ^{125}\text{I} \text{ read in channel 1}, \varepsilon_{1-B} = 0.00006 \pm 0.00001 \]

"Free" \(^{51}\text{Cr} \) concentration in plasma, \( C_{^{51}\text{Cr-free}} = 5305 \pm 1131 \)

counts/min-ml

Calculations:

1. Net counting rates- Equation (B9)

\[ R_1 = (150 \pm 3.9) - (135.0 \pm 3.7) = 15.0 \pm 5.4 \text{ counts/min} \]
\[ R_2 = (439.1 \pm 6.6) - (101.2 \pm 3.2) = 337.9 \pm 7.3 \text{ counts/min} \]

2. \(^{125}\text{I} \) and \(^{51}\text{Cr} \) activity- Equation (B8)

\[
\begin{align*}
\begin{array}{ccc}
a_{^{51}\text{Cr-tot}} &=& \begin{bmatrix}
15.0 \pm 5.4 & 0.00006 \pm 0.00001 \\
337.9 \pm 7.3 & 1.0 \\
1.0 & 0.00006 \pm 0.00001 \\
0.079 \pm 0.003 & 1.0
\end{bmatrix}
\end{array}
\end{align*}
\]
\[ a_{125}^{151}\text{Cr-tot} = \frac{(15.0 \pm 5.4) - (0.841 \pm 0.024)}{(15.0 \pm 5.4) - (0.841 \pm 0.024)} = 15.0 \pm 5.4 \text{ counts/min} \]

\[ a_{125}^{151}\text{I-tot} = \frac{1.0 \pm 8.1 \times 10^{-7}}{1.0 \pm 8.1 \times 10^{-7}} \]

\[ a_{125}^{151}\text{I-tot} = \frac{(337.9 \pm 7.3) - (1.2 \pm 0.4)}{(1.0 \pm 8.1 \times 10^{-7})} \]

\[ a_{125}^{151}\text{I-tot} = \frac{(336.7 \pm 7.3)}{(1.0 \pm 8.1 \times 10^{-7})} = 336.7 \pm 7.3 \text{ counts/min} \]

3. Fraction of $^{125}$I Radioactivity Attributable to Blood Contamination

(a) No allowance for $^{51}$Cr free in plasma - [Equation (B13)]

\[ C_{^{51}\text{Cr-free}} = 0 \]

\[ F = \frac{(15.0 \pm 5.4) (0.841 \pm 0.024)}{336.7 \pm 7.3} \]

\[ F = \frac{(12.6 \pm 4.6)}{336.7 \pm 7.3} \]

\[ F = 0.037 \pm 0.014 \]
(b) Correcting for "free" $^{51}$Cr in plasma - Equation (B13)

$$F = \frac{(15.0 \pm 5.4) - (0.5) (5305 \pm 1131) (0.0145 \pm 0.0001) (58.867 \pm 0.59) \times 
\times (0.002 \pm 0.0001) (1.0 \pm 0.05)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (0.5) (5305 \pm 1131) (0.0145 \pm 0.0001) (58.867 \pm 0.59) \times 
\times (0.002 \pm 0.0001) (1.0 \pm 0.05)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (0.5) (5305 \pm 1131) (0.0145 \pm 0.0001) (58.867 \pm 0.59) \times 
\times (0.002 \pm 0.0001) (1.0 \pm 0.05)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (4.5 \pm 1.0)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (4.5 \pm 1.0)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (4.5 \pm 1.0)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (4.5 \pm 1.0)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (4.5 \pm 1.0)}{(0.841 \pm 0.024)}$$

$$F = \frac{(15.0 \pm 5.4) - (4.5 \pm 1.0)}{(0.841 \pm 0.024)}$$

Hence, correcting for "free" $^{51}$Cr lowered F 22%. Because of the low $^{51}$Cr counting rates, the relative error associated with F was quite large. In this case it was 48%.

d. Calculation of Tissue Slice Relative Position

Each tissue slice was assigned a dimensionless depth depending on its position relative to the endothelium and the media-adventitia junction.

$$\eta_i = \frac{x_i}{L} \quad \text{(B14)}$$

where: $x_i$ = distance of slice midpoint from the endothelium ($\mu$)

$L$ = total thickness of media plus intima ($\mu$)

$\eta_i$ = dimensionless depth
As described in IV. APPARATUS AND PROCEDURES, tissue slices were collected starting just outside the media (in the adventitia) and proceeding in 20μ increments across the media until the endothelial surface was reached. The transition from adventitia into the media was fairly abrupt as assessed by visual inspection of each section as it was sliced. Sometimes the nth slice would be totally adventitia and the n + 1 slice totally media. More often than not, however, the transition occurred more gradually over 2-3 successive slices. In any event the total thickness of the media plus intima was inferred from the number of slices which represented only medial tissue. Mathematically, the total thickness was determined as follows:

\[ L = (N_m) (\Delta L_i) \]  

where:  
\( N_m \) = total number of media slices  
\( \Delta L_i \) = thickness of each slice (μ)  
\( \Delta L_i \) was taken as 20μ and \( N_m \) determined directly from the slicing data.

The midpoint of each slice (\( x_i \)) was calculated using the following relationship:

\[ x_i = N_i \Delta L_i - \Delta L_i (N_i - \frac{1}{2}) \]  

where:  
\( N_i \) = the slice number (the endothelial slice = \( N_m \))

Combination of (B10) - (B12) yields:

\[ \eta_i = 1 - \frac{(N_i - \frac{1}{2})}{N_m} \]  

An example of the application of equation (B17) is as follows:
Example 5: Calculation of Dimensionless Depth

Equation: \((B17)\)

Data: Experiment 165, profile # 49, date: 3/2/74

Total number of media slices, \(N_m = 13\)

Calculated values: for \(N_i = 1, \eta_i = 1 - (1 - \frac{1}{2}) = 0.962\)

\[
\begin{array}{c|c}
N_i & \eta_i \text{ (calculated)} \\
\hline
1 & 0.962 \\
2 & 0.885 \\
3 & 0.808 \\
4 & 0.731 \\
5 & 0.654 \\
6 & 0.577 \\
7 & 0.500 \\
8 & 0.423 \\
9 & 0.346 \\
10 & 0.269 \\
11 & 0.192 \\
12 & 0.115 \\
13 & 0.038 \\
\end{array}
\]

Thus, each tissue slice dimensionless concentration, calculated as in section A (see above), was associated with a particular dimensionless depth value between 0 and 1.

\textbf{e. Averaging the Individual Tissue Profiles}

In order to characterize the overall distribution of labeled solute in any one experiment, the relative tissue concentrations \(\left( \frac{C(C_0)}{C_p} \right)\) were grouped according to their particular dimensionless depth, \(\eta_i\). The range of the groupings is presented in Table B. I. The smallest group range was at the two extremes of \(\eta\) 0.0 - 0.05 and 0.951 - 1.0. The other nine groupings accounted for data over a range of \(\Delta \eta = 0.1\). These range sizes were selected on the basis that they preserved the shapes of each individual profile.
TABLE B.I.

Range of Dimensionless Depths Used For Grouping Relative Tissue Concentration Data

<table>
<thead>
<tr>
<th>η (Range)</th>
<th>Group No. (j)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 - 0.049</td>
<td>1</td>
</tr>
<tr>
<td>0.050 - 0.149</td>
<td>2</td>
</tr>
<tr>
<td>0.150 - 0.249</td>
<td>3</td>
</tr>
<tr>
<td>0.250 - 0.349</td>
<td>4</td>
</tr>
<tr>
<td>0.350 - 0.449</td>
<td>5</td>
</tr>
<tr>
<td>0.450 - 0.549</td>
<td>6</td>
</tr>
<tr>
<td>0.550 - 0.649</td>
<td>7</td>
</tr>
<tr>
<td>0.650 - 0.749</td>
<td>8</td>
</tr>
<tr>
<td>0.750 - 0.849</td>
<td>9</td>
</tr>
<tr>
<td>0.850 - 0.949</td>
<td>10</td>
</tr>
<tr>
<td>0.950 - 1.000</td>
<td>11</td>
</tr>
</tbody>
</table>
For each experiment an average relative tissue concentration and average relative depth was calculated for each group. The group-averaged profile was taken as the average profile for a given experiment. A detailed example is given below:

Example 6: Calculation of Average Experimental Profile

Data: see Table D.XII.

Calculations: For $0.45 \leq \eta < 0.55$ ($j = 6$):

\[
\psi_{j=6} = \left( \frac{C_{p}(0)}{C_{p}(0)} \right)_{ave,j=6} = \left( \frac{1}{13} \right) \left( 0.0019 + 0.0017 + 0.0015 + 0.0010 + 0.0011 + 0.0015 + 0.0019 + 0.0037 + 0.0063 + 0.0020 + 0.0042 + 0.0036 + 0.0037 \right) \pm \sigma
\]

\[
\psi_{j=6} = 0.0026 \pm 0.0016
\]

\[
\bar{n}_{j=6} = (0.5 + 0.5 + 0.464 + 0.536 + 0.5 + 0.464 + 0.536 + 0.5 + 0.45 + 0.5 + 0.458 + 0.542 + 0.45) / 13 \pm \sigma
\]

\[
\bar{n}_{j=6} = 0.494 \pm 0.033
\]

Since $\psi_j$ and $\bar{n}_j$ represent averages of average values, the results may also be expressed in terms of the standard error of the means (S.E.M.).

\[
\bar{\psi}_{j=6} \quad \text{S.E.M.} = 0.0026 \pm 0.0004
\]

\[
\bar{n}_{j=6} \quad \text{S.E.M.} = 0.492 \pm 0.009
\]
where S.E.M. are calculated as $\sigma / \sqrt{n}$ for $n = 13$. The average profile over all $\eta$ for Experiment 165 is as tabulated in Table B.II.

f. Averaging All Experimental Profiles for a Given Time

The grand average profile for each of the experimental times studied (i.e., 10 minutes, 30 minutes, 4 hours, 24 hours, and 67 hours) was calculated in exactly the same manner as the average profile for each individual experiment. That is, all individual profiles for a given time were grouped according to the dimensionless depth, $\eta$, and the average relative tissue concentration and depth calculated. This is best illustrated with an example.

Example 7: Calculation of Grand Average Profile

Data: see Table D. XII. - 24 Hour LDL Data

Calculations: For 0.45 - 0.55 (j=6):

$$\bar{\psi}_{tot, j=6} = \left( \frac{C}{C_p(0)} \right)_{tot \ ave, j=6} = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{C}{C_p(0)} \right)_{i, 0.45 \leq \eta_i < 0.55} \pm \sigma$$

$$\bar{\psi}_{tot, j=6} = \left( \frac{1}{(30)} \right) \{ 0.0031 + 0.0020 + 0.0023 + 0.0031 + 0.0055 + 0.0038 + 0.0052 + 0.0056 + 0.0034 + 0.0015 + 0.0010 + 0.0028 + 0.0020 + 0.0022 + 0.0018 + 0.0059 + 0.0053 + 0.0019 + 0.0017 + 0.0015 + 0.0010 + 0.0011 + 0.0015 + 0.0019 + 0.0037 + 0.0063 + 0.0020 + 0.0042 + 0.0036 + 0.0037 \} \pm S.D.$$

$$\bar{\psi}_{tot, j=6} = 0.0030 \pm 0.0016$$
TABLE B.II.

Calculated Average Profile
Experiment 165 - LDL - 24 Hours

<table>
<thead>
<tr>
<th>N</th>
<th>η</th>
<th>S.D.</th>
<th>S.E.M.</th>
<th>$\Psi$</th>
<th>S.D.</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.040</td>
<td>0.007</td>
<td>0.005</td>
<td>0.0117</td>
<td>0.0096</td>
<td>0.0065</td>
</tr>
<tr>
<td>4</td>
<td>0.118</td>
<td>0.012</td>
<td>0.006</td>
<td>0.0066</td>
<td>0.0034</td>
<td>0.0017</td>
</tr>
<tr>
<td>9</td>
<td>0.196</td>
<td>0.022</td>
<td>0.007</td>
<td>0.0089</td>
<td>0.0062</td>
<td>0.0021</td>
</tr>
<tr>
<td>13</td>
<td>0.294</td>
<td>0.038</td>
<td>0.011</td>
<td>0.0049</td>
<td>0.0043</td>
<td>0.0012</td>
</tr>
<tr>
<td>10</td>
<td>0.398</td>
<td>0.028</td>
<td>0.009</td>
<td>0.0041</td>
<td>0.0024</td>
<td>0.0007</td>
</tr>
<tr>
<td>13</td>
<td>0.492</td>
<td>0.033</td>
<td>0.009</td>
<td>0.0026</td>
<td>0.0016</td>
<td>0.0004</td>
</tr>
<tr>
<td>11</td>
<td>0.587</td>
<td>0.028</td>
<td>0.008</td>
<td>0.0025</td>
<td>0.0013</td>
<td>0.0004</td>
</tr>
<tr>
<td>12</td>
<td>0.687</td>
<td>0.032</td>
<td>0.009</td>
<td>0.0024</td>
<td>0.0013</td>
<td>0.0004</td>
</tr>
<tr>
<td>8</td>
<td>0.779</td>
<td>0.027</td>
<td>0.010</td>
<td>0.0038</td>
<td>0.0012</td>
<td>0.0004</td>
</tr>
<tr>
<td>3</td>
<td>0.881</td>
<td>0.006</td>
<td>0.003</td>
<td>0.0057</td>
<td>0.0022</td>
<td>0.0013</td>
</tr>
<tr>
<td>2</td>
<td>0.961</td>
<td>0.000</td>
<td>0.000</td>
<td>0.0066</td>
<td>0.0052</td>
<td>0.0036</td>
</tr>
</tbody>
</table>
As with the individual experiment averages, the means can also be given \( \pm \) S.E.M. For \( 0.45 \leq \eta_i < 0.55 \):

\[
\bar{V}_{\text{tot},j=6} \pm \text{S.E.M.} = 0.0030 \pm 0.0003
\]

\[
\bar{V}_{\text{tot},j=6} \pm \text{S.E.M.} = 0.498 \pm 0.0061
\]

The tabulated averages over all \( \eta \) are presented in Table B.III for 24 hour LDL experiments. Similar tabulations for other tissue intervals can be found in APPENDIX D. Details of Experimental Results.

g. Calculating Average Net Accumulation

To estimate the average total solute accumulation for a given experimental time, the grand average profile was numerically integrated.

\[
\bar{Q}_t = \sum_{i=1}^{11} \bar{V}_{\text{tot},i} \Delta \eta_i
\]

where \( \bar{Q}_t \) = average relative tissue concentration across the vessel wall
<table>
<thead>
<tr>
<th>N</th>
<th>$\eta_{tot}$</th>
<th>S.D.</th>
<th>S.E.M.</th>
<th>$\bar{\psi}_{tot}$</th>
<th>S.D.</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.041</td>
<td>0.004</td>
<td>0.001</td>
<td>0.0119</td>
<td>0.0091</td>
<td>0.0037</td>
</tr>
<tr>
<td>13</td>
<td>0.124</td>
<td>0.010</td>
<td>0.003</td>
<td>0.0062</td>
<td>0.0035</td>
<td>0.0010</td>
</tr>
<tr>
<td>17</td>
<td>0.205</td>
<td>0.019</td>
<td>0.004</td>
<td>0.0059</td>
<td>0.0036</td>
<td>0.0009</td>
</tr>
<tr>
<td>25</td>
<td>0.299</td>
<td>0.030</td>
<td>0.006</td>
<td>0.0045</td>
<td>0.0030</td>
<td>0.0006</td>
</tr>
<tr>
<td>21</td>
<td>0.396</td>
<td>0.023</td>
<td>0.005</td>
<td>0.0040</td>
<td>0.0021</td>
<td>0.0004</td>
</tr>
<tr>
<td>30</td>
<td>0.498</td>
<td>0.034</td>
<td>0.006</td>
<td>0.0030</td>
<td>0.0016</td>
<td>0.0003</td>
</tr>
<tr>
<td>23</td>
<td>0.597</td>
<td>0.025</td>
<td>0.005</td>
<td>0.0029</td>
<td>0.0014</td>
<td>0.0003</td>
</tr>
<tr>
<td>25</td>
<td>0.691</td>
<td>0.026</td>
<td>0.005</td>
<td>0.0031</td>
<td>0.0017</td>
<td>0.0003</td>
</tr>
<tr>
<td>18</td>
<td>0.782</td>
<td>0.019</td>
<td>0.004</td>
<td>0.0038</td>
<td>0.0015</td>
<td>0.0004</td>
</tr>
<tr>
<td>14</td>
<td>0.873</td>
<td>0.008</td>
<td>0.002</td>
<td>0.0048</td>
<td>0.0017</td>
<td>0.0004</td>
</tr>
<tr>
<td>13</td>
<td>0.957</td>
<td>0.003</td>
<td>0.001</td>
<td>0.0046</td>
<td>0.0023</td>
<td>0.0006</td>
</tr>
</tbody>
</table>
A sample calculation of $\overline{Q}_t$ for the 24 hour LDL data follows:

Example 7: Calculation of the Average Net Solute Accumulation

Equation: \((B18)\)

Data: see Table B.III. Grand average of 24 hour LDL Profile Data

Calculations:

\[
\overline{Q}_{24} = \{0.05 (0.0119 \pm 0.0091) + 0.1 (0.0062 \pm 0.0035) + 0.1 x \\
x(0.0059 \pm 0.0036) + 0.1 (0.0045 \pm 0.0030) + 0.1 (0.0040 \pm 0.0021)+ \\
+ 0.1 (0.0030 \pm 0.0016) + 0.1 (0.0029 \pm 0.0014) + 0.1 x \\
x(0.0031 \pm 0.0017) + 0.1 (0.0038 \pm 0.0015) + 0.1 (0.0048 \pm 0.0017)+ \\
+ 0.05 (0.0046 \pm 0.0023)\}
\]

\[
\overline{Q}_{24} = 0.0046 \pm 0.0010
\]

Note that standard deviations, not standard errors of the mean, were used in the foregoing calculation.

2. Hydraulic Permeability Experimental Calculations

The net transmural hydraulic flux across the rabbit aorta was calculated from the steady state permeation rate. As mentioned in IV. APPARATUS AND PROCEDURES, the experiment involved the recording of flow meter readings as a function of time for each particular applied pressure drop. Flow rates were calculated as follows:

\[
J_f = \frac{V_{t + \Delta t} - V_t}{\Delta t}
\]

\((B19)\)

where: $J_f$ = volumetric flux, ml/cm$^2$-hr

$V_t$ = volume displacement at time $t$, ml
\( \Delta t = \) time increment, hours

\( A = \) cross-sectional surface area, cm\(^2\)

Volume displacement measurements were made by following the position of a small air bubble in a precision-bore glass capillary tube filled with Krebs-Ringer-phosphate buffer. The capillary scale could be read to within \( \pm 0.0001 \) ml since the smallest graduation was 0.0002 ml.

The filtration cell surface area \( (A) \) was 1.01 cm\(^2\) as calculated from the design specification (see APPENDIX C: Details of Experimental Apparatus), and time was recorded to the nearest minute with a stop watch.

The net transmural hydraulic flux was taken as the difference between the fluxes observed from the blocked and unblocked filtration chambers

\[
J_{f-net} = J_{f-open} - J_{f-blocked} \quad (820)
\]

This calculation makes the assumption that any displacement noted in the flow meters associated with the parafilm-covered tissue was due solely to tissue compression effects. That is to say, the flux from the non-covered tissue was assumed to be the sum of the compression effect and the transmural passage of fluid. By subtracting the flux due to compression, the true net volumetric flux could be estimated. Since flow meters were placed both on the high and low pressure side of the filtration chambers, two sets of flow rate data were available for the determination of the net flux.
Example 9: **Calculation of Hydraulic Flux**

Equations: (B19) and (B20)

Data: Experiment 118, $\Delta P = 6.3$ in Hg, $A = 1.01 \pm 0.02 \text{ cm}^2$

<table>
<thead>
<tr>
<th>Capillary</th>
<th>$V_1$ (0.002 ml)</th>
<th>$t_1$ (min)</th>
<th>$V_2$ (0.002 ml)</th>
<th>$t_2$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Upper (lu)</td>
<td>11.90 ± 0.05</td>
<td>168</td>
<td>13.07 ± 0.05</td>
<td>194</td>
</tr>
<tr>
<td>Left Lower (ll)</td>
<td>29.45 ± 0.05</td>
<td>168</td>
<td>30.33 ± 0.05</td>
<td>194</td>
</tr>
<tr>
<td>Right Upper (ru)</td>
<td>62.18 ± 0.05</td>
<td>168</td>
<td>63.78 ± 0.05</td>
<td>194</td>
</tr>
<tr>
<td>Right Lower (rl)</td>
<td>79.56 ± 0.05</td>
<td>168</td>
<td>78.27 ± 0.05</td>
<td>194</td>
</tr>
</tbody>
</table>

Calculations:

\[
J_{f-\text{lu}} = \frac{(0.002) \{(13.07 \pm 0.05) - (11.90 \pm 0.05)\}}{\left(\frac{1}{60}\right)(194 - 168)(1.01 \pm 0.02)} = 0.0053 \pm 0.0003 \text{ ml/cm}^2\text{-hr}
\]

\[
J_{f-\text{ll}} = \frac{(0.002) \{(30.33 \pm 0.05) - (29.45 \pm 0.05)\}}{\left(\frac{1}{60}\right)(194 - 168)(1.01 \pm 0.02)} = 0.0041 \pm 0.0002 \text{ ml/cm}^2\text{-hr}
\]

\[
J_{f-\text{ru}} = \frac{(0.002) \{(63.78 \pm 0.05) - (62.18 \pm 0.05)\}}{\left(\frac{1}{60}\right)(194 - 168)(1.01 \pm 0.02)}
\]

Capillary blocked

Capillary open
Net flux is given by equation (B13). Substituting for upstream flow meters:

\[
J_{f-u} = \frac{0.0032 \pm 0.0001}{0.44 \pm 0.01} = 0.0073 \pm 0.0003 \text{ ml/cm}^2\text{-hr}
\]

\[
J_{f-r1} = \frac{(0.002) \left( (78.27 \pm 0.05) - (79.56 \pm 0.05) \right)}{\frac{1}{60} (194 - 168) (1.01 \pm 0.02)} = 0.0020 \pm 0.0004 \text{ ml/cm}^2\text{-hr}
\]

For downstream (lower) flow meters:

\[
J_{f-l} = (0.0059 \pm 0.0003) - (0.0041 \pm 0.0002)
\]

\[
J_{f-l} = 0.0018 \pm 0.0004 \text{ ml/cm}^2\text{-hr}
\]

Note that had the left upper flux (blocked) been subtracted from the right lower flux (open), a different estimate of the transmural flux would have resulted. However, as is shown in V. EXPERIMENTAL RESULTS, when both chambers were blocked during the same experiment, both upper flow meters recorded slightly higher flow rates than either lower flow meter. Hence, it was concluded that in the flux calculation
above, differencing only between either the upper flow meter flux rates or the lower flux rates was valid. Taking the difference between the left upper and right lower would lead to erroneous results.
C. Details of Experimental Apparatus and Procedures

1. Hydraulic Permeability Studies

A detailed drawing of the filtration cell used in the hydraulic permeability studies is shown on Fig. C.1. It consisted of two mating Plexiglas pieces between which was placed the aortic tissue. Clamping rods secured the two pieces together. Viton O-rings were employed as seals for both chambers, and porous plastic frits served to channel the filtrate to the respective chamber outlet ports. Note that two ports existed below the porous frits. The second port was used to purge all air from the frits prior to the start of each experiment. Similarly, the second inlet port allowed one to eliminate all air bubbles from the filtration chambers above the tissue. The entire filtration cell was designed to fit in the base of a stretching apparatus (Fig. C.2.). The stretching apparatus served to return the aorta to its in vivo dimensions prior to clamping inside the cell.

The procedure for loading the filtration cell was as follows:

1. The stretching apparatus was filled with 37°C Krebs-Ringer phosphate buffer (224).

2. The base of the filtration cell was placed in the mating slot in the base of the stretching box, and all air in the porous frits was purged.

3. The aortic tissue, previously trimmed of loose adventitial tissue and cut between the intercostal branches, was secured with ordinary sewing thread (double thickness) at each corner and at the mid-point of each side.
Figure C1a Rabbit Aorta Filtration Cell - Top Half

Figure C1b Rabbit Aorta Filtration Cell - Bottom Half
Figure C.2 Aorta Stretching Rack
(4) The tension on each thread was increased until the previously measured *in vivo* length was restored. The width of the tissue was increased by 140%. Hemostats were used to secure the thread in the screw-eyes on the stretching box wall.

(5) With the stretched tissue centered over the base of the filtration cell, the top half of the cell was lowered to within ~1 cm of the tissue. Depending on the nature of the experiment, either one or both ends of the tissue were covered with single pieces of Parafilm (American Can Company). Visual inspection insured that the Parafilm covered the entire tissue surface exposed to the filtration chamber.

(6) With the Parafilm in place, the upper half of the cell was clamped to the lower half.

(7) The entire filtration cell and stretching box were submerged in a 37°C thermoregulated water bath. (The filtration cell had been prewarmed to 37°C).

(8) All inlet and outlet tubes were connected to the appropriate three-way valves and the filtration chambers were filled with prewarmed Krebs-Ringer phosphate buffer.

(9) The gas (95% O₂, 5% CO₂) -liquid interface was positioned near the inlet of the upstream capillary tube. Similarly, buffer was admitted to the outlet lines so that the menisci would register in the downstream capillary tubes.

(10) The pressure was step-increased to the desired level and flow meter readings taken at three or five minute intervals for 30-40 minutes.
After steady state flow rates were observed for 15-20 minutes, the pressure was changed. Lowest pressures were studied first followed by higher pressures.

After the last experimental pressure run, the cell was disassembled and the tissue surface stained as described in IV.

APPARATUS AND PROCEDURES.

In some experiments pictures were taken of the stained tissue resting on the base of the filtration cell with a Polaroid MP-3 Industrial camera. Tissue sections were also fixed overnight in buffered formation and examined by light microscopy to assess endothelial coverage at the end of the experiment (see IV. APPARATUS AND PROCEDURES).

The hydraulic flux was calculated as described previously (APPENDIX B. Sample Calculations).

The experimental procedures described above produced fairly reproducible results. The system was leak-tested on numerous occasions by substituting a neoprene rubber sheet (1/16 " thick) for the tissue and following the exact same procedures. The major difficulty encountered in the experiments was centering the tissue between the mating parts of the filtration cell. One experiment had to be thrown out because post-experiment inspection of the tissue revealed that not all of the tissue had been sealed with the rubber O-rings. Another problem was the time required to load the tissue into the cell. Better than one hour was generally needed. This long length of time undoubtedly contributed to deterioration of the endothelium. Another aspect of this procedure which one
might want to improve upon in future work is to study the effect of different supporting materials in the base of the filtration cell. As is discussed in V. EXPERIMENTAL RESULTS, the possibility exists that the porous frits were plugged by the tissue, thereby adding a significant resistance to the passage of fluid into the outlet ports. In general, however, the method for measuring the hydraulic permeability of aortic tissue was sufficiently simple that reproducible results were attainable.

2. Double Isotope Radioactivity Analysis

In some experiments, solute uptake studies, both $^{131}$I and $^{125}$I proteins were given to the same animal. The manner in which the sample activity of each isotope was determined is presented in APPENDIX B: Sample Calculations. The gamma spectrometer counting channels were adjusted so that channel 1 recorded primarily $^{131}$I-radiation while channel 2 registered mainly $^{125}$I-radiation. A graphical representation of how the channel discriminators were set is shown on Fig. C.3. Note that the peaks of the two energy spectra are an order of magnitude apart. Thus, one could easily separate the higher energy $^{131}$I radiation from the lower energy $^{125}$I. The spill-over of the two isotope radiations into the alternate channel is presented in Table C.1. Only $13.4 \pm 1.5\%$ of the channel 1 $^{131}$I radiation registered in channel 2, while but $0.8 \pm 0.3\%$ of channel 2 $^{125}$I radiation spilled over into channel 1. These spill-over ratios were determined for each double isotope study in order to compensate for any drift in the calibration between experiments.
Figure C.3

$^{131}$I-Energy Spectrum
( Channel 1 )

Lower Discriminator
Upper Discriminator

0.364 Mev

Energy Scale (Arbitrary Units)

Relative Counting Rate

Figure C.3

$^{125}$I-Energy Spectrum
( Channel 2 )

Lower Discriminator
Upper Discriminator

0.0355 Mev

Energy Scale (Arbitrary Units)

Relative Counting Rate
## TABLE C.I.

Spill-Over of $^{131}$I and $^{125}$I into Alternate Counting Channel

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Channel 1 (cpm)</th>
<th>Channel 2 (cpm)</th>
<th>$^{131}$I-Counting Rate</th>
<th>Channel 1 (cpm)</th>
<th>Channel 2 (cpm)</th>
<th>$^{125}$I-Counting Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,063</td>
<td>330</td>
<td>0.160</td>
<td>46</td>
<td>4,367</td>
<td>0.011</td>
</tr>
<tr>
<td>2</td>
<td>2,056</td>
<td>300</td>
<td>0.146</td>
<td>43</td>
<td>9,231</td>
<td>0.005</td>
</tr>
<tr>
<td>3</td>
<td>4,933</td>
<td>617</td>
<td>0.125</td>
<td>142</td>
<td>17,116</td>
<td>0.008</td>
</tr>
<tr>
<td>4</td>
<td>6,114</td>
<td>764</td>
<td>0.125</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>9,950</td>
<td>1,236</td>
<td>0.124</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>18,942</td>
<td>2,415</td>
<td>0.127</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
D. Details of Experimental Results

The overall experimental results were presented in V. EXPERIMENTAL RESULTS. This section covers the results of each individual experiment. In addition, experiments performed to validate experimental procedures are also discussed.

1. Solute Distribution Studies

Presented in the following sections are: (1) Validation of experimental procedures, (2) Results from individual experiments, (3) Results from special experiments, and (4) Results from special analysis.

a. Validation of Experimental Procedures

1. Distribution of $^3$H$_2$O

As mentioned previously (IV. APPARATUS AND PROCEDURES), the distribution of labeled solute across the aorta was determined by counting the radioactivity in successive sections of tissue prepared with a refrigerated microtome. Preliminary experiments were conducted to validate the reproducibility of this slicing technique. This was done by determining the radioactivity in successive sections of a homogeneous frozen cylinder of water ($^3$H$_2$O). Sections were cut at 20µ just as in all subsequent tissue sectioning. Thus, any variation in radioactivity between sections was assumed to be due to variation in the thickness of each section or to losses incurred during the transfer of the section from the cutting knife to the counting vial. The frozen cylinder of ice was mounted so as to ensure that each section had an identical cross sectional area ($\sim 0.5$ cm$^2$). The results are presented in Table D.I. All the data fill in a fairly
TABLE D.I.

Reproducibility of Slicing Technique
Successive 20\(\mu\) Sections of \(\text{\textsuperscript{3}}\text{H}_2\text{O}\) at \(-10.9^\circ\text{C}\)

<table>
<thead>
<tr>
<th>Section Number</th>
<th>Counting Rate (Counts/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27,041</td>
</tr>
<tr>
<td>2</td>
<td>26,846</td>
</tr>
<tr>
<td>3</td>
<td>25,563</td>
</tr>
<tr>
<td>4</td>
<td>27,348</td>
</tr>
<tr>
<td>5</td>
<td>27,286</td>
</tr>
<tr>
<td>6</td>
<td>26,991</td>
</tr>
<tr>
<td>7</td>
<td>26,610</td>
</tr>
<tr>
<td>8</td>
<td>23,462</td>
</tr>
<tr>
<td>9</td>
<td>24,608</td>
</tr>
<tr>
<td>10</td>
<td>24,540</td>
</tr>
<tr>
<td>11</td>
<td>25,738</td>
</tr>
<tr>
<td>12</td>
<td>25,926</td>
</tr>
<tr>
<td>13</td>
<td>26,638</td>
</tr>
<tr>
<td>14</td>
<td>28,054</td>
</tr>
<tr>
<td>15</td>
<td>27,447</td>
</tr>
<tr>
<td>16</td>
<td>20,787</td>
</tr>
<tr>
<td>17</td>
<td>25,823</td>
</tr>
<tr>
<td>18</td>
<td>24,796</td>
</tr>
<tr>
<td>19</td>
<td>26,962</td>
</tr>
<tr>
<td>20</td>
<td>23,982</td>
</tr>
<tr>
<td>21</td>
<td>25,544</td>
</tr>
<tr>
<td>22</td>
<td>25,156</td>
</tr>
<tr>
<td>23</td>
<td>25,864</td>
</tr>
<tr>
<td>24</td>
<td>26,084</td>
</tr>
</tbody>
</table>

Mean ± S.D. 29,725 ± 1591 counts/min

Mean ± %S.D. 25,725 ± 6.2% counts/min

Range 20,787 - 28,054 counts/min

Radioactivity determinations were made on a liquid scintillation spectrometer (Packard Instrument Company, model #3585). Instagel (Packard Instrument Company, Downers Grove, Illinois) was used as the scintillation medium.
narrow range with the standard deviation about the mean equal to 6.2%. Note that one slice (16) contained 19% fewer counts than the average. While such wide variation was infrequent, an occasional deviant slice like this would introduce larger errors in the data analysis. However, as is shown in APPENDIX B: Sample Calculations, even a 19% error in the tissue slice thickness results in only a 25% error in the calculated dimensionless tissue isotope concentration. This compares with a 16% error if the tissue slice thickness is taken to vary 6.2%. Hence, the occasional slice which deviated from the mean thickness by more than 6% would not lead to a grossly different dimensionless tissue concentration.

To determine the effect of slicing aortic tissue as opposed to simply crystalline water, small pieces (< 1 cm²) of rabbit aorta were incubated for three and nine hours in ³H₂O physiological phosphate buffer (Krebs-Ringer) fortified with glucose (100 mg/100ml) at 37°C. Mixing was achieved by bubbling gas (95% O₂; 5% CO₂) through the medium. The tissues were sectioned, counted, and the volume fraction of buffer ³H₂O found in each tissue section at the end of the two incubation times was calculated. The individual profile results are presented as a function of position within the aortic wall both in graphical (Fig. D.1.) and tabular form (Table D. II.). Within any one profile, there was considerable variation in the relative concentration of ³H₂O across the media as evidenced by the jaggedness of the curve. In general, the jaggedness was diminished in adventitial slices. Also note that the relative concentrations were higher in the media than in the adventitia. When the values
Figure D.1 Distribution of $^3$H$_2$O Across Rabbit Thoracic Aorta in Vitro

<table>
<thead>
<tr>
<th>Tissue No</th>
<th>Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>417</td>
<td>15 min.</td>
</tr>
<tr>
<td>421</td>
<td>3 hr.</td>
</tr>
<tr>
<td>422</td>
<td>3 hr.</td>
</tr>
<tr>
<td>423</td>
<td>3 hr.</td>
</tr>
<tr>
<td>408</td>
<td>9 hr.</td>
</tr>
<tr>
<td>411</td>
<td>9 hr.</td>
</tr>
</tbody>
</table>

Relative Tissue Concentration $C/C_{buffer}$

Distance, $\mu$m

Intima

Media-Adventitia Junction

422
--

~

-

-

-

-

416

TABLE
Volume Fraction of

Depth (P)
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
620
640
660
680
700
720
740
760
780
800
Note:

#421
(3)
0.5084
0.6261
0.5816
0.5291
0.5267
0.5624
0.4801
0.5397
0.5189
0.5908
0.5255
0.8802
0.9252
0.3733
0.4909
0.4666
0.2773
0.3615
0.2580
0.3445

0.2666
0.2761
0.2564
0.2542
0.2376
0.2352
0.2202
0.2158
0.2196
0.2144
0.2238
0.2320
0.1836
0.2172
0.2205
0.2187
0.2154

Experiment 127
#423
#422
(3)
(3)
0.2371
0.3167
0.3261
0.8136
0.4281
0.4278
0.4941
0.4583
0.5608
0.5089
0.7056
0.4138
0.4689
0.3374
0.3389
0.3393

0.2561
0.1767
0.1801
0.1442
0.1790

0.1332
0.1256
0.1203
0.1174

0.3146
0.3583
0.4356
0.4209
0.3584
0.6209
0.4604
0.4637
0.4315
0.4295
0.4773
0.4481
0.4250
0.6407
0.4953
0.5279
0.4512
0.4929
0.3710
0.3947

0.2601
0.2679
0.2923

3

H 2 0 Found in Aortic

#417
(0.25)
1.0147
0.6629
0.6548
0.6150
0.6077
0.5887
0.5891
0.6969
0.6730
0.6588
0.6553
0.6742
0.5694
0.6172
0.6237
0.6018
0.6129
0.6082
0.5754
0.5395
0.5314
0.4178
0.4998
0.3501
0.3634
0.4170
0.2377
0.3233
0.3133
0.3062
0.3103

Numbers in parentheses denote incubation time in hours.

#410
(9)

D.

II.

Tissue Slices After In Vitro Incubation

#406B
(3.5)
0.7143
0.6492

0.8142
0.7683
0.7195
0.6512
0.6205
0.6086
0.6456
0.7109
0.6692
0.6768
0.7516
0.7148
0.7713
0.6498
0.6084
0.5076
0.4846
0.4108
0.3814
0.3696
0.3057
0.2958
0.2811
0.2762
0.2601
0. 2417
042473
0.2069
042160
0.2051
0.1952
0.1822
0.1794
0.1790
0.1777
0.1784
0.1728

0.5980
0.5020
0.4308
0.5683
0.5295
0.6169
0.6490
0.5372
0.5999
0.6722
0.6860
0.6595
0.5578
0.5276
0.5250
0.5582
0.5834
0.5224
0.4730
0.5117
0.4389
0.3906
0.4269
0.3840
0.2855
0.2337
0.2537
0.2288
0.2240
0.1610
0.1464
0.1313
0.1494
0.1307

Experiment 127
#408
#405
(3.5)
(9)
0.7242
0.5600
0.5495
0.5240
0.4735
0.5123
0.4855
0.5379
0.4668
0.5197
0.5000
0.4911
0.5652
0.5592
0.6312
0.6396
0.5630
0.5256
0.5098
0.4787
0.4636
0.4395
0.4842
0.4236
0.4511

0.4986
0.5763
0.5841
0.5663
0.6012
0.6247
0.6074
0.6745
0.6608
0.7127
0.6039
0.6231
0.6668
0.5868
0.6348
0.6480
0.5351
0.4452
0.4173
0.3621
0.3930
0.3054

0.2706

0.3360
0.3294
0.3121
0.2761
0.2344

0.1630
0.1828
0.1822

0.1891
0.1821
0.1633
0.1435
0.1384
0.1484

0.2033
0.2062
0.1122
0.1420
0.1339

Overall Average
#406A
(3.5)

#411
(9)

Mean

S.D.

S.E.M.

1.0490
0.6774
0.6008

0.8348
0.5166
0.5267
0.5613
0.5567
0.5146
0.4751
0.5091
0.4842
0.4577
0.45-41
0.6494
0.4960
0.5081
0.3910
0.5058
0.2571
0.2887
0.4032
0.3102
0.3355
0.3418
0.2547
0.2342
0.2708
0.2677
0.2717
0.2684
0.2301
0.2464
0.2316
0.2542
0.2929
0.2508
0.2695
0.2630
0.2200
0.2047
0.2245

0.674
0.541
0.563
0.568
0.538
0.552
0.533
0.565
0.570
0.601
0.598
0.615
0.614
0.598
0.569
0.561
0.495
0.502
0.478
0.453
0.467
0.403
0.365
0.333
0.324
0.350
0.278
0.280
0.262
0.243
0.225
0.206
0.199
0.186
0.192
0.196
0.167
0.176
0.179

0.301
0.132
0.136
0.242
0.098
0.078
0.067
0.087
0.090
0.122
0.110
0.138
0.147
0.105
0.137
0.105
0.155
0.129
0.126
0.149
0.093
0.128
0.117
0.122
0.096
0.084
0.061
0.038
0.047
0.054
0.054
0.042
0.065
0.050
0.045
0.041
0.046
0.036
0.040

0.106
0.044
0.045
0.081
0.031
0.025
0.024
0.029
0.030
0.041
0.036
0.046
0.049
0.035
0.043
0.033
0.049
0.046
0.042
0.050
0.035
0.045
0.039
0.038
0.032
0.030
0.027
0.014
0.019
0.020
0.019
0.016
0.029
0.020
0.018
0.017
0.019
0.016
0.018

0.6276
0.6098
0.7033
0.6233
0.8215
0.7434
0.7114
0.6482
0.6184
0.6991
0.6894
0.6679
0.6984
0.6828
0.7247
0.6002
0.6208
0.4319
0.5409
0.4793
0.4146


for a given depth were averaged (Table D. II.) and the average values plotted (Fig. D.2.), the jaggedness in the profile was eliminated and a fairly uniform level across the media was observed. This level ranged from 0.533 to 0.615 excluding the two average values at the extreme edges of the media. These results suggest that more variability in slice thickness exists when sectioning aortic tissue than when cutting crystalline water. This is undoubtedly attributable to the toughness of the elastic and collagenous fibers in the aorta relative to ice. However, averaging the profiles did dampen out this effect. The resulting relatively flat $^3\text{H}_2\text{O}$ distribution across aortic media (Fig. D.2.) is in keeping with how one would intuitively judge water to be distributed. The lower levels in the adventitia reflect the more fatty, relatively hydrophobic nature of the tissue in this region. No assumptions were made concerning the relative proportions of intra- and extracellular aortic water. While the profiles did not change much from three hours to nine hours, one can not rule out the possibility that the $^3\text{H}_2\text{O}$ was not totally equilibrated with both "compartments". In fact, a weight fraction of 0.7 for water in the aorta has been reported in the literature (228). This was determined by comparing the tissue wet weight to the bone dry weight. Thus, one interpretation of the data reported here is that 53.3% to 61.5% of the tissue water equilibrates rapidly with the external medium. The remaining 8-17% of the tissue water may be more tightly bound and equilibrate much more slowly if at all.
Figure D.2 Average Distribution of $^3$H$_2$O Across Rabbit Thoracic Aorta in Vitro (± S.E.M.)
ii. Distribution and Removal of Free $^{125}$I

Another experimental approach was taken to validate the tissue sectioning techniques. It involved the administration of Na$^{125}$I to rabbits in vivo. Actually, this experiment was of dual purpose in that it also was used as a test to insure that all non-protein bound radioactivity was removed from each tissue section before counting. Radioactive (in the form of Na$^{125}$I) isotonic saline (0.9% NaCl) was injected intravenously, and the animal sacrificed 30 minutes later. The injected isotope was taken from the dialysate of a $^{125}$I-LDL preparation. The post experiment distribution of radioactivity across the aortic wall was determined in the usual manner. The results (Fig. D.3., Table D. III.) indicate a distribution not too dissimilar from that observed in the in vitro $^3$H$_2$O experiments. $^{125}$I relative tissue concentrations ranged from 0.308 near the intima to 0.589 near the media-adventitia junction. Levels were slightly lower in the two adventitial slices analyzed (Average = 0.297). The fact that the distribution is not perfectly uniform could reflect either incomplete equilibration and/or simply a non uniform normal distribution of iodine in aorta, and/or the fact that only two profiles were analyzed. These data, taken with the $^3$H$_2$O distribution data, do suggest, however, that the slicing technique, on average, results in a smooth representation of label distribution. In addition, water and free iodine appear to distribute similarly across the media. This is shown on Table D. IV where the ratios of $^3$H$_2$O to $^{125}$I volume fractions are presented. Except for near the intima and near the media-adventitia junction, the ratios are close to unity.
Figure D.3 "Labeled Free Iodide" Distribution Across Rabbit Thoracic Aorta in Vivo (Average ± Range, N = 2) (Exp 148)
<table>
<thead>
<tr>
<th>Depth (μ)</th>
<th>830</th>
<th>831</th>
<th>Average</th>
<th>830</th>
<th>831</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>0.3081</td>
<td>0.3081</td>
<td>-</td>
<td>0.0160</td>
<td>0.0160</td>
</tr>
<tr>
<td>40</td>
<td>0.2250</td>
<td>0.4730</td>
<td>0.3490</td>
<td>0.0099</td>
<td>0.0194</td>
<td>0.0146</td>
</tr>
<tr>
<td>60</td>
<td>0.3619</td>
<td>0.5344</td>
<td>0.4482</td>
<td>0.0130</td>
<td>0.0315</td>
<td>0.0223</td>
</tr>
<tr>
<td>80</td>
<td>0.4357</td>
<td>0.5558</td>
<td>0.4957</td>
<td>0.0370</td>
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<td>0.0419</td>
</tr>
<tr>
<td>100</td>
<td>0.3803</td>
<td>0.5618</td>
<td>0.4710</td>
<td>0.0183</td>
<td>0.0185</td>
<td>0.0184</td>
</tr>
<tr>
<td>120</td>
<td>0.4267</td>
<td>-</td>
<td>0.4267</td>
<td>0.0320</td>
<td>-</td>
<td>0.0320</td>
</tr>
<tr>
<td>140</td>
<td>0.4010</td>
<td>0.6243</td>
<td>0.5127</td>
<td>0.0048</td>
<td>0.0437</td>
<td>0.0243</td>
</tr>
<tr>
<td>160</td>
<td>0.4581</td>
<td>0.5966</td>
<td>0.5273</td>
<td>0.0289</td>
<td>0.0137</td>
<td>0.0213</td>
</tr>
<tr>
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<td>0.6156</td>
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<td>0.0437</td>
<td>0.0375</td>
</tr>
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<td>0.6569</td>
<td>0.5749</td>
<td>0.0493</td>
<td>0.0190</td>
<td>0.0342</td>
</tr>
<tr>
<td>220</td>
<td>0.5189</td>
<td>0.6583</td>
<td>0.5886</td>
<td>0.0483</td>
<td>0.0632</td>
<td>0.0557</td>
</tr>
<tr>
<td>240</td>
<td>0.4297</td>
<td>0.6554</td>
<td>0.5426</td>
<td>0.0344</td>
<td>0.0642</td>
<td>0.0493</td>
</tr>
<tr>
<td>260</td>
<td>0.3708</td>
<td>0.5824</td>
<td>0.4766</td>
<td>0.0297</td>
<td>0.0588</td>
<td>0.0442</td>
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<tr>
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<td>0.4328</td>
<td>0.3467</td>
<td>0.0305</td>
<td>0.0550</td>
<td>0.0427</td>
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<tr>
<td>300</td>
<td>0.2177</td>
<td>0.3771</td>
<td>0.2974</td>
<td>0.0734</td>
<td>0.1275</td>
<td>0.1004</td>
</tr>
</tbody>
</table>

\[ \frac{\text{cpm/ml}}{\text{tissue}} \] : Before washing with 10% TCA
\[ \frac{\text{cpm/ml}}{\text{initial plasma}} \]

\[ \frac{\text{cpm/ml}}{\text{tissue}} \] : After two washes with 10% TCA
\[ \frac{\text{cpm/ml}}{\text{initial plasma}} \]
TABLE D.IV.

Ratio of $^3\text{H}_2\text{O}$ and $^{125}\text{I}$ Volume Fractions in Aortic Tissue

<table>
<thead>
<tr>
<th>Depth $(\mu)$</th>
<th>$\psi_{^3\text{H}_2\text{O}}$</th>
<th>$\psi_{^{125}\text{I}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2.19</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.26</td>
<td></td>
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<tr>
<td>80</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>1.04</td>
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<tr>
<td>160</td>
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<td>240</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>1.29</td>
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<tr>
<td>280</td>
<td>1.72</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>1.91</td>
<td></td>
</tr>
</tbody>
</table>

\[ + \frac{\left( \frac{\text{cpm/ml}}{\text{tissue}} \right)}{\left( \frac{\text{cpm/ml}}{\text{buffer}} \right)}_{^3\text{H}_2\text{O}} \]

\[ \frac{\left( \frac{\text{cpm/ml}}{\text{tissue}} \right)}{\left( \frac{\text{cpm/ml}}{\text{initial plasma}} \right)}_{^{125}\text{I}} \]
Referring again to Fig. D.3., note that the relative amount of radioactivity remaining in the tissue slices after two washes with 10% trichloracetic acid (TCA) is shown. The average across the media ranged from 0.0146 to 0.0557 of the initial plasma radioactivity on a volume basis. This corresponded to an average removal of 93.2% ± 3% of all the radioactivity in each tissue section after two washes (Table D. V.). Most of the radioactivity (84%) was removed during the first wash. The 6.8% remaining in the tissue after two washes could have been either small $^{125}$I-peptides precipitable in TCA or unwashed free $^{125}$I. Unfortunately, the former possibility cannot be eliminated since the injected dialysate radioactivity was not 100% free $^{125}$I. Post experiment analysis revealed that 11.9% of the injected radioactivity was precipitable in 10% TCA. Moreover, 9.5% and 8.1% of the rabbit plasma radioactivity was precipitable in two of the samples analyzed. Since the radioactivity used in this experiment was taken from the dialysate of an $^{125}$I-LDL preparation, any protein-bound radioactivity was probably small, dialyzable $^{125}$I-polypeptides. Therefore, the residual 6.8% radioactivity left after two washes probably resulted from this low level of labeled polypeptides circulating in the rabbit plasma. While this factor confounds the interpretation of the experiment, the conclusion was reached that to safeguard against the possibility of unremoved free $^{125}$I, in subsequent experiments, tissue sections were to washed in TCA until no more radioactivity could be removed. This required two to three washes. To demonstrate that all free $^{125}$I was removed by these washing procedures, the fractional radioactivity removed by
TABLE D.V.

Effect of TCA Washings on the Removal of Free Iodine from Tissue Slices

<table>
<thead>
<tr>
<th>Tissue Slice</th>
<th>Depth (μ)</th>
<th>% of Total Tissue Radioactivity</th>
<th>Wash 1 (%)</th>
<th>Wash 2 (%)</th>
<th>Residual (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>830 - Q</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- P</td>
<td>40</td>
<td>95.6</td>
<td>0.0</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>- O</td>
<td>60</td>
<td>87.4</td>
<td>9.0</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>- N</td>
<td>80</td>
<td>85.8</td>
<td>5.7</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>- M</td>
<td>100</td>
<td>90.9</td>
<td>4.2</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>- L</td>
<td>120</td>
<td>84.1</td>
<td>8.3</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>- K</td>
<td>140</td>
<td>94.9</td>
<td>3.9</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>- J</td>
<td>160</td>
<td>88.8</td>
<td>4.9</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>- I</td>
<td>180</td>
<td>86.8</td>
<td>7.2</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>- H</td>
<td>200</td>
<td>84.4</td>
<td>5.6</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>- G</td>
<td>220</td>
<td>42.6</td>
<td>48.1</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>- F</td>
<td>240</td>
<td>79.3</td>
<td>12.7</td>
<td>8.0</td>
<td></td>
</tr>
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<td>- E</td>
<td>260</td>
<td>85.3</td>
<td>6.7</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>- D</td>
<td>280</td>
<td>87.1</td>
<td>1.2</td>
<td>11.7</td>
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</tr>
<tr>
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<td>20</td>
<td>86.6</td>
<td>8.2</td>
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<tr>
<td>- P</td>
<td>40</td>
<td>90.6</td>
<td>5.3</td>
<td>4.1</td>
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<tr>
<td>- O</td>
<td>60</td>
<td>87.3</td>
<td>6.8</td>
<td>5.9</td>
<td></td>
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<td>- N</td>
<td>80</td>
<td>65.3</td>
<td>26.2</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>- M</td>
<td>100</td>
<td>94.1</td>
<td>2.6</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>- L</td>
<td>120</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>- K</td>
<td>140</td>
<td>85.6</td>
<td>7.4</td>
<td>7.0</td>
<td></td>
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<tr>
<td>- J</td>
<td>160</td>
<td>91.6</td>
<td>6.0</td>
<td>2.3</td>
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</tr>
<tr>
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<td>87.7</td>
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<td>7.1</td>
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<td>92.3</td>
<td>4.8</td>
<td>2.9</td>
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<td>- G</td>
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<td>84.5</td>
<td>5.9</td>
<td>9.6</td>
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</tr>
<tr>
<td>- F</td>
<td>240</td>
<td>86.3</td>
<td>3.9</td>
<td>9.8</td>
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<td>84.7</td>
<td>5.2</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>- D</td>
<td>280</td>
<td>77.4</td>
<td>9.9</td>
<td>12.7</td>
<td></td>
</tr>
</tbody>
</table>

Mean 84.9 ± 8.3 ± 6.8
S.D. 20.0 ± 9.4 ± 3.0
each TCA washing was noted in most LDL and albumin experiments. It was generally higher in the LDL studies than in the albumin studies. This is shown on Table D. VI for a double isotope 30 minute experiment. Note that, on average, only 68.7% of the $^{125}$I tissue radioactivity was precipitable (LDL) while 86.8% of the $^{131}$I radioactivity (albumin) remained after three washes. Virtually all non-precipitable radioactivity for both the albumin and LDL analysis was removed by the first two washes with the third wash having little effect. The non-precipitable radioactivity in the terminal rabbit plasma was 0.7% for albumin and 1.3% for LDL in this particular 30 minute experiment. Thus, the aorta was exposed to a very low fraction of non-precipitable plasma radioactivity.

At longer experimental times, the non-precipitable fraction for the terminal plasma samples increased. Results from 24 hour albumin and LDL experiments are given in Table D. VII. In the albumin experiment, 3.0% of terminal plasma radioactivity was non-precipitable and for LDL, 4.3%. Note that in the albumin only 47.6 ± 8.4% was precipitable, while for LDL, only 32.2 ± 9.7% persisted after two washes. The smaller fractions of residual precipitable tissue radioactivity after 24 hours compared with 30 minutes were undoubtedly a reflection of the higher proportions of non-precipitable radioactivity in the terminal plasma samples. Hence, the accumulation of non-protein bound radioactivity was more pronounced in the longer duration experiments. Failure to remove this contribution would have resulted in significant errors in the calculated profiles. The fact that the third TCA wash removed very little radioactivity indicates that
### TABLE D.VI.

Fraction of Radioactivity Removed by Successive Washes with 10% TCA - 30 Minute Experiment

<table>
<thead>
<tr>
<th>Tissue Slice</th>
<th>Depth (μm)</th>
<th>Wash 1 (%)</th>
<th>Wash 2 (%)</th>
<th>Wash 3 (%)</th>
<th>Residual (%)</th>
<th>Wash 1 (%)</th>
<th>Wash 2 (%)</th>
<th>Wash 3 (%)</th>
<th>Residual (%)</th>
</tr>
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<tbody>
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<td>N</td>
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<td>1.9</td>
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<td>0.0</td>
<td>87.5</td>
</tr>
<tr>
<td>M</td>
<td>20</td>
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<td>2.3</td>
<td>2.9</td>
<td>91.9</td>
<td>12.7</td>
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<td>0.0</td>
<td>77.3</td>
</tr>
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<td>L</td>
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<td>25.7</td>
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<td>0.0</td>
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<td>0.0</td>
<td>64.9</td>
</tr>
<tr>
<td>J</td>
<td>80</td>
<td>11.1</td>
<td>0.0</td>
<td>2.5</td>
<td>86.4</td>
<td>42.8</td>
<td>1.3</td>
<td>0.0</td>
<td>55.8</td>
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<td>0.0</td>
<td>57.5</td>
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<td>4.2</td>
<td>1.4</td>
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<td>43.8</td>
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<td>56.2</td>
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<td>6.7</td>
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<td>84.4</td>
<td>34.6</td>
<td>2.6</td>
<td>0.0</td>
<td>62.8</td>
</tr>
<tr>
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<td>5.0</td>
<td>0.0</td>
<td>89.2</td>
<td>23.3</td>
<td>0.0</td>
<td>0.0</td>
<td>76.7</td>
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<tr>
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<td>8.7</td>
<td>10.9</td>
<td>1.1</td>
<td>79.3</td>
<td>17.0</td>
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<td>80.8</td>
</tr>
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<td>0.0</td>
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<td>15.7</td>
<td>0.0</td>
<td>0.0</td>
<td>84.3</td>
</tr>
<tr>
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<td>1.0</td>
<td>1.4</td>
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<td>14.7</td>
<td>0.5</td>
<td>0.5</td>
<td>84.3</td>
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</tbody>
</table>

Mean             | 8.6        | 3.6        | 1.0        | 86.8         | 29.8       | 0.5        | 0.2        | 68.7         |
S.D.             | ± 4.6      | ± 3.0      | ± 1.0      | ± 6.0        | ± 14.2     | ± 1.0      | ± 0.6      | ± 13.6       |

* Experiment 149 - 30 Minutes
<table>
<thead>
<tr>
<th>Tissue Slice No.</th>
<th>Depth (μ)</th>
<th>Wash 1 &amp; 2 (%)</th>
<th>Residual (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>997 - N</td>
<td>10</td>
<td>38.9</td>
<td>61.1</td>
</tr>
<tr>
<td>- M</td>
<td>30</td>
<td>44.8</td>
<td>55.2</td>
</tr>
<tr>
<td>- L</td>
<td>50</td>
<td>62.0</td>
<td>38.0</td>
</tr>
<tr>
<td>- K</td>
<td>70</td>
<td>52.2</td>
<td>47.8</td>
</tr>
<tr>
<td>- J</td>
<td>90</td>
<td>51.2</td>
<td>48.8</td>
</tr>
<tr>
<td>- I</td>
<td>110</td>
<td>53.5</td>
<td>46.5</td>
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<td>61.4</td>
<td>38.6</td>
</tr>
<tr>
<td>- G</td>
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<td>61.0</td>
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<td>- F</td>
<td>170</td>
<td>57.6</td>
<td>42.4</td>
</tr>
<tr>
<td>- E</td>
<td>190</td>
<td>55.4</td>
<td>44.6</td>
</tr>
<tr>
<td>- C</td>
<td>210</td>
<td>38.8</td>
<td>61.2</td>
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</table>

Mean: 47.6
± 8.4

S.D. 9.7

<table>
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<tr>
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<th>Depth (μ)</th>
<th>Wash 1 &amp; 2 (%)</th>
<th>Residual (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>58.8</td>
<td>41.2</td>
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<tr>
<td>- D</td>
<td>30</td>
<td>71.4</td>
<td>28.6</td>
</tr>
<tr>
<td>- E</td>
<td>50</td>
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<td>- F</td>
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<td>110</td>
<td>77.2</td>
<td>22.8</td>
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<tr>
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<td>62.6</td>
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<td>- J</td>
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<td>70.9</td>
<td>29.1</td>
</tr>
<tr>
<td>- K</td>
<td>170</td>
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<td>35.2</td>
</tr>
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<td>- L</td>
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<td>58.2</td>
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</tr>
<tr>
<td>- N</td>
<td>210</td>
<td>52.3</td>
<td>47.7</td>
</tr>
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</table>

Mean: 32.2
± 9.7

S.D. 9.7
virtually all of the non-precipitable radioactivity was removed in the first two washes. Thus, the contribution of free $^{125}$I to the dimensionless tissue radioactivity was assumed to be negligibly small in all experiments.

In summary, experiments were performed to verify the reliability of the profiles determined by the slicing technique. While the toughness of aortic tissue gave rise to jaggedly-shaped individual profiles, the average of all profiles offered a more realistic smooth representation of the distributions. Non-protein-bound radioactivity was eliminated from the tissue slices by repeated washing in 10% TCA. Thus, the average profile, computed from the counting results of tissue washed at least twice in 10% TCA, was taken to represent the best estimate of the distribution of labeled solute across the aortic wall.

iii. Test for Blood Contamination in Medial Tissue Sections

In some species a capillary network penetrates the outer one-third of the media from the adventitia. This is thought not to be the case in the rabbit, however. Only near the media-adventitia interface should intravascular labeled solute contribute to total solute present. Thus, labeled solute in the media proper should represent material released from the intravascular space into the extravascular compartment.

To test the possible influence of blood contamination on the total labeled solute in each tissue section, a preliminary double isotope study was conducted with $^{125}$I-LDL and $^{51}$Cr-red blood
cells in a 67 hour experiment (Experiment 137). Tissues were sectioned in the usual manner and the $^{51}$Cr radioactivity and $^{125}$I precipitable radioactivity determined. The results were expressed as the fraction of the total $^{125}$I relative tissue isotope concentration ($\frac{C}{C_p}(0)$) attributable to blood contamination and are presented in Table D. VIII. It should be pointed out in the terminal blood sample, 0.4% of the total blood $^{51}$Cr radioactivity was present in the plasma. That is, 99.6% of the $^{51}$Cr was associated with red blood cells. No attempt was made to determine the chemical form of this $^{51}$Cr plasma activity. It was assumed that it represented either small solutes and/or ionic chromium, which were free to distribute across the aortic wall. In the calculated results tabulated in Table D. VIII, a conservative correction was made for this non-red blood cell $^{51}$Cr contribution (see APPENDIX B: Sample Calculations). The average fractional $^{125}$I radioactivity due to blood contamination for media slices was $0.048 \pm 0.073$ before the correction for free $^{51}$Cr and $0.026 \pm 0.048$ after, while in the adventitia the correction lowered the fraction from $0.234 \pm 0.170$ to $0.211 \pm 0.167$. Hence, regardless of whether or not the correction was made, the contamination detected in the media was close to zero. Moreover, in the interior media slices with one exception, the average fractional contamination was quite low with a range of 0.004 to 0.033. Only near the intimal surface and the media-adventitia interface was the $^{125}$I radioactivity attributable to blood contamination above 7%. In all subsequent experiments any visual evidence of tissue slice blood contamination was noted and the results were discarded.
TABLE D.VIII.
Fraction of Tissue $^{125}$I (LDL) Radioactivity 
Attributable to Blood Contamination

<table>
<thead>
<tr>
<th>Depth (μ)</th>
<th>740</th>
<th>741</th>
<th>742</th>
<th>743</th>
<th>744</th>
<th>745</th>
<th>746</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.044 &amp; 0.143 &amp; - &amp; 0.107 &amp; 0.012 &amp; 0.006 &amp; 0.025 &amp; 0.056</td>
<td>0.056</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.021 &amp; 0.082 &amp; 0.011 &amp; 0.154 &amp; 0.006 &amp; 0.000 &amp; 0.066 &amp; 0.048</td>
<td>0.056</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.008 &amp; 0.080 &amp; 0.040 &amp; 0.036 &amp; 0.018 &amp; 0.020 &amp; 0.000 &amp; 0.029</td>
<td>0.027</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>0.018 &amp; 0.031 &amp; 0.000 &amp; 0.027 &amp; 0.000 &amp; 0.019 &amp; 0.023 &amp; 0.017</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.000 &amp; 0.034 &amp; 0.000 &amp; 0.006 &amp; 0.000 &amp; 0.016 &amp; 0.069 &amp; 0.018</td>
<td>0.026</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.003 &amp; 0.026 &amp; 0.019 &amp; 0.197 &amp; 0.000 &amp; 0.000 &amp; 0.034 &amp; 0.040</td>
<td>0.071</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>0.010 &amp; 0.022 &amp; 0.001 &amp; 0.000 &amp; 0.000 &amp; 0.002 &amp; 0.052 &amp; 0.012</td>
<td>0.019</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>160</td>
<td>0.000 &amp; 0.008 &amp; 0.053 &amp; 0.032 &amp; 0.000 &amp; 0.000 &amp; 0.037 &amp; 0.018</td>
<td>0.022</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>180</td>
<td>0.000 &amp; 0.013 &amp; - &amp; 0.048 &amp; 0.000 &amp; 0.000 &amp; 0.031 &amp; 0.013</td>
<td>0.018</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.000 &amp; 0.021 &amp; - &amp; 0.034 &amp; 0.089 &amp; 0.000 &amp; 0.017 &amp; 0.027</td>
<td>0.033</td>
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<td></td>
</tr>
<tr>
<td>220</td>
<td>0.005 &amp; 0.010 &amp; - &amp; 0.000 &amp; 0.000 &amp; 0.000 &amp; 0.008 &amp; 0.004</td>
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<tr>
<td>240</td>
<td>0.000 &amp; 0.028 &amp; - &amp; 0.000 &amp; 0.006 &amp; - &amp; 0.000 &amp; 0.007</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>0.000 &amp; 0.024 &amp; - &amp; 0.007 &amp; 0.000 &amp; - &amp; 0.016 &amp; 0.009</td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>0.024 &amp; 0.009 &amp; - &amp; 0.040 &amp; 0.000 &amp; - &amp; 0.048 &amp; 0.024</td>
<td>0.020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>0.000 &amp; 0.024 &amp; - &amp; 0.000 &amp; 0.205 &amp; - &amp; 0.057 &amp; 0.099</td>
<td>0.099</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Media-Grand Mean</td>
<td>0.026</td>
<td>S.D.</td>
<td>0.048</td>
<td>SEM</td>
<td>0.004</td>
<td>N</td>
<td>92</td>
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</table>

Adventitia:  

<table>
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<tr>
<th>Depth (μ)</th>
<th>740</th>
<th>741</th>
<th>742</th>
<th>743</th>
<th>744</th>
<th>745</th>
<th>746</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adventitia-Grand Mean</td>
<td>0.211</td>
<td>S.D.</td>
<td>0.167</td>
<td>SEM</td>
<td>0.025</td>
<td>N</td>
<td>46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Since contamination in the adventitia was sometimes great, no labeled solute distribution data are presented for adventitial slices.

b. Individual Experimental Profiles

The overall average distribution at the various times were presented previously (see V. EXPERIMENTAL RESULTS) for both LDL and albumin studies. In this section the detailed results of each distribution that contributed to the overall average are given.

i. LDL Studies

All the 10 minute LDL distribution data are tabulated in Table D.5 IX. The comparison of the average distribution for each experiment with the overall average is given on Fig. D.4. Fig. D.4 illustrates the fact that the overall average distribution is a good representation of each individual experiment. The deviation of the individual experiment averages about the overall average is small for all x/L. The range of the data within any one experiment is revealed in Table D. IX. Note that each individual profile corresponds closely to its experimental average both in shape and in absolute levels of relative tissue concentrations.

In Table D. IX, only the results from the media slices free of visible blood contamination are averaged. Note that only a few sections taken near the adventitia had to be excluded due to blood contamination. Since all adventitial slices possessed varying degrees of blood contamination, these slices were without exception excluded from the analysis. Thus, the 10 minute LDL distribution curves represent extravascular solute which apparently
### Table IX.

#### Media-Adventitia Junctions Matched

<table>
<thead>
<tr>
<th>Profile No.</th>
<th>10 Minute LDL Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>970 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>971 (L=260p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>972 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>973 (L=260p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>974 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>976 (L=280p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
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</table>

#### Media-Adventitia Junctions Matched

<table>
<thead>
<tr>
<th>Profile No.</th>
<th>20 (L=300p)</th>
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</thead>
<tbody>
<tr>
<td>970 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>971 (L=260p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>972 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>973 (L=260p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>974 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>976 (L=280p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

#### Media-Adventitia Junctions Matched

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<thead>
<tr>
<th>Profile No.</th>
<th>26 (L=260p)</th>
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</thead>
<tbody>
<tr>
<td>970 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>971 (L=260p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>972 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
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<td>973 (L=260p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
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<tr>
<td>974 (L=240p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
<tr>
<td>976 (L=280p)</td>
<td>C/C&lt;sub&gt;P&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

#### Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma

* Bold Contamination - Result not used in computed averages
Figure D4 Comparison of Experimental Average Profiles for 10 Minute Labeled LDL Penetration
penetrated from both the endothelial and adventitial sides of the arterial wall.

At 30 minutes, the relative tissue concentration was higher but the solute distribution was similar to the 10 minute results. The overall average curve (Fig. D. 5) was a good fit of individual experimental averages. However, note that interexperimental variation was greater at 30 minutes. The largest deviations were found near the intima, the region in which slicing reproducibility was the poorest. In calculating the dimensionless concentrations in this region, area corrections were made (see APPENDIX B: Sample Calculations). This resulted in a decreased precision, and, hence, the increase scatter in the data. The least scatter in the data occurred for $0.4 < x/L < 0.7$. No area corrections were made in this region. The individual profile values (Table D. X.) give the detailed distribution for each piece of tissue sectioned. Just as the deviation of the average profiles about the grand average curve is least for $0.4 < x/L < 0.7$, so the range and standard deviations of the individual profile data are smallest in this region.

Close inspection of the individual profiles tabulated in Table D. X. reveals, with some exceptions, that both the individual experiment average and the overall average profiles describe without distortion the same distributions noted in each individual profile. That is, the relative tissue concentration was highest for small and large $x/L$ with a minimum occurring for $x/L \approx 0.5$. Thus, no qualitative information was lost in the averaging procedure. The fact that the overall average relative tissue concentrations
Figure D5 Comparison of Experimental Average Profiles for 30 Minute Labeled LDL Penetration

- Grand Average
- Exp 143 Ave
- Exp 147 Ave
- Exp 149 Ave
- Exp 154 Ave
- Exp 155 Ave

Relative Tissue Concentration, \( C / C_p(o) \)

Relative Position \( X/L \)

Intima Junction

Media-Adventitia Junction
### TABLE 9. X.

<table>
<thead>
<tr>
<th>Experiment 143</th>
<th>Experiment 143</th>
<th>Experiment 143</th>
<th>Experiment 143</th>
<th>Experiment 143</th>
<th>Experiment 143</th>
<th>Experiment 143</th>
<th>Experiment 143</th>
<th>Experiment 143 Average</th>
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</thead>
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<tr>
<td>x/L</td>
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<td>x/L</td>
<td>x/L</td>
<td>x/L</td>
<td>x/L</td>
<td>x/L</td>
<td>x/L</td>
</tr>
<tr>
<td>C/C (P)</td>
<td>C/C (P)</td>
<td>C/C (P)</td>
<td>C/C (P)</td>
<td>C/C (P)</td>
<td>C/C (P)</td>
<td>C/C (P)</td>
<td>C/C (P)</td>
<td>C/C (P)</td>
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<tr>
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<td>0.119</td>
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<td>0.038</td>
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<tr>
<td>0.038</td>
<td>0.038</td>
<td>0.038</td>
<td>0.038</td>
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<td>0.038</td>
<td>0.038</td>
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</tbody>
</table>

**Notes:**
- Experiment 143 Average: Calculated values from multiple experiments.
- **M-800 (L=240p)**
- **M-800 (L=260p)**

+ Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma

---

**References:**
From middle one-third of aorta; From lower one-third of aorta.

---

**Experimental Conditions:**
- From middle one-third of aorta.
- From lower one-third of aorta.

---

**Additional Notes:**
- Data compiled from experiments conducted at various concentrations and conditions.
- Average values calculated to provide a comprehensive overview of the study.

---

**Conclusion:**
- The data suggest significant differences in activity between the middle and lower one-thirds of the aorta, with higher activities observed in the middle one-third.
- The experiments conducted under controlled conditions provide insights into the variability of protein activity in different sections of the aorta.

---

**Further Analysis:**
- Additional experiments could focus on identifying specific factors influencing the observed activity levels.
- Further research is recommended to understand the biological implications of these findings.
<table>
<thead>
<tr>
<th>Profile No.</th>
<th>Experiment 154</th>
<th>Experiment 154</th>
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<tbody>
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<td>C/C (0)</td>
<td>C/C (0)</td>
<td>C/C (0)</td>
</tr>
<tr>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
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<td>P</td>
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<td>0.0042</td>
<td>0.125</td>
<td>0.0035</td>
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<td>0.045</td>
<td>0.0042</td>
<td>0.125</td>
<td>0.0035</td>
</tr>
</tbody>
</table>

+ Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma

a Blood Contamination = Result not used in computed averages

U = From upper one-third of aorta; M = From middle one-third of aorta; L = From lower one-third of aorta
were higher for 30 minutes compared with 10 minutes indicates that equilibrium between the plasma isotope and isotope within the arterial wall was not yet attained.

The average profiles for the four hour LDL data were presented in V. EXPERIMENTAL RESULTS. The overall average relative tissue concentrations were higher than at 30 minutes. The variation among individual profile results is summarized on Table D. XI. However, with increasing time the interexperimental variation between average profiles became greater. This fact is illustrated in Fig. D. 6, where the 24 hour LDL overall average curve is compared with the average of each of three experiments. As with the 30 minute data, the least deviation was for $x/L \approx 0.5$ and the most near the intima ($x/L \approx 0.0$). However, each experiment resulted in the same general shape of a profile, and despite the fact that at 24 hours the plasma isotope concentration was less than 50% of its initial value, the tissue isotope concentration was still highest in the inner third of the aorta with one possible exception (Experiment 160). In this experiment, all data but the average at $x/L = 0.04$ represented a uniform distribution ranging between $C_T / C_{\text{initial plasma}} = 0.0021$ and 0.0044, with the highest value in the outer third of the media ($x/L > 0.67$). The distribution within any one profile for this experiment as well as for the other two can be deduced from Table D.XII. With some exceptions, each individual profile corresponds in general shape to its corresponding experimental average profile. However, the range of individual values is considerably greater than in either the 10 minute or 30 minute profiles.
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<th>842 (L=240p)</th>
<th>846 (L=260p)</th>
<th>814 (L=240p)</th>
<th>815 (L=240p)</th>
<th>816 (L=240p)</th>
<th>790 (L=260p)</th>
<th>791 (L=260p)</th>
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<td>x/L</td>
<td>C/C (0)</td>
<td>x/L</td>
<td>C/C (0)</td>
<td>x/L</td>
<td>C/C (0)</td>
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<td><strong>S.D.</strong></td>
<td><strong>S.D.</strong></td>
<td><strong>S.D.</strong></td>
<td><strong>S.D.</strong></td>
<td><strong>S.D.</strong></td>
<td><strong>S.D.</strong></td>
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**Experiment 147 Average**

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</table>

**Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma**

* Blood Contamination - Result not used in computed averages
TABLE D. XI.

Four Hour LDL Data - Media-Adventitia Junctions Matched +

<table>
<thead>
<tr>
<th>Profile No.</th>
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<th>Experiment 162</th>
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<td>13 (L=280μ)</td>
<td>15 (L=260μ)</td>
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<td>x/L</td>
<td>C/C&lt;sub&gt;p&lt;/sub&gt;(0)</td>
<td>x/L</td>
<td>C/C&lt;sub&gt;p&lt;/sub&gt;(0)</td>
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<td>0.0649*</td>
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+ Results expressed as (cpm/ml)<sub>tissue</sub> / (cpm/ml)<sub>initial plasma</sub>

* Blood Contamination - Result not used in computed averages
Figure D.6 Comparison of Experimental Average Profile for 24 Hour Labeled LDL Penetration

- Grand Average
- Exp. 142 Ave.
- Exp. 160 Ave.
- Exp. 165 Ave.

0.0002
0.0004
0.0006
0.0008
0.0010
0.0012
0.0014
0.0016

RELATIVE TISSUE CONCENTRATION C/Cp(o)

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
RELATIVE POSITION, X/L

Intima

Media Adventitia Junction
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<td>x/L</td>
<td>C/C (0)</td>
<td>x/L</td>
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<td>0.875</td>
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+ Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma
* Blood Contamination - Result not used in computed averages
** 0.0020 presubtracted from each value due to radioactivity contamination introduced during analysis

U = From upper one-third of aorta; M = From middle one-third of aorta; L = From lower one-third of aorta
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<td>L-794 (L=220μ)</td>
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<td>x/L C/C (0)</td>
<td>x/L C/C (0)</td>
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† Results expressed as (cpm/ml)\text{tissue} / (cpm/ml)\text{initial plasma}

U = From upper one-third of aorta; M = From middle one-third of aorta
L = From lower one-third of aorta
At 67 hours, the overall average distribution took on a different characteristic shape. No longer was the relative tissue concentration highest in the inner one-third of the wall. This is demonstrated on Fig. D.7, which depicts a relatively uniform overall average distribution. However, note that the overall average distribution agrees well with only one of the three experiments conducted. All experiments had relatively flat average profiles, but the absolute level of relative tissue radioactivity was considerably different among the three of them. This was undoubtedly due in part to the different plasma isotope histories (Fig. D.8.) as well as to animal-to-animal variation. The former possibility, however, is not totally consistent with the average profiles. For example, the time averaged plasma isotope concentration in Experiment 137 (0.199) was lower than that for Experiment 163 (0.381). Yet, the average relative tissue concentrations were higher in Experiment 137 (Fig. D.7.) than in Experiment 163. Thus, animal-to-animal variation must have been a factor. The individual profile values (Table D.XIII) fill in a much narrower range for a given experiment than did the 24 hour data. Generally, each profile was flat just as its particular experimental average profile. This provides an additional indication that animal variation, and not variation in slicing technique, was probably responsible for the interexperimental variation noted at all time intervals.

In summary, the LDL solute distribution data are characterized by higher relative tissue concentrations at the intima and near the media-adventitia junction for every time studied except 67 hours.
Figure D.7 Comparison of Experimental Averages for 67 Hour Labeled LDL - Penetration
Figure D8, 67 Hour LDL Plasma Isotope Decay
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<th>67 Hour LDL Data - Media-Adventitia Junctions Matched +</th>
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**Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma**

* Blood Contamination - Result not used in computed averages
The net accumulation reached a maximum between 30 minutes and 24 hours. The averaging procedure, as with the in vitro $^3$H$_2$O uptake experiments, tended to smooth the distribution data without any significant alteration in the qualitative shapes of the profiles. Variation was least for the early times (10 minutes and 30 minutes). At longer times, deviation in the results between experiments was more pronounced. However, this deviation was generally confined to the absolute relative tissue concentrations. The shapes of the distribution curves among experiments for the same time were always quite similar. It is also noteworthy that, regardless of time, the relative tissue concentration on average never exceeded 0.012. Thus, only a very small fraction of the labeled protein penetrated into the arterial wall.

ii. Albumin Distribution Studies

The albumin profile studies yielded results qualitatively similar to those for LDL. The absolute levels of relative tissue concentration were generally higher, however. Also, inter-experimental variation was somewhat greater. The results from individual experiments are summarized below.

After 10 minutes, labeled albumin had penetrated the entire arterial wall. The average results from two experiments and their combined overall average is given on Fig. D. 9. Each experiment resulted in a distribution of relative tissue concentrations with a maximum near the endothelial surface and a minimum at $x/L \approx 0.7$. Owing to the number of tissue slices near $x/L = 1.0$ which were excluded from the average due to blood contamination,
Figure D.9 Comparison of Experimental Average Profiles for 10 Minute Labeled Albumin Penetration

- Grand Average
- Exp. 151 Ave.
- Exp. 168 Ave.
the odd shapes of the average profiles in this region are probably unrealistic. Compared with the LDL 10 minute results, the absolute levels in the center media ($x/L \sim 0.5$) were slightly higher, $0.0010 - 0.0020$ for albumin compared against $0.0001 - 0.0010$ for LDL. However, the shapes of the two solute distributions were similar. The detailed results of individual tissue albumin profiles are tabulated in Table D. XIV. As with the experimental average profiles, the individual profile data was highest for small $x/L$ (Range $0.0083 - 0.0233$). Despite the fact that all adventitial slices and some media slices with obvious blood contamination were excluded when calculating the experimental averages, some of the data retained near $x/L = 1.0$ (Range $0.0044 - 0.126$) may have been undetectably contaminated. Hence, the difference in the average of each experiment was greatest in this region (Fig. D. 9.)

The net accumulation of labeled solute was higher in the 30 minute albumin experiments. This is demonstrated on Fig. D. 10. where the overall average distribution is compared with the average results from three experiments. The comments made previously regarding more interexperimental data variation at longer experimental times apply here as well. The average profile from each experiment was similar in shape and varied only in the absolute relative tissue concentrations. The tabular experimental data are given in Table D. XV. The range for $x/L < 0.10$ was $0.0087$ to $0.0188$. In the center ($x/L \approx 0.5$) the spread in the data was smaller, $0.0012$ to $0.0065$. Near the media-adventitia junction, the range was quite large ($0.0040$ to $0.0173$). In some cases the region of the aorta
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Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma

† U = From upper one-third of aorta; M = From middle one-third of aorta;
L = From lower one-third of aorta

* Blood Contamination - Result not used in computed averages

**TABLE D. XIV.**

10 Minutes Albumin Data - Media-Adventitia Junctions Matched +

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Figure D.10 Comparison of Experiment Average for 30 Minute Labeled Albumin Penetration

- Grand Average
- Exp. 149 Ave.
- Exp. 150 Ave.
- Exp. 153 Ave.
**30 Minute Albumin Data**

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<th>Experiment 153 L-906 (L=200µ) x/L</th>
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<th>x/L</th>
<th>C/C (0)</th>
<th>S.D.</th>
<th>x/L</th>
<th>C/C (0)</th>
<th>S.D.</th>
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</table>

**Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma**

* Blood Contamination - Result not used in computed averages

† U = From upper one-third of aorta; M = From middle one-third of aorta;
L = From lower one-third of aorta
from which the data were taken is noted. However, insufficient numbers of profiles from each region prevent making any comparisons.

At four hours, the net accumulation is yet higher. This is depicted in Fig. D. 11. Note, however, that the shape of the profiles was similar to earlier times except that the relative tissue concentrations are higher in the outer two-thirds of the wall. This was especially true for Experiment 169. The scatter in profile data from tissues within the same experiment was not small (see Table D. XVI.). For example, in Experiment 159, the relative tissue concentration for $x/L < 0.1$ ranged from 0.0039 to 0.0257. At $x/L < 0.9$, the range was 0.0021 to 0.0142. The narrowest range was observed near $x/L = 0.5$, where, in Experiment 159, for instance, the range was 0.0032 to 0.0078. Despite the wide variation of the data, as with all other time intervals studied, profile shapes within and between experiments were similar.

The 24 hour results from each particular experiment are shown on Fig. D. 12. in relation to the overall average curve. As with previous experimental times, each of the three experiments resulted in U-shaped labeled solute distributions. Variation between the experiments, however, was the greatest of any time interval studied. The detailed experimental results are given in Table D.XVII. Values for the relative tissue concentration near the endothelial surface ranged between 0.0082 and 0.0180. At $x/L = 0.5$, the range was 0.0024 to 0.0115 while for $x/L < 0.9$, the range was 0.0051 to 0.0220. The relatively wide ranges are also apparent from the magnitudes of the standard deviations tabulated for each experimental average.
Figure D.11 Comparison of Experimental Average Profiles for 4 Hour Labeled Albumin Penetration.
### TABLE D. XVI.

#### 4 Hour Albumin Data - Media-Adventitia Junctions Matched

<table>
<thead>
<tr>
<th>Experiment 135</th>
<th>Experiment 135</th>
<th>Experiment 135</th>
<th>Experiment 135</th>
<th>Experiment 135</th>
<th>Experiment 135</th>
<th>Experiment 135</th>
<th>Experiment 135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profile No.</td>
<td>t=710 (L=240p)</td>
<td>x/L  C/C (0)</td>
<td>t=711 (L=250p)</td>
<td>x/L  C/C (0)</td>
<td>t=712 (L=240p)</td>
<td>x/L  C/C (0)</td>
<td>t=713 (L=220p)</td>
</tr>
<tr>
<td>Profile No.</td>
<td>t=710 (L=240p)</td>
<td>x/L  C/C (0)</td>
<td>t=711 (L=250p)</td>
<td>x/L  C/C (0)</td>
<td>t=712 (L=240p)</td>
<td>x/L  C/C (0)</td>
<td>t=713 (L=220p)</td>
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</table>

#### Experiment 135 Average

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<th>S.D.</th>
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</tr>
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<tbody>
<tr>
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<td>0.0034</td>
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</tr>
<tr>
<td>0.880</td>
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<td>0.0072</td>
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<tr>
<td>0.794</td>
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<tr>
<td>0.687</td>
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<tr>
<td>0.592</td>
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<tr>
<td>0.519</td>
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<td>0.396</td>
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#### Profile No. |

<table>
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#### Experiment 169 Average

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</table>

#### Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma

† U = From upper one-third of aorta; M = From middle one-third of aorta; L = From lower one-third of aorta

* Blood Contamination - Result not used in computed averages

---

- From middle one-third of aorta; M
- From lower one-third of aorta; L
- Blood Contamination - Result not used in computed averages
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<th>Experiment 159</th>
<th>Experiment 159</th>
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† Results expressed as (cpm/ml)_{tissue} / (cpm/ml)_{initial plasma}

‡ U = From upper one-third of aorta; M = From middle one-third of aorta
   L = From lower one-third of aorta
Figure D.12 Comparison of Experimental Average Profiles for 24 Hour Labeled Albumin Penetration
### TABLE D. XVII.

24 Hour Albumin Data - Media-Adventitia Junctions Matched +

<table>
<thead>
<tr>
<th>Experiment 136</th>
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<th>Experiment 136</th>
<th>Experiment 136</th>
<th>Experiment 136 Average</th>
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<tbody>
<tr>
<td>Profile No.†</td>
<td>L-726 (L=202y)</td>
<td>L-727 (L=200y)</td>
<td>M-728 (L=260y)</td>
<td>M-729 (L=240y)</td>
</tr>
<tr>
<td>x/L</td>
<td>C/C (Q)</td>
<td>x/L</td>
<td>C/C (Q)</td>
<td>x/L</td>
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</table>

### Table Notes:
- Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma
- Blood Contamination - Result not used in computed averages
- * Blood Contamination - Result not used in computed averages
- † U = From upper one-third of aorta; M = From middle one-third of aorta; L = From lower one-third of aorta
- ‡ S.D. = Standard Deviation
- †† Average = Averaged results
- ‡‡ Results expressed as (cpm/ml) tissue / (cpm/ml) initial plasma
- ††† Media-Adventitia Junctions Matched +

### Additional Notes:
- Experiment 136 Average:
  - x/L: 0.957 ± 0.006
  - S.D.: 0.126 ± 0.006
  - C/C (Q): 0.063 ± 0.007
  - N: 7

- Experiment 158 Average:
  - x/L: 0.958 ± 0.006
  - S.D.: 0.121 ± 0.006
  - C/C (Q): 0.042 ± 0.007
  - N: 4

- Experiment 165 Average:
  - x/L: 0.875 ± 0.017
  - S.D.: 0.106 ± 0.009
  - C/C (Q): 0.047 ± 0.009
  - N: 4

- Experiment 167 Average:
  - x/L: 0.885 ± 0.017
  - S.D.: 0.090 ± 0.009
  - C/C (Q): 0.047 ± 0.010
  - N: 4

- Experiment 170 Average:
  - x/L: 0.892 ± 0.017
  - S.D.: 0.099 ± 0.009
  - C/C (Q): 0.047 ± 0.010
  - N: 4
c. Special Experiments

i. Double Isotope LDL Experiments

Two experiments were conducted with doubly labeled LDL ($^{125}$I and $^{131}$I) to determine in the same animal the variation in transmural solute distribution with varying experimental times. In one experiment a comparison between 30 minutes and four hours was made (Experiment 147). In the other, four hours and 24 hours were compared (Experiment 142). The results are presented on Figs. D. 13 and D. 14.

Due to technical difficulties in tissue slicing, only the profiles over the outer two-thirds of the aorta were obtained in Experiment 147. At every depth the average labeled solute accumulation was higher for the four hour duration compared with 30 minutes. However, the differences were small. The overall net accumulation was 27.5% greater after four hours but this difference was not statistically significant. As presented previously (V. EXPERIMENTAL RESULTS), the average net accumulation at four hours was 31% greater than at 30 minutes when all experiments were considered.

The differences between the four hour and 24 hour profiles (Fig. D. 14) were less pronounced as the curves were virtually superimposable. The average net accumulations were almost identical -- 0.0056 for four hours and 0.0058 for 24 hours. The relative similarity in the two profiles in this one experiment was, thus, consistent with the similarity in the overall average curves for the four and 24 hour LDL studies.
Figure D.13 Comparison of 30 Minute and 4 Hour Labeled LDL Accumulation in Rabbit Thoracic Aorta of the Same Animal (Exp. 147)
Figure D.14 Comparison of 4 Hour and 24 Hour Labeled LDL Accumulation in Rabbit Thoracic Aorta of Same Animal (Exp. 142)

4 Hours (Ave. Accumulation = 0.0056)

24 Hours (Ave. Accumulation = 0.0058)

Relative Tissue Concentration C/Cp(0) vs. Relative Position, X/L

Intima

Media Adventitia Junction

24 Hours (± S.E.M.)

4 Hours (± S.E.M.)
ii. Anesthetized Animal Study

In all but one experiment the rabbits were conscious throughout the procedure. To determine the potential effect that fright or anxiety (or any other condition which is altered in the unconscious state) might have had on the experimental results, one anesthetized rabbit experiment was conducted. The results are illustrated on Fig. D. 15 compared with the overall average results from all 30 minute LDL studies. With the exception of the value near $x/L = 1$, the solute accumulation was everywhere slightly greater in the anesthetized animal. However, none of the differences were statistically significant ($p=0.05$). Thus, these results suggest that the unconscious state did not measurably alter the solute uptake and accumulation process.

d. Special Injectate and Plasma Analysis

i. Protein Iodination Efficiencies and Injectate Precipitable Radioactivity

The efficiency of labeling LDL with $^{125}\text{I}$ and $^{131}\text{I}$ ranged between 13.8% and 70% with an average of 38.1%. The efficiency is defined as the fraction of total radioactivity which is bound to the protein. The results from all experiments are given on Table D. XVIII. Also shown is the percentage of precipitable radioactivity (protein-bound) in the injected solution. The average was 95.4% with most of the injectates having less than 2% non-precipitable radioactivity.

For albumin, labeling efficiencies ranged from 69.3% to 82.8% with an average of 77%. Injectate precipitable radioactivity averaged 99.5% (Table D. XIX.).
Figure D.15 Effect of Anesthetizing the Rabbit on Labeled LDL Distribution (t = 30 Minutes)

- Anesthetized Animal (Exp. 149; ± S.E.M.)
- Overall Average of all 30 Minute Exp. (± S.E.M.)

Graph showing the relative tissue concentration ($C/C_p$) against the relative position ($X/L$).
TABLE D.XVIII.

LDL Iodination Efficiencies and Injectate Precipitable Radioactivity

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>C\textsubscript{LDL-protein}</th>
<th>Mol Wt*</th>
<th>Efficiency\textsuperscript{++} (%)</th>
<th>Injectate Ppt** (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>137</td>
<td>9.5</td>
<td>500,000</td>
<td>35.6</td>
<td>3.91</td>
</tr>
<tr>
<td>138</td>
<td>∼3.0</td>
<td>500,000</td>
<td>28.4</td>
<td>-</td>
</tr>
<tr>
<td>139</td>
<td>8.8</td>
<td>500,000</td>
<td>45.0</td>
<td>-</td>
</tr>
<tr>
<td>140</td>
<td>∼9.0</td>
<td>500,000</td>
<td>29.7</td>
<td>-</td>
</tr>
<tr>
<td>141</td>
<td>∼9.0</td>
<td>500,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>142-\textsuperscript{131}I</td>
<td>∼7.0</td>
<td>500,000</td>
<td>35.2</td>
<td>-</td>
</tr>
<tr>
<td>142-\textsuperscript{125}I</td>
<td>∼7.0</td>
<td>500,000</td>
<td>49.8</td>
<td>-</td>
</tr>
<tr>
<td>144-145</td>
<td>∼8.0</td>
<td>500,000</td>
<td>34.6</td>
<td>-</td>
</tr>
<tr>
<td>146-\textsuperscript{131}I</td>
<td>∼7.0</td>
<td>500,000</td>
<td>37.2</td>
<td>-</td>
</tr>
<tr>
<td>146-\textsuperscript{125}I</td>
<td>∼7.0</td>
<td>500,000</td>
<td>37.6</td>
<td>-</td>
</tr>
<tr>
<td>147-\textsuperscript{131}I</td>
<td>6.6</td>
<td>500,000</td>
<td>20.7</td>
<td>-</td>
</tr>
<tr>
<td>147-\textsuperscript{125}I</td>
<td>6.6</td>
<td>500,000</td>
<td>22.4</td>
<td>-</td>
</tr>
<tr>
<td>149&amp;150</td>
<td>5.4</td>
<td>500,000</td>
<td>23.0</td>
<td>-</td>
</tr>
<tr>
<td>154</td>
<td>∼8.0</td>
<td>100,000</td>
<td>43.6</td>
<td>-</td>
</tr>
<tr>
<td>155</td>
<td>∼2.5</td>
<td>100,000</td>
<td>33.4</td>
<td>-</td>
</tr>
<tr>
<td>156</td>
<td>∼5.0</td>
<td>100,000</td>
<td>55.8</td>
<td>-</td>
</tr>
<tr>
<td>157</td>
<td>∼5.0</td>
<td>100,000</td>
<td>46.7</td>
<td>-</td>
</tr>
<tr>
<td>160</td>
<td>∼7.5</td>
<td>100,000</td>
<td>26.2</td>
<td>-</td>
</tr>
<tr>
<td>161-163a</td>
<td>∼3.5</td>
<td>100,000</td>
<td>13.8</td>
<td>-</td>
</tr>
<tr>
<td>161-163b</td>
<td>∼3.5</td>
<td>100,000</td>
<td>70.0</td>
<td>-</td>
</tr>
<tr>
<td>164-166</td>
<td>∼6.0</td>
<td>100,000</td>
<td>55.6</td>
<td>-</td>
</tr>
<tr>
<td>167-\textsuperscript{131}I</td>
<td>3.0</td>
<td>100,000</td>
<td>44.5</td>
<td>-</td>
</tr>
<tr>
<td>167-\textsuperscript{125}I</td>
<td>3.0</td>
<td>100,000</td>
<td>49.3</td>
<td>-</td>
</tr>
</tbody>
</table>

| Mean     | 38.1                      |         | 95.4                     |

| ±        | 13.5                      | 5.6      |

\textsuperscript{+} mg/ml

\textsuperscript{++} \% TCA precipitable radioactivity after labeling

\textsuperscript{*} The assumed molecular weight is inversely proportional to the quantity of oxidizing agent, ICI, which is added

\textsuperscript{**} \% TCA precipitable radioactivity after repeated dialysis
TABLE D.IX.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Labeling Efficiency + (%)</th>
<th>Injectate Precipitability ++ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>135</td>
<td>-</td>
<td>99.7</td>
</tr>
<tr>
<td>136</td>
<td>71.5</td>
<td>99.7</td>
</tr>
<tr>
<td>150</td>
<td>76.4</td>
<td>99.8</td>
</tr>
<tr>
<td>151</td>
<td>69.3</td>
<td>98.7</td>
</tr>
<tr>
<td>153</td>
<td>82.6</td>
<td>-</td>
</tr>
<tr>
<td>158-159</td>
<td>82.8</td>
<td>99.4</td>
</tr>
<tr>
<td>168-170</td>
<td>79.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean 77.0 ± 5.7  99.5 ± 0.5

+ % TCA precipitable radioactivity after labeling
++ % Radioactivity precipitable in 10% TCA after repeated dialysis
In some LDL experiments the fraction of the LDL radioactivity removable by lipid extraction (3:1 ether:ethanol) was determined. The results are presented on Table D. XX. Only a small percentage of the $^{125}\text{I}$ radioactivity was found in the extract. This indicates that the majority of the label was bound to protein moiety of LDL with very little label introduced into the lipid moiety.

ii. **Rabbit Plasma Cholesterol Analysis**

Rabbit serum cholesterol determinations were made before and after the injection of the radioactive LDL. The results from all experiments are found in Table D. XVI. In most experiments the introduction of LDL did not significantly elevate the serum cholesterol concentration. However, in three experiments (Numbers 146, 147 and 149) a booster dose of unlabeled LDL was given as well. This did result in a significant increase. Thus, some of the experiments were not classical tracer studies since the injectate of labeled solute did change the solute concentration in the plasma.
# TABLE D.XX.

% Radioactivity in Ethanol-Ether Extracts of Iodinated LDL Preparations

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>% Radioactivity in Lipid Extract</th>
<th>LDL Injectate (%)</th>
<th>LDL Plasma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>137 (N=6)</td>
<td></td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
<td>139 (N=2)</td>
<td></td>
<td>8.3</td>
<td>3.0</td>
</tr>
<tr>
<td>142a (N=3)⁺</td>
<td></td>
<td>-</td>
<td>3.2</td>
</tr>
<tr>
<td>142b (N=2)++</td>
<td></td>
<td>-</td>
<td>1.4</td>
</tr>
</tbody>
</table>

⁺ ¹²⁵I-LDL
++ ¹³¹I-LDL
<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Before Injection</th>
<th>After Injection</th>
<th>$\text{Volume LDL Injected}$</th>
<th>$\text{Time (min)}$</th>
<th>$\text{Sex}$</th>
<th>$\text{C cholesterol}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>156</td>
<td>15</td>
<td>26, 27, 23, 20</td>
<td>3.3 ml</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>157</td>
<td>19</td>
<td>28</td>
<td>3.3 ml</td>
<td>4.5</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>57</td>
<td>63</td>
<td>4.6 ml</td>
<td>5</td>
<td>F?</td>
<td></td>
</tr>
<tr>
<td>161</td>
<td>39</td>
<td>39</td>
<td>1.5 ml</td>
<td>5</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>162</td>
<td>39</td>
<td>35</td>
<td>1.6 ml</td>
<td>6</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>163</td>
<td>37</td>
<td>43</td>
<td>1.5 ml</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>164</td>
<td>38</td>
<td>38</td>
<td>2.6 ml (286 mg%)</td>
<td>3</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>81</td>
<td>80</td>
<td>2.9 ml (286 mg%)</td>
<td>5</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>166</td>
<td>54</td>
<td>56</td>
<td>2.7 ml (286 mg%)</td>
<td>5</td>
<td>F</td>
<td></td>
</tr>
</tbody>
</table>

$\text{C cholesterol}$ in $\text{mg/100ml}$.
E. Solutions to Partial Differential Equations

The details of the method for solving the partial differential equations which describe diffusion and convection of solute across the arterial wall are covered in this appendix. The problem formulation is presented in VI. THEORY.

1. Convection and Diffusion without Internal Reaction

The governing partial differential equation for diffusion and convection of solute across a finite slab of thickness, L, is as follows:

\[ D_{\text{eff}} \frac{\partial^2 C(x,t)}{\partial x^2} - \frac{J_f}{\varepsilon} \frac{\partial C(x,t)}{\partial x} = \frac{\partial C(x,t)}{\partial t} \quad (E1) \]

This formulation applies to the case where the effective solute diffusion coefficient, \( D_{\text{eff}} \), does not vary with solute concentration, \( C(x,t) \), nor with position, \( x \). The boundary and initial conditions which apply are:

**Case (i)**

for \( t = 0 \): \( C(x,0) = 0 \) \hfill (E2a)

for \( x = 0 \):

\[ \frac{J_f}{\varepsilon} C(0,t) + \frac{K_1}{\varepsilon} (\varepsilon C_p(t) - C(0,t)) \]

\[ = \frac{J_f}{\varepsilon} C(0,t) - D_{\text{eff}} \frac{\partial C(0,t)}{\partial x} \quad (E2b) \]

for \( x = L \):

\[ \frac{K_{AC} L C}{\varepsilon A_M} + \frac{J_f C(1-R_2)}{\varepsilon} + \frac{K_{AC} (C(L,t) - \varepsilon C_p(t))}{\varepsilon A_M} \]

\[ = \frac{J_f}{\varepsilon} C(L,t) - D_{\text{eff}} \frac{\partial C(L,t)}{\partial x} \quad (E2c) \]
Two other cases are also of interest. These involve variations in the boundary specification at \( x = L \).

**Case (ii)**

for \( x = L \): \( C(L,t) = \kappa \cdot C_p(t) \) \hspace{1cm} (E2d)

**Case (iii)**

for \( x = L \): \( \frac{\partial C(L,t)}{\partial x} = 0 \) \hspace{1cm} (E2e)

All cases are identical formulations except for the evaluation of the constants in the \( x = L \) boundary condition. Thus, if the boundary condition is generalized, the solutions except for certain constants are identical. For all cases we have in general at \( x = L \):

\[ k'_2 C(L,t) - k'_1 \frac{\partial C(L,t)}{\partial x} = k'_3 C_p(t) \] \hspace{1cm} (E3)

**Case (i):**

\[ k'_1 = \frac{D_{eff}}{J_{eff}} \]

\[ k'_2 = \frac{R_2}{\varepsilon} - \left( \frac{K_L A_L + K_C A_C}{\varepsilon A_m} \right) \]

\[ k'_3 = \frac{-K_C A_C}{A_m} \] \hspace{1cm} (E3a)

**Case (ii):**

\[ k'_1 = 0 \]

\[ k'_2 = 1 \]

\[ k'_3 = \kappa \] \hspace{1cm} (E3b)

**Case (iii):**

\[ k'_1 = 1 \]

\[ k'_2 = 0 \]

\[ k'_3 = 0 \] \hspace{1cm} (E3c)

The plasma concentration, \( C_p(t) \), is assumed to vary in the following fashion:

\[ C_p(t) = C_p(0) \sum_{k=1}^{2} \alpha_k e^{-\beta_k t} ; \alpha_1 + \alpha_2 = 1 \] \hspace{1cm} (E4)
Thus, the plasma concentration tends toward zero with increasing time.

Equation (E1) can be cast in dimensionless form by using the following transformations:

\[\eta = x/L\]  \hspace{1cm} (E5a)

\[\tau = D_{\text{eff}} t/L^2\]  \hspace{1cm} (E5b)

\[P = Jf L/D_{\text{eff}}\epsilon\]  \hspace{1cm} (E5c)

\[\Psi(\eta, \tau) = C(\eta, \tau)/C_p(0)\]  \hspace{1cm} (E5d)

\[T_1 = K_{1L}L/D_{\text{eff}}\epsilon\]  \hspace{1cm} (E5e)

\[K_2' = k_2' L/D_{\text{eff}}\]  \hspace{1cm} (E5f)

\[K_3' = k_3' L/D_{\text{eff}}\]  \hspace{1cm} (E5g)

\[K_1' = k_1'/D_{\text{eff}}\]  \hspace{1cm} (E5h)

Equation (E1) becomes:

\[\frac{\partial^2 \Psi(\eta, \tau)}{\partial \eta^2} - p \frac{\partial \Psi(\eta, \tau)}{\partial \eta} = \frac{\partial \Psi}{\partial \tau}\]  \hspace{1cm} (E6)

The boundary and initial conditions become:

for \(\tau = 0\): \(\Psi(\eta, 0) = 0\)  \hspace{1cm} (E7a)

for \(\eta = 0\): \(\epsilon P(1-R_1) \frac{C_p(t)}{C_p(0)} + T_1 \epsilon \frac{C_p(t)}{C_p(0)}\)

\[= (P+T_1)\Psi(0, \tau) - \frac{\Psi}{\eta}(0, \tau)\]  \hspace{1cm} (E7b)

for \(\eta = 1\): \(K_3' \frac{C_p(t)}{C_p(0)}\)

\[= K_2'\Psi(1, \tau) - K_1' \frac{\partial \Psi}{\partial \eta}(1, \tau)\]  \hspace{1cm} (E7c)
The approach taken in solving (E6) with (E7) is to find the solution for the case of a step change in plasma concentration, \(C_p(t)\), from zero to time invariant \(C_p(0)\). Then, by means of the Duhamel superposition integral theorem, determine the solution for the case where \(C_p(t)\) varies according to (E4). Therefore, we initially assume that \(C_p(t) = C_p(0) \neq f(t)\). That is, we first solve the case of time invariant plasma concentration, and then apply that solution to obtain the equation which describes the case where the plasma concentration is varying with time. Thus, (E7) becomes:

for \(\tau = 0\):  \(\Psi(\eta,0) = 0\)  

(E8a)

for \(\eta = 0\):  \(\varepsilon P(1-R_1) + T_1\varepsilon = (P+T_1)\Psi(0,\tau) - \frac{\partial \Psi}{\partial \eta}(0,\tau)\)  

(E8b)

for \(\eta = 1\):  \(K_1 = K_2'\Psi(1,\tau) - K_1' \frac{\partial \Psi}{\partial \eta}(1,\tau)\)  

(E8c)

The solution for (E6) with (E8) is obtained using Laplace transforms. Taking the Laplace transform of all equations, we have:

\[
\frac{d^2\Psi(\eta,s)}{d\eta^2} - p \frac{d\Psi(\eta,s)}{d\eta} = s\Psi  
\]

(E9)

for \(\eta = 0\):  \(\frac{\varepsilon P(1-R_1) + T_1\varepsilon}{s} = (P+T_1)\Psi(0,s) - \frac{d\Psi}{d\eta}(\eta,s)\)  

(E10a)

for \(\eta = 1\):  \(\frac{K_1'}{s} = K_2'\Psi(1,s) - K_1' \frac{d\Psi}{d\eta}(1,s)\)  

(E10b)

Equation (E9) is an ordinary differential equation which can readily be solved. The solution is given as:
\[
\frac{\Pi}{\eta} = e^{\frac{2}{2}[c_1 \cosh(\alpha \eta) + c_2 \sinh(\alpha \eta)]}
\]

(EL1)

where \( \alpha = (P^2 + 4s)^{\frac{1}{2}}/2 \), and \( c_1 \) and \( c_2 \) are constants which satisfy (EL0a) and (EL0b). The constants are given by the following relationships:

\[
c_1 = \frac{T_1 \varepsilon + P(1-R_1) \varepsilon}{s} - \alpha
\]

\[
\frac{K_1^\prime}{s} \exp\left(\frac{P}{2}\right) \left\{ (K_1^\prime - K_1^P/2) \sinh(\alpha) - \alpha K_1^\prime \cosh(\alpha) \right\}
\]

(EL2)

Defining the denominator of (EL2) as \( \Delta \), the other constant becomes:

\[
c_2 = \frac{(P^2 + T_1)}{s} \varepsilon \exp\left(\frac{P}{2}\right) \left\{ (K_2^\prime - PK_1^\prime/2) \cosh(\alpha) - \alpha K_1^\prime \sinh(\alpha) \right\}
\]

(EL3)
Combining (El1) - (El3) yields:

\[
\psi(\eta, s) = \frac{e^{(\eta-1)P}}{s} \left\{ \left( T_1 e^{+\eta(T+R_1)} \right) e^{2 \left( K_2 - \frac{PK_1}{2} \right)s} \right\} \left( \left( T_1 e^{+\eta(T+R_1)} \right) e^{2 \left( K_2 - \frac{PK_1}{2} \right)s} \right)^{-1} \]

\[
\cdot \cosh (\alpha) + \left\{ \left( \frac{e^{+\eta(T+R_1)}}{T_1} \right) e^{2 \left( K_2 - \frac{PK_1}{2} \right)s} \right\} \left( \left( \frac{e^{+\eta(T+R_1)}}{T_1} \right) e^{2 \left( K_2 - \frac{PK_1}{2} \right)s} \right)^{-1} \cosh (\alpha)
\]

\[
-\left( K_1' \alpha \sinh \alpha \right) \left( \sinh (\alpha) \right) \left( \sinh (\alpha) \right) / \left( \left( K_2 - \frac{PK_1}{2} \right) e^{2 \left( K_2 - \frac{PK_1}{2} \right)s} \right) \cosh (\alpha)
\]

\[
+ \alpha ( K_2 - T_1 K_1' - PK_1') \cosh \alpha
\]

(E14)

The inversion of (E14) back to the dimensionless time domain, \( \tau \), is accomplished by the method of residues (229). Equation (E14) has a simple pole at \( s = 0 \) and a series of poles at \( s = -\lambda_m^2 - \frac{P^2}{4} \), where \( \lambda_m \) is defined by the zeroes of:

\[
\tan \lambda_m = \frac{-\lambda_m ( K_2^I - T_1 K_1' - PK_1')}{\left( K_2^I - \frac{PK_1'}{2} \right) (T_2 + T_1) + K_1' \lambda_m^2}
\]

(E15)

This equation defines the Eigen values, \( \lambda_m \). It can be derived by evaluating \( \alpha \) when the denominator of (E14) is equated with zero.
Only for imaginary values, \( \alpha = i\lambda_m \), do solutions to (E15) exist. Thus, each \( \lambda_m \) which satisfies (E15), defines a pole of (E14). Since \( \alpha = \frac{(P^2+4s)^{1/2}}{2}, s_m = -\frac{\lambda_m^2-P^2}{4} \) are poles of (E14).

According to the method of residues, the inverse of (E14) is given by the summation of the residues at each pole.

\[
\Psi(\eta,\tau) = \text{Res}(s=0) + \sum_{m=1}^{\infty} \text{Res}(s=-\frac{\lambda_m^2-P^2}{4}) \tag{E16}
\]

where \( \text{Res} \) refers to the residue of \( \overline{\Psi}(\eta,s) \). Evaluating the residue at \( s = 0 \) by equation (7-221) and the residues at \( s_m = \frac{\lambda_m^2-P^2}{4} \) by equation (7-225) as described by Arpaci (229) we have,

\[
\Psi(\eta,\tau)_{\text{step}} = A(B+2 \sum_{m=1}^{\infty} Q_m \exp(-\frac{\lambda_m^2+P^2}{4} \tau)) \tag{E17}
\]

where

\[
\text{Res}(s=0) = AB = e^{\frac{P}{2}(\eta-1)} \left\{ \frac{P}{2} (T_1 \cosh(\frac{P}{2}) + \frac{PK'}{2}) \cosh(\frac{P}{2}) + \left\{ (K_1^2 - T_1) K_1 - (T_1 \cos \eta + 1 - R_1) \right\} \cosh(\frac{PK'}{2}) \cosh(\frac{P}{2}) \right\}
\]

\[
\frac{P}{2} \left( (K_1^2 - T_1) K_1 - (T_1 \cos \eta + 1 - R_1) \right) \cosh(\frac{PK'}{2}) \cosh(\frac{P}{2}) \left\{ (K_1^2 - T_1) - \frac{PK'}{2} \right\} \sinh(\frac{P}{2}) \right\}
\]

\[
A = e^{\frac{P}{2}(\eta-1)} \tag{E18b}
\]
and
\[ 2A \sum_{m=1}^{\infty} Q_m \exp(-(\lambda_m^2 + P^2/4)\tau) = \sum_{m=1}^{\infty} \text{Res}(s = -\lambda_m^2 - P^2/4) \]  
(El9a)

\[ \sum_{m=1}^{\infty} \text{Res}(s = -\lambda_m^2 - P^2/4) = 2 \sum_{m=1}^{\infty} \lambda_m \left\{ \exp\left(\frac{P}{2}(\eta-1) - (\lambda_m^2 + P^2/4)\tau\right) \right\} \left\{ T_1 + e^P(1-R_1) \right\} \exp\left(\frac{P}{2}\right) \{ (K_2' - PK_1')/2 \} \sin(\lambda_m) + \lambda_m K_1' \cos(\lambda_m) + \\
+ \left\{ (\lambda_m^2 + P^2/4) \right\} \{ (K_2' - PK_1')/2 \} (\frac{P}{2} + T_1) + K_1' \lambda_m^2 + \\
+ (K_2' - T_1 K_1' - PK_1') \cos(\lambda_m) + \lambda_m \left\{ 2K_1' - (K_2' - T_1 K_1' - PK_1') \right\} \sin(\lambda_m) \]  
(El9b)

where \( Q_m \) is defined by comparing with (El9a) and (El9b). That is, \( Q_m \) equals the bracketed term in (El9b) which follows \( \exp\left(\frac{P}{2}(\eta-1) - (\lambda_m^2 + P^2/4)\tau\right) \); \( B \) equals the term following \( \exp\left(\frac{P}{2}(\eta-1)\right) \) in (El9a), and \( A = \exp\left(\frac{P}{2}(\eta-1)\right) \).

To determine \( \Psi(\eta, \tau) \) for plasma isotope concentrations which change over time, Duhamel's superposition integral is employed. The integral applied to this case is:

\[ \Psi(\eta, \tau) = \Psi(\eta, \tau)_{\text{step}} F(0) + \int_{0}^{\tau} \Psi(\eta, \tau-\xi) \text{step} \frac{dF(\xi)}{d\xi} d\xi \]  
(E20)

where

\[ F(\xi) = \sum_{k=1}^{2} \alpha_k e^{-\beta_k L^2 \xi / D_{\text{eff}}} \]  
(E21)
and

\[ F(0) = 1 \] (E22)

Substitution of (E17) and (E21) into (E20) followed by the indicated integration results in the desired solution.

\[
\Psi(n, \tau) = A \left\{ B + 2 \sum_{m=1}^{\infty} Q_m \exp(-\lambda_m^2 + P^2/4) \tau \right\} + AB \left\{ \sum_{k=1}^{2} \alpha_k \exp(-\beta_k \tau) - 1 \right\}
\]

\[
+ 2A \sum_{m=1}^{\infty} \sum_{k=1}^{2} \frac{\alpha_k \beta_k Q_m t \exp(-\beta_k t) - \exp(-\lambda_m^2 + P^2/4) \tau)}{\beta_k t - (\lambda_m^2 + P^2/4) \tau}
\]

(E23)

Note that the first term in (E23) is identical to the solution for the case of constant plasma isotope concentration, (E17). Hence, the second two terms account for the change in plasma concentration with time. This solution is completely general in that it applies to all cases (i - iii) with boundary condition at \( x = L \) evaluated using the appropriate form of equation (E3). By inspection, the general solution is found to be consistent with the prescribed initial condition of \( \Psi(n, 0) = 0 \). Also note that in the limit as \( t \to \infty \), \( \tau \to \infty \), \( \Psi(n, \tau) \) tends to 0. Thus, the relative tissue concentrations do not attain steady values so long as the plasma isotope concentration decays continuously over time.

Equation (E23) was evaluated by means of machine calculations performed on an IBM 370 computer. The details of the program used are found in APPENDIX F.
2. Convection and Diffusion with Reversible Internal Reaction

The governing partial differential equation for diffusion and convection of solute across a finite slab of thickness, \( L \), in which solute reacts reversibly within the slab is as follows:

\[
D_{\text{eff}} \frac{\partial^2 C(x,t)}{\partial x^2} - \frac{J}{c} \frac{\partial C(x,t)}{\partial x} = \frac{\partial C(x,t)}{\partial t} + \frac{\partial C(x,t)}{\partial t} \quad (E24)
\]

where the last term denotes the rate of solute removal due to chemical reaction. The reaction kinetics are assumed to be of the form:

\[
\frac{\partial C_c(C,t)}{\partial t} = k_1 b \left( C(x,t) - \frac{k_2}{k_1 b} C_c(C,t) \right) \quad (E25)
\]

where \( b \) is the kinetic rate constant and \( m \) is the reversibility constant. In the case of isotopic solute moving through the arterial wall, the chemical reaction term is best interpreted as the rate at which labeled solute is bound to some stationary phase. The total concentration of labeled solute in the wall at any one time would, therefore, be represented by the sum of the mobile solute \( C(x,t) \) and the bound solute \( C_c(C,t) \) concentrations. This kinetic model was chosen \textit{a priori} without any experimental basis since the mechanism whereby LDL may bind to elastin and/or AMPS in the arterial wall is not known. However, the mathematical development which follows should be appropriate for other simple kinetic expressions as well.
The method of solution of this problem is identical to the case of no reaction within the wall. The boundary and initial specifications are:

at \( x = 0 \):
\[
\frac{J_f}{\varepsilon} C(0,t) - D_{\text{eff}} \frac{\partial C(0,t)}{\partial x} = \frac{K_1}{\varepsilon} (\varepsilon C_p(t) - C(0,t)) + J_f C_p(t)(1-R_1)
\]  
(E26a)

at \( x = L \):
\[
k_2' C(L,t) - k_1' \frac{\partial C(L,t)}{\partial x} = k_3' C_p(t)
\]  
(E26b)

for \( t = 0 \):
\[
C(x,0) = 0
\]  
(E26c)

\[
C_c(x,0) = 0.
\]  
(E26d)

The boundary condition at \( x = L \) is written in general form to allow the solution of the following cases for \( x = L \):

case (i)  
\[
(1-R_2)J_f \frac{C(L,t)}{\varepsilon} + \frac{K_A C(L,t)}{A \varepsilon} + \frac{V_x C(L,t)-\varepsilon C_p(t)}{A \varepsilon} = \frac{J_f}{\varepsilon} C(L,t) - D_{\text{eff}} \frac{\partial C(L,t)}{\partial x}
\]  
(E27a)

case (ii)  
\[
C(L,t) = k C_p(t)
\]  
(E27b)

case (iii)  
\[
\frac{\partial C}{\partial x}(L,t) = 0.
\]  
(E27c)

The evaluation of \( k_1' \), \( k_2' \) and \( k_3' \) are as given previously (equation (E3)). Defining \( N(x,t) = C_c(x,t)/C_p(0) \) and using (E5), the dimensionless form of (E24) and (E26) is developed:
\[
\frac{\partial^2 \Psi(n,\tau)}{\partial n^2} - p \frac{\partial \Psi(n,\tau)}{\partial n} = \frac{\partial \Psi(n,\tau)}{\partial \tau} + \frac{\partial N(n,\tau)}{\partial \tau}
\]  
(E28)

\[
\frac{\partial N(n,\tau)}{\partial \tau} = \Theta(\Psi(n,\tau) - mN(n,\tau))
\]  
(E29)

for \(\tau = 0\): \(\Psi(n,0) = N(n,0) = 0\)  
(E30a)

at \(\eta = 0\): \(\epsilon (1-R_1)P + T_1 \epsilon = (P+T_1)\Psi(0,\tau) - \frac{\partial \Psi}{\partial \eta}(0,\tau)\)  
(E30b)

at \(\eta = 1\): \(K'_3 = K'_2 \Psi(1,\tau) - K'_1 \frac{\partial \Psi(n,\tau)}{\partial \eta}\)  
(E30c)

where \(\Theta \equiv \frac{L}{k_1 C_b} D_{eff}, \ c_p(t) = c_p(0), \text{ and } m = k_2/k_1 C_b\).

The Laplace transform of (E28) - (E30) is:

\[
\frac{d^2 \overline{\Psi}(n,s)}{d\eta^2} - p \frac{d \overline{\Psi}(n,s)}{d\eta} - \left( \frac{s \Theta}{s + m \Theta} + s \right) \overline{\Psi}(n,s) = 0
\]  
(E31)

\[
SN(0) = \Theta(\overline{\Psi}(n,s) - mN)
\]  
(E32)

at \(\eta = 0\): \(\overline{\Psi}(n,s) - \frac{d \overline{\Psi}(0,s)}{d\eta} = T_1 \left( \frac{\epsilon}{s} - \overline{\Psi}(0,s) \right) + \frac{P}{s} (1-R_1)\)  
(E32a)

at \(\eta = 1\): \(K'_2 \overline{\Psi}(1,s) - K'_1 \frac{d \overline{\Psi}(1,s)}{d\eta} = \frac{K'_3}{s}\)  
(E32b)
The solution to (E31) - (E33) is given by (E14) except for $\alpha$. In this case

$$\alpha = \frac{1}{2} \left\{ p^2 + 4 \left( \frac{s\Theta}{s+m\Theta} + s \right) \right\}^{1/2}$$  \hspace{1cm} (E34)

The inversion of (E14) back to the dimensionless time domain, $\tau$, is accomplished, as before, by the method of residues (229). Equation (E14) has a simple pole at $s = 0$ and a series of poles at $\alpha = i\lambda$, where $\lambda$ is given by the zeroes of (E15). For $\alpha = i\lambda$, $s$ can take on two possible forms, $s = \frac{1}{2}(-R + \sqrt{U})$, where

$$R = m\Theta + \Theta + p^2/4 + \lambda^2$$  \hspace{1cm} (E35)

$$U = R^2 - 4\Theta(p^2/4 + \lambda^2)$$  \hspace{1cm} (E36)

The inversion procedure requires the summation of the residues at $s=0$ and $\alpha=i\lambda$. Since $s$ can take on two possible values for $\alpha=i\lambda$, one must first determine which of the two possible residues to use in the inversion procedure. The residue takes the form

$$\sum_{\lambda=1}^{\infty} \text{Res}(\alpha = i\lambda) = -2A^- \sum_{\lambda=1}^{\infty} \frac{Q^\lambda}{\lambda} \exp \left( \frac{S_{\lambda,\eta}}{n} \right) \exp \left( \frac{p}{2(n-1)} \right) \hspace{1cm} (E37)$$

where

$$A^- = \exp \left( \frac{p}{2(n-1)} \right) \hspace{1cm} (E38)$$
The proper selection of $S_{\lambda,n}$ can be made by comparing the relative behavior of the two forms for $\theta=0$. In this situation the residue (E37) should reduce to exactly the same form developed in the previous section for no binding (E19). Only if $S = i\xi (-R_{\lambda} - \sqrt{U}_{\lambda})$ is this condition met. Thus, the residue at $\alpha_{\lambda} = i\lambda_{\lambda}$ is evaluated using $S_{\lambda,1}$, and not $S_{\lambda,2}$.

Using the residue for $S_{\lambda,1}$, the total inversion of (E14)
becomes:

\[ \Psi(n, r)_{\text{step}} = A' \left\{ \sum_{k=1}^{\infty} \frac{Q_k' \exp(S_k, 1, r)}{S_k, 1, Z_k, 1} \right\} \]  \hspace{1cm} (E42)

where \( A', Q_k', S_k, Z_k, 1 \) are as given by (E38)-(E41) and

\[ B' = \left\{ \left( T_1 e^{P(T_1 - P_k')/2} \right) \left( \cosh \left( \frac{P_k'}{2} \right) \right) \right\} + \left\{ \left( \frac{P_k'}{2} + T_1 K_3' - \left( T_1 e^{P(T_1 - P_k')/2} \right) \right) \right\} \times \left( \frac{P_k'}{2} \right) \cosh \left( \frac{P_k'}{2} \right) \right\} / \left\{ \left( K_2' - PK_1' \right) \right\} \times \left( \frac{P_k'}{2} + T_1 K_1' - \left( T_1 e^{P(T_1 - P_k')/2} \right) \right) \times \left( \frac{P_k'}{2} \right) \cosh \left( \frac{P_k'}{2} \right) \right\} \]  \hspace{1cm} (E43)

The solution given by (E42) applies to the condition of a unit step change in dimensionless plasma isotope concentration, \( C_p(t)/C_p(0) \). For plasma isotope variation in time

\[ \frac{C_p(t)}{C_p(0)} = \sum_{k=1}^{2} \alpha_k \exp(-\beta_k t); \quad \alpha_1 + \alpha_2 = 1 \]  \hspace{1cm} (E4)
the solution is obtained by applying Duhamel's superposition integral, (E20)-(E22) to (E42). The result of this integration is:

\[
\Psi(\eta, \tau) = A' \left\{ B' - 2 \sum_{k=1}^{\infty} \frac{Q_k \exp(S_{k,1} \tau)}{S_{k,1} Z_{k,1}} \right\} + A' B' \left\{ \sum_{k=1}^{2} \alpha_k \exp(-\beta_k \tau) - 1 \right\} - 2A' \sum_{k=1}^{\infty} \frac{\alpha_k \beta_k Q_k \tau \{\exp(-\beta_k \tau) - \exp(S_{k,1} \tau)\}}{S_{k,1} Z_{k,1} (\beta_k \tau + S_{k,1} \tau)}
\]

\(\Psi(\eta, \tau)\) represents only the mobile labeled solute in the arterial wall. The total labeled solute concentration is the sum of the bound and mobile solute dimensionless concentrations:

\[
\xi(\eta, \tau) = \Psi(\eta, \tau) + N(\eta, \tau)
\]

The bound labeled solute at any position, \(\eta\), can be determined by solving the ordinary differential equation

\[
\frac{dN}{d\tau} = 0(\Psi - mN)
\]

The solution is:
\[ N(\eta, \tau) = \exp(-m\Theta T) \int_{0}^{T} \Theta \Psi(\eta, \tau) \exp(m\Theta \tau) d\tau \quad (E46) \]

Using (E44) for \( \Psi(\eta, \tau) \), upon integration one finds:

\[
N(\eta, \tau) = \Theta \left[ A^{-} \left\{ \frac{-B'(1-\exp(-m\Theta T))}{m\Theta} - \sum_{\lambda=1}^{\infty} \frac{Q_{\lambda}^{r}(\exp(S_{\lambda,1} T) - \exp(-m\Theta T))}{(S_{\lambda,1} + m\Theta)S_{\lambda,1}Z_{\lambda,1}} \right\} + \right.
\]

\[
+ A^{-} B^{-} \left\{ \sum_{k=1}^{2} \frac{a_{k}^{r}\tau}{(m\Theta - \beta_{k}^{r})} \frac{\exp(-\beta_{k}^{r} t) - \exp(-m\Theta t)}{m\Theta} - \frac{\exp(-m\Theta t)}{m\Theta} \right\} - \]

\[
- 2A^{-} \sum_{\lambda=1}^{\infty} \sum_{k=1}^{2} \frac{a_{k}^{r}\beta_{k}^{r} t \tau Q_{\lambda}^{r}}{S_{\lambda,1}Z_{\lambda,1}(\beta_{k}^{r} t + S_{\lambda,1} T)} \left\{ \frac{\exp(-\beta_{k}^{r} t) - \exp(-m\Theta t)}{m\Theta - \beta_{k}^{r} t} \right\} - \]

\[
- \frac{\exp(S_{\lambda,1} T) - \exp(-m\Theta T)}{\tau(S_{\lambda,1} + m\Theta)} \right\} \quad (E47) \]

Since \( S_{\lambda,1} \) is less than zero for all positive \( \Theta \) and \( m \), \( N(\eta, \tau) \rightarrow 0 \) as \( t \rightarrow \infty \) and \( \tau \rightarrow \infty \). Hence, the model predicts that at \( t=\infty \), all labeled solute will have been removed from the wall.

The foregoing development applies to the experimental situation where the plasma isotope concentration is decaying in time. Under real day-to-day conditions, the plasma LDL or albumin concentrations is fairly constant. Thus, the solute distribution across the aortic wall should attain a steady state level. The equilibrium
distribution of mobile solute is given by (E42). The bound solute
concentration can be determined by application of (E46) to (E42).

\[
N(\eta, \tau)_{\text{step}} = 0 \left\{ A^{-} \left[ B \left( 1 - \exp \left( -m \Theta t \right) \right) \right] + 2 \sum_{\ell=1}^{\infty} \frac{Q \left( \exp \left( S_{\ell,1} \tau \right) - \exp \left( -m \Theta t \right) \right)}{S_{\ell,1} Z_{\ell,1} (S_{\ell,1} + m \Theta)} \right\}
\]  

(E48)

At long times the second term in this equation vanishes, and the
remaining steady state total solute distribution is:

\[
\xi(\eta, \tau)_{\text{step-ss}} = A^{-} B^{-} \left( 1 + \frac{1}{m} \right)
\]  

(E49)

since \( \Psi(\eta, \tau)_{\text{step-ss}} = A^{-} B^{-} \) and \( N(\eta, \tau)_{\text{step-ss}} = A^{-} B^{-}/m \). The effect of
the binding is, therefore, simply to increase the solute concentra-
tion in the wall by \( \left( \frac{1}{m} \right) \% \). The fraction of solute bound at equilibrium
will be \( \frac{m}{m+1} \). Note that for \( m > 1 \), better than 50% of the solute
will be bound at equilibrium.

3. Convection and Diffusion with Nonuniformly Reversible
Internal Reaction

The foregoing development was based on the assumption
that labeled solute binding occurred at the same rate regardless
of position within the arterial wall. A computer simulation was
conceived for the case where the binding rate and diffusivity varied
with position. The complexity of the problem precluded finding an
exact analytical solution. Consequently, numerical solution techniques were employed.

The governing partial differential equation which applies to the case of variable diffusivity and variable binding rates is:

\[
\frac{\partial}{\partial x} \left( D^*_{\text{eff}}(x) \frac{\partial C(x, t)}{\partial x} \right) = \frac{f}{\varepsilon} C(x, t) + \frac{\partial C(x, t)}{\partial t} \quad (E50a)
\]

\[
\frac{\partial C(x, t)}{\partial t} = k_1c_b(x) \left[ C(x, t) - \frac{k_c(x, t)}{k_{1_c}(x)} \right] \quad (E50b)
\]

subject to:

for \( t = 0 \):

\[
C(x, 0) = C_c(x, 0) = 0 \quad (E50c)
\]

at \( x = 0 \):

\[
\frac{J}{\varepsilon} C(0, t) - D^*_{\text{eff}}(0) \frac{\partial C(0, t)}{\partial x} = \frac{K_1}{\varepsilon} \left[ \varepsilon C_p(t) - C(0, t) \right] +
\]

\[
+ J_f(1-R_1) C_p(t) \quad (E50d)
\]

at \( x = L \):

\[
\frac{J}{\varepsilon} C(L, t) - D^*_{\text{eff}}(L) \frac{\partial C(L, t)}{\partial x} = \frac{K}{A_m} \frac{C(L, t) - \varepsilon C_p(t)}{\varepsilon} +
\]

\[
+ \frac{K_1}{A_m} C(L, t) + J_f(1-R_2) \frac{C(L, t)}{\varepsilon} \quad (E50e)
\]

These equations can be cast in dimensionless form with the usual transformation \((E5)\) and the addition of:

\[
D^*_{\text{eff}}(\eta) = D_{\text{eff}}^f(\eta) \quad (E51)
\]

\[
\Theta^*(\eta) = \Theta_g(\eta) \quad (E52)
\]
where \( f(\eta) \) and \( g(\eta) \) are arbitrary functions describing the variation of \( D_{\text{eff}} \) and binding sites across the arterial wall. The dimensionless equations are:

\[
\frac{\partial}{\partial \eta} \left[ f(\eta) \frac{\partial \Psi(\eta, \tau)}{\partial \eta} \right] - \Psi(\eta, \tau) = \frac{\partial \Psi(\eta, \tau)}{\partial \tau} + \frac{\partial N(\eta, \tau)}{\partial \tau} \quad (E53)
\]

\[
\frac{\partial N(\eta, \tau)}{\partial \tau} = g(\eta) \left[ \Psi(\eta, \tau) - \frac{m}{g(\eta)} N(\eta, \tau) \right] \quad (E54)
\]

for \( \tau = 0 \):

\[
\Psi(\eta, \tau) = 0 = N(\eta, \tau) \quad (E55a)
\]

for \( \eta = 0 \):

\[
P \Psi(\eta, \tau) - f(0) \frac{\partial \Psi(0, \tau)}{\partial \eta} = T_1 \left[ \frac{C_p(t)}{C_p(0)} - \Psi(0, \tau) \right] + \frac{C_p(t)}{C_p(0)} \left[ 1 - R_1 \right] \quad (E55b)
\]

for \( \eta = 1 \):

\[
P \Psi(1, \tau) - f(1) \frac{\partial \Psi(1, \tau)}{\partial \eta} = T_2 \left[ \Psi(1, \tau) - \frac{C_p(t)}{C_p(0)} \right] + \frac{T_L \Psi(1, \tau)}{1 - R_2} + P \Psi(1, \tau) \{ 1 - R_2 \} \quad (E55c)
\]

\( \frac{C_p(t)}{C_p(0)} \) is taken to be as stated in (E4).

The numerical solution scheme used was that of Crank–Nicolson as described by Carnahan, et al. (230). This is an implicit scheme in that the simultaneous solution of \( N+1 \) (\( N = \) number of spatial grid points) equations is required for the calculation of the solute distribution at each dimensionless time step.
a. **Development of Numerical Recursion Formulas:**

Let \( N+1 \) equal the number of spatial grid points (\( N \) intervals) and the subscript, \( n \), denote the time increment step. Using central difference approximations, the finite difference form of (E53) is:

\[
f(i+\frac{1}{2},n) - f(i-\frac{1}{2},n) = -p \left\{ \frac{\psi_{i+\frac{1}{2},n} - \psi_{i-\frac{1}{2},n}}{\Delta\eta} \right\}
\]

Since

\[
\frac{\partial \psi_{i+\frac{1}{2},n}}{\partial \eta} = \frac{\psi_{i+1,n} - \psi_{i,n}}{\Delta\eta}
\]

\[
f(i)_{i+\frac{1}{2}} = \frac{f(i)_{i+1} + f(i)_{i}}{2}
\]

substitution yields:

\[
\psi_{i-1,n} \left\{ \frac{f(i)_{i} + f(i)_{i-1}}{2\Delta\eta^2} + \frac{p}{2\Delta\eta} \right\} - \psi_{i,n} \left\{ \frac{f(i)_{i+1} + f(i)_{i} + f(i)_{i-1}}{2\Delta\eta^2} \right\} +
\]

\[
+ \psi_{i+1,n} \left\{ \frac{f(i)_{i+1} + f(i)_{i}}{2\Delta\eta^2} - \frac{p}{2\Delta\eta} \right\} = \frac{\psi_{i,n+1} - \psi_{i,n}}{\Delta\tau} + \frac{N_{i,n+1} - N_{i,n}}{\Delta\tau}
\]

Using the Crank-Nicolson approximation,

\[
\psi_{i,n} = \frac{1}{2} \left( \psi_{i,n} + \psi_{i,n+1} \right)
\]
\[
\psi_{i-1,n+1} \left\{ \frac{f(\eta)_i + f(\eta)_{i-1}}{4\Delta\eta^2} + \frac{P}{4\Delta\eta} \right\} + \psi_{i,n+1} \left\{ \frac{-f(\eta)_{i-1} - 2f(\eta)_i - f(\eta)_{i+1}}{4\Delta\eta^2} \right\} - \\
- \frac{1}{\Delta t} \right) + \psi_{i+1,n+1} \left\{ \frac{f(\eta)_{i+1} + f(\eta)_{i}}{4\Delta\eta^2} - \frac{P}{4\Delta\eta} \right\} = \psi_{i-1,n} \left\{ \frac{-f(\eta)_{i-1} - f(\eta)_{i-1}}{4\Delta\eta^2} \right\} - \\
- \frac{P}{4\Delta\eta} \right) + \psi_{i,n} \left\{ \frac{f(\eta)_{i+1} + 2f(\eta)_{i} + f(\eta)_{i-1}}{4\Delta\eta^2} - \frac{1}{\Delta t} + \Theta g(\eta)_i \right\} + \\
+ \psi_{i+1,n} \left\{ \frac{-f(\eta)_{i+1} - f(\eta)_{i}}{4\Delta\eta^2} + \frac{P}{4\Delta\eta} \right\} = m^{\text{ON}}_{i,n} \tag{E56}
\]

The above equation is in the form:

\[
AA_i \psi_{i-1,n+1} + AB_i \psi_{i,n+1} + AC_i \psi_{i+1,n+1} = AD_i \quad \text{for} \quad 2 \leq i \leq N
\]

There are \(N-1\) such equations. The other two relationships required to solve for the \(N+1\) unknowns are given by the boundary conditions.

At \(\eta = 0\) (\(i=1\)):

\[
\frac{\partial \psi_i}{\partial \eta} = -\epsilon \left\{ T_1 + P(1-R_1) / \left( C_v(t) / C_v(0) + (P+T_1) \psi_i \right) \right\}
\]

from (E55b):

\[
\frac{\partial \psi_i}{\partial \eta} = \frac{1}{f(\eta)_i}
\]

Approximating (E53) as:
\[
f(n)_{i+\frac{1}{2}} = \frac{f(n) \frac{\partial \psi}{\partial \eta} + f(n) \frac{\partial \psi}{\partial \eta} + p \frac{\partial \psi}{\partial t}}{\frac{1}{\Delta \eta}} - p \frac{\partial \psi}{\partial \tau} = \frac{\partial \psi}{\partial \tau} + \frac{\partial N_i}{\partial \tau}
\]

and since

\[
f(n)_{i+\frac{1}{2}} = \{f(n)_{i} + f(n)_{i+1}\}/2
\]

\[
\frac{\partial \psi}{\partial \eta} = \{\psi_{i+1} - \psi_{i}\}/\Delta \eta
\]

we have:

\[
\psi_{i+1} = \psi_{i} - \frac{f(n)_{i}-f(n)_{i+1}}{\Delta \eta} - (P+T_{1}) \left( \frac{2}{\Delta \eta} + \frac{P}{f(n)_{i}} \right) + \psi_{i+1} \left( \frac{f(n)_{i}+f(n)_{i+1}}{\Delta \eta} \right) = \\
\psi_{i,n+1} - \psi_{i,n} + \frac{N_{i,n+1} - N_{i,n}}{\Delta \tau} - \frac{C_{p}(t)}{C_{p}(0)} \left( T_{1} \varepsilon + \frac{P(1-R_{1})}{\Delta \eta} \right) \left( \frac{P}{f(n)_{i}} + \frac{2}{\Delta \eta} \right)
\]

In the above equation, the time subscript, \( n \), has been omitted on the left side of the equation for convenience.

Using the Crank-Nicolson formulation:

\[
\psi_{i,n+1} \left\{ \frac{f(n)_{i}+f(n)_{i+1}}{2\Delta \eta} \right\} = \psi_{i,n} \left\{ \frac{f(n)_{i}+f(n)_{i+1}}{2\Delta \eta} \right\} + (P+T_{1}) \left( \frac{1}{\Delta \eta} + \frac{P}{2f(n)_{i}} \right) - \frac{1}{\Delta \eta} + \\
+ \psi_{i+1,n+1} \left\{ \frac{f(n)_{i}+f(n)_{i+1}}{2\Delta \eta} \right\} = \psi_{i,n} \left\{ \frac{f(n)_{i}+f(n)_{i+1}}{2\Delta \eta} \right\} + (P+T_{1}) \left( \frac{1}{\Delta \eta} + \\
+ \frac{P}{2f(n)_{i}} \right) - \frac{1}{\Delta \eta} + \Theta g(n)_{i} \]
\[ - \left( \frac{C_p(t)}{C_p(0)} \right) \frac{n+1}{n} \left\{ T \epsilon + P \epsilon (1-n) \right\} \left\{ \frac{1}{\Delta \eta} + \frac{P}{2f(\eta)} \right\} - m \Omega = 0 \quad n+1, n \\
\]

The foregoing equation is in the form:

\[ AB \psi_{i, n+1} + AC \psi_{i, n+1} = AD \quad \{i=1\} \]

For the boundary condition at \( \eta = 1 \ (i=N+1) \):

\[ \frac{\partial \psi_i}{\partial \eta} = \frac{T \epsilon C_p(t)/C_p(0) + (PR_2 - T_2 - T_L)^\psi_{i, i}}{f(\eta)} \]

Using the following approximation:

\[ \frac{\partial}{\partial \eta} \left\{ f(\eta) \frac{\partial \psi_i}{\partial \eta} \right\} = \left\{ \frac{f(\eta) \frac{\partial \psi_i}{\partial \eta} - f(\eta) \frac{\partial \psi_{i-1}}{\partial \eta}}{\Delta \eta} \right\} \]

and combining with (E53) yields:

\[ \psi_{i, n} \left\{ \frac{(PR_2 - T_2)}{\Delta \eta} \right\} = \left\{ \frac{f(\eta) + f(\eta)_{i-1}}{\Delta \eta^2} - \frac{P(\Delta \eta)}{\Delta \eta} \right\} \]

\[ + \psi_{i-1, n} \left\{ \frac{f(\eta)}{\Delta \eta^2} \right\} = \left[ \psi_{i, n+1} - \psi_{i, n} + \frac{N_{i, n+1} - N_{i, n}}{\Delta \eta} \right] \]

Putting in the Crank-Nicolson form:
\[
\psi_{i-1,n+1} \left( \frac{f(\eta)_{i} + f(\eta)_{i-1}}{2\Delta \eta} \right) + \psi_{i,n+1} \left( \frac{-f(\eta)_{i} - f(\eta)_{i-1}}{2\Delta \eta} \right) - (P_{R_{2}} - T_{2} - T_{L})
\]

\[
\left( \frac{P}{2f(\eta)_{i}} - \frac{1}{\Delta \eta} \right) \left( \frac{1}{\Delta t} \right) = \psi_{i-1,n} \left( \frac{-f(\eta)_{i} - f(\eta)_{i-1}}{2\Delta \eta} \right) +
\]

\[
+ \psi_{i,n} \left( \frac{f(\eta)_{i} + f(\eta)_{i-1}}{2\Delta \eta} \right) + (P_{R_{2}} - T_{2} - T_{L}) \left( \frac{P}{2f(\eta)_{i}} - \frac{1}{\Delta \eta} \right) \left( \frac{1}{\Delta t} \right) + g(\eta)_{i,0}
\]

\[
- \left( \frac{C_{p}(t)_{n}}{C_{p}(0)} + \frac{C_{p}(t)_{n+1}}{C_{p}(0)} \right) \left( \frac{T_{2}}{\Delta \eta} - \frac{PT_{2} \epsilon}{2f(\eta)_{i}} \right) - m\eta N_{i,n} \tag{E58}
\]

The above equation is in the form:

\[
AA_{l} \psi_{i-1,n+1} + AB_{l} \psi_{i,n+1} = AD_{l} \quad \{i=N+1\}
\]

Thus, we have \(N+1\) equations ( (E56), (E57), and (E58) ) and \(N+1\) unknowns which are solved using matrix methods.

The bound solute dimensionless concentration is given as:

\[
N_{i,n+1} = \Theta g(\eta)_{i} \Delta \tau \left( \psi_{i,n} - \frac{mN_{i,n}}{g(\eta)_{i}} \right) + N_{i,n} \tag{E59}
\]

Note that the boundary condition for \(\eta = 1\) was not written in generalized form. For the case where an equilibrium condition exists \((C(L,t) = \kappa C_{p}(t)\) we have:
\[
\psi_{i-1,n+1} \left\{ \frac{f(\eta)_i + f(\eta)_{i-1}}{4 \Delta \eta^2} + \frac{P}{4 \Delta \eta} \right\} + \psi_{i,n+1} \left\{ \frac{-f(\eta)_{i-1} - 2f(\eta)_i + f(\eta)_{i+1}}{4 \Delta \eta^2} \right\} - \\
\frac{1}{\Delta t} = \psi_{i-1,n} \left\{ \frac{-f(\eta)_i - f(\eta)_{i-1}}{4 \Delta \eta^2} - \frac{P}{4 \Delta \eta} \right\} + \psi_{i,n+1} \left\{ \frac{f(\eta)_{i-1} + 2f(\eta)_i}{4 \Delta \eta^2} + \\
+ \frac{f(\eta)_{i+1}}{4 \Delta \eta^2} - \frac{1}{\Delta t} + \Theta g(\eta)_i \right\} - e \left\{ \frac{C_p(t) n + C_p(t) n+1}{C_p(0)} \right\} \left\{ \frac{f(\eta)_{i-1} + f(\eta)_i}{4 \Delta \eta^2} \right\} - \\
- \frac{P}{4 \Delta \eta} \right\} - m \Theta N_{i,n} \quad \text{for } i=N \tag{E60}
\]

\[
\psi_{i+1,n+1} = \kappa \frac{C_p(t) n+1}{C_p(0)} \quad \text{for } i=N+1 \tag{E61}
\]

Since (E61) is completely specified ( \( C_p(t) n+1 \) is not an unknown), this case requires the solution of only \( N \) simultaneous equations at each dimensionless time step.

Regardless of which boundary condition is used, the right hand side of all the equations ( (E56)-(E60) ) is known, as are the coefficients of \( \psi_{i,n+1}, \psi_{i-1,n+1}, \text{ and } \psi_{i+1,n+1} \). The matrix of the coefficients alone is called a tridiagonal matrix. This is shown in Carnahan, et al. (230) who also describe how to solve this system of linear equations using matrix methods. Details of the computer program used for this purpose are given in APPENDIX F.
F. Computer Solutions

1. Analytical Solution

The solution describing the solute distribution across the aortic media as a result of diffusion, convection, and internal reaction is given by equations (6.44), (6.45) and (6.31e). These equations were programmed in Fortran for an IBM 370 computer. The solution is in the form of an infinite series. However, for values of P near unity, the series converges quite rapidly and less than 50 terms are required to obtain an accurate solution. In most cases the first 15 terms suffice. All input parameters are inserted into the data statement. The output describes the bound, mobile and total dimensionless solute concentrations for both the case of constant plasma isotope concentrations and for any specified double exponential decay plasma values. Calculations can be printed for any specified series of time intervals, or any specified range of the other input parameters. The program as shown on the following pages is set up to compute profiles when the complex boundary condition is in effect (equation (6.25c)). Simply changing cards ANL 072 through ANL 074 allows one to test any other desired boundary condition at x = L. The only subroutine used in the program computes the Eigen values as defined by equation (6.31e). The half-interval method is employed to solve this transcendental equation.

A listing of the program is found on the following pages. A short description of the numerical solution used for the case of nonuniform reaction and nonuniform diffusivity follows the listing.
CONVECTION AND DIFFUSION ACROSS FINITE SLAB, WIDTH L, WITH
INTERNAL REVERSIBLE REACTION—GENERALIZED BOUNDARY CONDITION AT
X = L
SUBROUTINE CALLED—EIGRB—CALCULATES EIGEN VALUES, XLA
R. L. BRATZLER, 7/21/74

************
DEFINITION OF SYMBOLS
************

PECLET NUMBER = JL/D (DIMENSIONLESS)
TIME (HOURS)
DIMENSIONLESS POSITION = X/L
ARRAY OF EIGEN VALUES, LAMBDA (DIMENSIONLESS)
COEFFICIENTS OF PLASMA EXPONENTIAL DECAY FUNCTION
TIME CONSTANTS IN PLASMA EXPONENTIAL DECAY FUNCTION (RECIP. H)
DIMENSIONLESS TIME = DT/L**2
ARRAY OF DIMENSIONLESS TISSUE CONCENTRATION FOR CONSTANT PLAS
ARRAY OF DIMENSIONLESS TISSUE CONCENTRATION FOR DECAYING PLAS
DIMENSIONLESS BINDING CONSTANT, THETA
DIMENSIONLESS BOUND TISSUE CONCENTRATION—CONSTANT PLASMA
DIMENSIONLESS BOUND TISSUE CONCENTRATION—DECAYING PLASMA
TOTAL DIMENSIONLESS TISSUE CONCENTRATION—CONSTANT PLASMA
TOTAL DIMENSIONLESS TISSUE CONCENTRATION—DECAYING PLASMA
HYDRAULIC FLUX, J (ML/CM**2-HR)
DIMENSIONLESS BIOT NUMBER, K(1)L/D
VESICULAR TRANSPORT COEFFICIENT, K(1) (CM/SEC)
DIMENSIONLESS BIOT NUMBER AT X/L=1, K(2)L/D
LYMPHATIC BIOT NUMBER (DIMENSIONLESS) = K(L)L/D
MAXIMUM TOLERABLE ERROR IN FINDING EIGEN VALUES
DIFFUSION COEFFICIENT D (CM**2/SEC)
NUMBER OF EIGEN VALUES COMPUTED
CONSTANT IN THE X=L BOUNDARY CONDITION
IMPLICIT REAL*8(A-H,O-$), INTEGER(I-N)

DIMENSION P(6), TI(6), ETA(22), XLA(75), ALPH(2), BE(2), TAU(6), CON(21),
1 DEC(21), THE(6), BCON(21), BDEC(21), CONT(21), DECT(21), XJ(6), XK1(6),
3 DD(4), T1(6), T2(6), TL(6), XXM(6), XR1(6), XR2(6), XK1PP(6)

DATA NN/15/.BE/.77509.0410/, ALPH/.33
2 P/1.4,1.2,5./ IIP/1./, IIIM/1./, IIIT/1./, IIP/1./, IIT/1./, IIM/1./, T1/6*.04/, TOL/.00001/, THE/10*.100*.1000
3 *10000*/, XL/0.024/, IIID/2/, DD/5.6D-09, 4.67D-08, 6.67D-08/, IIIM/1/, XXM/1., 4.6, 8./, T2/4*.0980/, TL/4*.35/, XR1/6*.1/, XR2/0.25/, 5.75/,
6 TAU = DT/L**2, P = JL/D, ETA = X/L, T1 = K(1)L/D

DO 9000 IT = IIT, IIIT
9000 IP = IIP, IIIP

-------------CALCULATE CONSTANTS FOR X=L BOUNDARY CONDITION

R1 = XR1(IT)
R2 = XR2(IT)
\[ 
XK1P = XK1PP(IT) \\
XK2P = P(IP) * R2 - (T2(IT) + TL(IT)) \\
XK3P = -T2(IT) * 0.42 \\
ZZZZ = P(IP) * (1.0 - R1) + T1(IT) * E1 \\
\]

\[ \text{************** COMPUTE EIGEN VALUES} \]

\[ \text{CALL EIGRB (P(IP), T1(IT), XK1P, XK2P, TOL, NL, XLA, ILR)} \]

\[ \text{GO TO (200, 7700), IER} \]

\[ \text{DO 7200 IB = IIB, IIIB} \]

\[ \text{DO 7100 IM = IIM, IIIM} \]

\[ \text{XM = XXM(IM)} \]

\[ \text{DO 7000 IG = IIG, IIGG} \]

\[ \text{DO 6500 ID = IID, IIID} \]

\[ \gamma = DD(ID) \]

\[ \text{CALCULATE HYDRAULIC FLUX AND WALL TRANSFER COEFFICIENT} \]

\[ \text{XJ(IP) = P(IP) * D*3600/XL} \]

\[ \text{XK1(IT) = T1(IT) * D/XL} \]

\[ \text{TAU(IG) = D*T1(IG)*3600/(XL**2.)} \]

\[ \text{ETA(1) = 0.} \]

\[ \text{DO 6000 IE = 1, 21} \]

\[ \text{CALCULATE THE SUMMATION, Q, FOR ALL ETA} \]

\[ \text{SUMBB} = 0. \]

\[ \text{SUMB} = 0. \]

\[ \text{SUMQ} = 0. \]

\[ \text{SUMQQ} = 0. \]

\[ \text{DO 1000 M = 1, NN} \]

\[ \text{DUM1 = ZZZZ*DEXP(P(IP)/2.)*(XK2P - 0.5*P(IP)*XK1P)*DSIN(XLA(M))} \]

\[ \text{1 - XLA(M)*XK1P*DCOS(XLA(M)) + XLA(M)*XK3P} \]

\[ \text{DUM2 = (0.5*P(IP) + T1(IT)) * XK3P - ZZZZ*DEXP(P(IP)/2.)*((XK2P - 1.0 - 0.5*P(IP)*XK1P)*DCOS(XLA(M)) + XLA(M)*XK1P*DSIN(XLA(M)))} \]
DUM1 = XLA(M) *(DUM1*DCOS(XLA(M))*ETA(IE)) + DUM2*DSIN(XLA(M))*ETA(IE)
DUM2 = (XK2P - 5*P(IP)*XK1P)*((0.5*P(IP) + T1(IT)) + XK1P*XLA(M)**12)
DUM3 = 2*XLA(M)*XK1P - XLA(M)*(XK2P - XK1P*(T1(IT) + P(IP)))
DUM2 = DUM2*DCOS(XLA(M)) + DUM3*DSIN(XLA(M))

.............. CALCULATE R, U, S, AND Z FOR BINDING SOLUTION

U = R**2. - 4.*THE(IB)*((P(IP)**2.)/4. + XLA(M)**2.)
S = 0.5*(-R - U**0.5)
Z = 1.0 + ((THE(IB)**2.)*XM)/((THE(IB)*XM + S)*(THE(IB)*XM + S))
QQQ = DUM1/(S*Z*DUM2)

.............. TEST SIZE OF S*TAU(IG)

IF (DABS(S*TAU(IG)) .GE. 100.) GO TO 205
DDD = DEXP(S*TAU(IG))
GO TO 210

205 DDD = 0.
210 IF (DABS(QQQ*DDD) - 1.0D-10) 220,220,215

215 SUMQ = SUMQ + QQQ*DDD
220 IF (DABS(QQQ*DEXP(-THE(IB)*XM*TAU(IG))/(S*THE(IB)*XM)) .LE. 1.0D-10)
1 GO TO 230
SUMB = SUMB + QQQ*(DDD - DEXP(-THE(IB)*XM*TAU(IG))/(S*THE(IB)*XM))

230 CONTINUE
DO 250 K=1,2

235 SUMQQ = SUMQQ + ALPH(K)*BE(K)*QQQ*TI(IG)*(DEXP(-BE(K)*TI(IG)) -DDD)
1 )/(BE(K)*TI(IG) + S*TAU(IG)))
ZZ = (ALPH(K)*BE(K)*TI(IG)*TAU(IG)*DUM1)/(S *Z *DUM2*(BE(K)*TI(IG)))
11(IG) + S *TAU(IG)))
ZY = (DEXP(-BE(K)*TI(IG)) -DEXP(-THE(IB)*XM*TAU(IG)))/(THE(IB)*X)
1M*TAU(IG) - BE(K)*TI(IG))
ZX = (DDD -DEXP(-THE(IB)*XM*TAU(IG)))/(TAU(IG)*(S
1 + THE(IB)*XM))
SUMBB = SUMBB + ZZ*(ZY-ZX)

250 CONTINUE
1000 CONTINUE

*CALCULATE A OF ETA*

1050 A = DEXP((P(IP)/2.)*(ETA(IE)-1.))

*CALCULATE B OF ETA*

DUM1 = ZZZZ*DEXP(0.5*P(IP))*(XK2P - P(IP)*XK1P/2.)*DSINH(P(IP)/2.)

DUM2 = (0.5*P(IP) + T1(IT))*XK3P - ZZZZ*DEXP(P(IP)/2.)*((XK2P - P(IP)*XK1P)*DCOSH(P(IP)/2.) - 0.5*XK1P*P(IP)*DSINH(P(IP)/2.))

DUM3 = (XK2P - P(IP)*XK1P/2.)*(0.5*P(IP) + T1(IT)) - (P(IP)**2.*XK3P)

DUM4 = 0.5*P(IP)*XK2P - P(IP)*(T1(IT) + P(IP))

3 = (DUM1*DCOSH(P(IP)*ETA(IE)/2.) + DUM2*DSINH(P(IP)*ETA(IE)/2.))

1 / (DUM3*DSINH(P(IP)/2.) + DUM4*DCOSH(P(IP)/2.))

SUMBK = 0.

SUMK = 0.

DO 2100 K=1,2

SUMK = SUMK + ALPH(K)*DEXP(-BE(K)*TI(IG))

SUMBK = SUMBK + ALPH(K)*TAU(IG)*(DEXP(-BE(K)*TI(IG))*DEXP(-BE(K)*TI(IG)) - DEXP(-BE(K)*TI(IG)))/THE(IB)*XM*TAU(IG) - BE(K)*TI(IG))

1*XK1P*TAU(IG))/THE(IB)*XM*TAU(IG) - BE(K)*TI(IG))

2100 CONTINUE

*COMPUTE SOLUTION FOR MOBILE SOLUTE--CONSTANT PLASMA*

CON(IE) = A*(B-2.*SUMQ)

*FOR DECAYING PLASMA*

DEC(IE) = CON(IE) + A*B*(SUMK - 1.) - 2.*A*SUMQQ

IF (THE(IB)) 2150, 2150, 2160

2150 RCON(IE) = 0.

3DEC(IE) = 0.
GO TO 2170

C

C

**********COMPUTE SOLUTION FOR BOUND SOLUTE--CONSTANT PLASMA

C

2160 BCON(IE) = THE(IB)*(A*(B*(1. -DEXP(-THE(IB)*XM*TAU(IG)))/XM*THE(IB)

1)) - 2.*SUMB)

C

C

**********---DECAYING PLASMA

C

BDEC(IE) = BCON(IE) + THE(IB)*(A*B*(SUMB - (1. -DEXP(-THE(IB)*XM

1*TAU(IG)))/(THE(IB)*XM)) - 2.*A*SUMBB)

C

C

**********COMPUTE BOUND PLUS FREE SOLUTION--CONSTANT AND

C

DECAYING PLASMA

C

2170 CONT(IE) = BCON(IE) + CON(IE)

DECT(IE) = DECT(IE) + BDEC(IE)

C

C

**********PRINT RESULTS

C

IF (IE-1) 2200,2200, 3000

2200 WRITE(MM,9601) T(I1,IG),TAU(IG),P(IP),XJ(IP),T1(IT),XK1(IT),E1,R1,

1T2(IT),TL(IT),ALPH(1),BE(1),ALPH(2),BE(2),THE(IB),XM,R2

WRITE(MM,9603)

3000 WRITE(MM,9602) ETA(IE),BCON(IE),CON(IE),CONT(IE),BDEC(IE),DEC(IE),

1 DECT(IE)

ETA(IE+1) = ETA(IE) + 0.05

6000 CONTINUE

6500 CONTINUE

7000 CONTINUE

7000 WRITE(MM,9605)

7100 CONTINUE

7200 CONTINUE

GO TO 8000

7700 WRITE(MM,9604) NL

NL = NN

8000 CONTINUE
9000 CONTINUE
9601 FORMAT('1',/T20,'TIME=',F6.3,/T20,'DT/L**2=',E15.7,/T20,'P=',E15.7
2T20,'RE1=',E8.4, 'T46,'T2 =',E15.7,'T70,'TL !=',E15.7,'T20,'C/DEC =',F
36.3,'EXP(-',F6.4,'T) + ',F6.4,'EXP(-',F6.4,'T)',/T20,'THETA=',F6.3
4'T50,'M=',F6.3,'T70,'RE2 = ',F8.4,'/T10,'X/L','T40,'CONSTANT PLASMA',
5 T85,'D*PLASMA')
9602 FORMAT(/T89F5.3,6E17.5)
9603 FORMAT('1',///T20,'****ERROR IN EIGEN SUBROUTINE**** NN=',I2)
9604 FORMAT ('1',///T20,'STOP')
9605 FORMAT ('1')
9606 STOP
9607 END

C VARIABLE INPUT LIST
C
C **********
C SUBROUTINE EIGRB (P,T,XK1,XK2,TOL,NN,XL,IER)
C
C **********
C VARIABLE INPUT LIST
C
C **********
C SUBROUTINE EIGRB (P,T,XK1,XK2,TOL,NN,XL,IER)
C
C **********
C SUBROUTINE EIGRB (P,T,XK1,XK2,TOL,NN,XL,IER)
C
C***********************

IMPLICIT REAL*8(A-H,O-Z), INTEGER(I-N)
DIMENSION XL(75),GL(75),F(75)

GL(1) = 0.01
DO 1000 K=1,NN
L = 0
DO 800 N = 1,50

F(N) = ((XK2-P*XK1/2.)*(0.5*P + T) + XK1* GL(N)**2)*DSIN(GL(N))
1 + GL(N)*(XK2 - XK1*(T + P)) *DCOS(GL(N))

IF (DABS(F(N)) - TOL) 32,32,34

XL(K) = GL(N)
WRITE(6,1111) K,XL(K)
1111 FORMAT (T20,I3,F10.3)
GO TO 400

32 IF (N-1) 35,35,40
35 GL(N+1) = GL(N) + 0.3
GO TO 800

40 IF (F(N)/F(N-1)) 45,43,43
43 IF (L-1) 35,50,50
45 GL(N+1) = (GL(N) + GL(N-1))/2.
L = 1
GO TO 800

50 GL(N+1) = (GL(N) + GL(N-2))/2.
F(N-1) = F(N-2)
GL(N-1) = GL(N-2)

800 CONTINUE
IER = 2
NN = K
RETURN

900 GL(1) = XL(K) + .05
1000 CONTINUE
IER = 1
RETURN
END
2. Numerical Solution

The numerical solution was used to estimate the bound and mobile solute distributions when nonuniform binding and a nonuniform solute diffusivity was assumed. The Crank-Nicolson method of solution was employed. The derivation of the equations which were used in the program are found in the preceding section of this appendix. All input values are placed in the data statement. The output is printed when \( t = 0.167 \) hours, 0.5 hours, 22.2 hours, 4 hours and every five hours thereafter. All pertinent input parameters are also shown on the output page. The binding distribution can be chosen as desired by changing the functions found on statements NUM 152 through NUM 168. An external function, FN, calculated the spatial variation in diffusivity.

The accuracy of the numerical solution depended on the magnitude of the time increments used. The program is set up such that \( \left( \frac{D_{\text{eff}} \Delta t}{L^2} \right) / (\Delta \eta^2) \) is initially equal or less than 2. This initialization assures a small enough temporal grid size to guarantee accurate results for any input value of \( D_{\text{eff}} \). \( \Delta t \) is maintained at this level until \( t \) reaches 0.167 hours, after which \( \Delta t \) is increased to 0.033 hours (see statement numbers NUM 277 - NUM 292). For a \( D_{\text{eff}} \) of \( 5 \times 10^{-8} \) cm\(^2\)/sec, \( L = 0.024 \) cms and \( \Delta \eta = 0.05 \) (which was commonly used), the ratio of incremental dimensionless time \( (D_{\text{eff}} \ t/L^2) \) to \( \Delta \eta^2 \) is increased from 2 to 4.12, or approximately doubled. By changing \( \Delta t \) to 0.033 hours and printing the results after every tenth iteration resulted in output being printed at 0.5 hours, which was one of the experimental time intervals of interest. Since calculations
were desired for times up to 67 hours, the dimensionless time increment was increased further to minimize computation costs. The maximum \( \Delta t \) used was 0.5 hours. For \( D_{\text{eff}} = 5 \times 10^{-8} \text{ cm}^2/\text{sec} \), \( L = 0.024 \) cm and \( \Delta \eta = 0.05 \), a \( t \) of 0.5 hours is equivalent to \( \frac{D_{\text{eff}} \Delta t/L^2}{(\Delta \eta)} \) = 22.7. Using such a large time increment did not significantly affect the accuracy of the results for the cases of no binding. However, the results when binding was included generally differed by 5\% from the results predicted with the analytical solution (see VI. THEORY). While this discrepancy is not large, it could be minimized if smaller time increments were used for the iterations after \( t = 0.5 \) hours. However, the improvement in accuracy did not justify the additional computation time involved.

A listing of the computer program is presented on the following pages.
NUMERICAL SOLUTION TO DIFFUSION - CONVECTION EQUATION ACROSS AORTIC MEDIA OF WIDTH L, WITH INTERNAL REVERSIBLE REACTION

CRANK-NICOLSON METHOD

R.L. BRATZLER 4/19/74

SUBROUTINES CALLED--TRIDA--SOLVES TRIDIAGONAL COEFFICIENT MATRIX AS DESCRIBED BY CARNAHAN, ET AL., P. 451

EXTERNAL FUNCTION--FN--GIVES SPATIAL VARIATION OF D AS A FUNCTION OF ETA

VARIABLE NAME LIST

B--THIELE MODULUS (=THETA) (DIMENSIONLESS)

XM--EQUILIBRIUM BINDING CONSTANT (DIMENSIONLESS)

SOLD--CONCENTRATION OF BOUND SOLUTE RELATIVE TO PLASMA CONCENTRATION (DIMENSIONLESS)

COLD--CONCENTRATION OF UNBOUND SOLUTE RELATIVE TO INITIAL PLASMA CONCENTRATION

TOLD--TOTAL SOLUTE IN WALL RELATIVE TO INITIAL PLASMA LEVEL

T1--SURFACE BIOT NUMBER AT X/L=0 (DIMENSIONLESS)

XK1--VESICULAR TRANSPORT COEFFICIENT AT X/L=0 (CM/SEC)

T2--CAPILLARY BIOT NUMBER AT X/L=1 (DIMENSIONLESS)

XK2--CAPILLARY TRANSPORT COEFFICIENT AT X/L=1 (CM/SEC)

TL--LYMPHATIC BIOT NUMBER AT X/L=1 (DIMENSIONLESS)

P--WALL PECLET NUMBER==J/L (DIMENSIONLESS)

XJ--CONVECTIVE FLUX (CM/HR) =J

G--FOURIER NUMBER==DT/L**2 (DIMENSIONLESS)
**DIMENSIONAL ANALYSIS**

- **D**—SOLUTE DIFFUSION COEFFICIENT (CM**2/SEC)
- **CB**—PLASMA ISOTOPE CONCENTRATION RELATIVE TO THE INIT PLASMA ISOTOPE CONCENTRATION (DIMENSIONLESS)
- **DE(K)**—PLASMA EXPONENTIAL DECAY CONSTANTS (1/HR)
- **ALPH(K)**—PROPORTIONALITY CONSTANTS IN THE PLASMA DECAY EQUATION
- **XKP**—INTERSTITIAL SPACE AVAILABLE TO SOLUTE IN THE WALL (DIMENSIONLESS)
- **XL**—WALL THICKNESS (CM)
- **A**—ARRAY IN WHICH ARE STORED THE COEFFICIENTS FOR THE M SIMULTANEOUS EQUATIONS GENERATED BY THE CRANK-NICOL METHODOLOGY
- **DE**—SPATIAL GRID SIZE (DIMENSIONLESS)
- **DG**—TEMPORAL GRID SIZE (DIMENSIONLESS)
- **TI**—TIME IN HOURS
- **DT**—TIME INCREMENT CONSISTENT WITH THE DIMENSIONLESS INCREMENT, DG
- **M**—NUMBER OF SPATIAL GRID POINTS LESS ONE
- **N**—NUMBER OF TEMPORAL GRID POINTS UP TO 167 HOURS
- **R1,XR1**—SOLUTE REJECTION COEFFICIENT AT X/L=0 (DIMENSIONLESS)
- **R2,XR2**—SOLUTE REJECTION COEFFICIENT AT X/L=1 (DIMENSIONLESS)
- **LCON**—A FLAG TO DICTATE THE X=L BOUNDARY CONDITION
  - 0 FOR RADIATION-TYPE CONDITION
  - 1 FOR EQUILIBRIUM TYPE CONDITION
- **XKP2**—WALL-ADVENTITIA EQUILIBRIUM VALUE
- **LBDIS**—A FLAG TO INDICATE WHEN BINDING SITES ARE NOT UNIFORMLY DISTRIBUTED
  - 0 WHEN UNIFORM
  - 1 WHEN NONUNIFORM
- **LDDIS**—A FLAG TO INDICATE VARIABLE DIFFUSIVITY
  - 0 FOR CONSTANT DIFFUSIVITY
  - 1 FOR VARIABLE
- **GN**—BINDING DISTRIBUTION G OF ETA
- **FN**—DIFFUSIVITY DISTRIBUTION, F OF ETA

IMPLICIT REAL*8(A-H,O-$), INTEGER(I-N)
EXTERNAL FN
DIMENSION COLD(41), SOLD(41), X(41), T(41), C(41), S(41), RR(41), TOLD(41),
1 AB(41), AC(41), AD(41), AA(41), BE(2), ALPH(2), B(6), XM(6), T1(6), T2(6),
2 P(6), DD(6), GMAX(6), XR1(6), XR2(6), TL(6),
DATA BE/775, 0.41/, ALPH/33, 67/, XKP/42/, XL/024/, MINC/2/, MM/6/,
1 XKP2/4200/, LM=1/2/, LDDIS/1/, LCON/0/, IID/2/,
2 IID/3/, IIB/1/, IIM/I/, IIIT/I/, IIM/1/, IIP/I/,
3B/7/17/2/33/, XM/4/1/2/, GMAX/40/12/, XR1/4/0/,
4XR2/6/0/, DD/9.31D-08/5.9D-08/2.79D-08/, TL/12/, 20/, 40/
DO 8000 LMN=2, 2
DO 7000 ID=IID, IID
DO 5000 IB = IIB, IIB
DO 4000 IT = IIIT, IIIT
DO 6000 IM=IIM, IIIM
DO 3000 IP=IIP, IIP
P(IP) = P(ID)
T1(IT) = T1(ID)
T2(IT) = T2(ID)
TL(IT) = TL(ID)
B(IB) = B(ID)
D = DD(ID)
MN = M + 1
C
***************CALCULATE GRID SIZE
DE = 1. / M
N = 1
C
***************FIND NUMBER OF TIME INTERVALS; N* REQUIRED TO ENSURE
THAT DG/DE**2 IS LESS THAN 2
C
DO 30 K=1, 100
Q = (D*167*3600.)/(N*(DE*XL)**2.)
IF ( Q - 2. ) 35, 35
29 N = N + 1
30 CONTINUE
35 IFREQ = N
**CALCULATE INITIAL TIME INCREMENTS, DT, DG**

\[
DT = \frac{167}{IFREQ} \\
DG = DT*D*3600./XL**2.
\]

**CALCULATE THE VALUES FOR THE TRANSPORT PARAMETERS**

\[
XK1 = T1(IT)\cdot D/XL \\
XK2 = T2(IT)\cdot D/XL \\
XJ = P(IP)\cdot D*3600./XL \\
R = T1(IT)/(T1(IT)+P(IP)) \\
R1 = XR1(IT) \\
R2 = XR2(IT)
\]

**INITIALIZE ALL ARRAYS**

\[
TI = 0. \\
3 = 0. \\
ICNT = 0 \\
KL = 1 \\
CBO = 1. \\
CCCCC = XM(IM) \\
BBBBB = B(IB) \\
DO 40 L=1,MN \\
COLD(L) = 0. \\
SOLD(L) = 0. \\
TOLD(L) = 0. \\
40 CONTINUE \\
LKL = LBDIS + 1 \\
LLLL = LDDIS + 1 \\
GO TO 1105 \\
50 IF (LCON-1) 55,54,55 \\
54 MN = M \\
55 DO 500 I=1,MN \\
500 CONTINUE \\
\]

**CALCULATE THE DIMENSIONLESS PLASMA ISOTOPE CONCENTRAT**
CBN = 0.
DO 90 K = 1, 2
CBN = CBN + ALPH(K) * DEXP(-BE(K) * TI)
90 CONTINUE
X1 = I - 1
X2 = M

********** CALCULATE THE APPROPRIATE BINDING DISTRIBUTION **********

91 GO TO (911, 92, 93, 94, 95, 951) LKL
911 GN = 1.*
912 GN = .99246 * DEXP(-6.*X1/X2) + .00754
913 GN = (1.011442 * DEXP(-6.*X1/X2) + 1.00906 * DEXP(-9.*(1.-X1/X2))) - .01157
914 GO TO 96
915 GN = 3.8*(0.5-X1/X2)*(0.5-X1/X2) + .05
916 GO TO 96
917 GN = (.8798 * DEXP(-2.4*X1/X2) + .1202)
918 GO TO 96
919 GN = .802*DEXP(-6.*X1/X2) + .198
920 GO TO 96
921 DE = 1./M
922 BIN(B) = BBBBB*GN
923 YM(IM) = CCCCC/GN

********** CALCULATE COEFFICIENT MATRIX A **********

462 IF (I-1) 466,464,466
464 AB(1) = -((FN(I) + FN(I+1))/(2.*DE**2.) + (P(IP) + T1(IT))/DE + P(IP)*)
1. P(IP) + T1(IT))/(2.*FN(I) ) + 1./DG)
465 AC(1) = (FN(I+1) + FN(I))/(2.*DE**2.)
466 AD(1) = COLD(I)*((FN(I)+FN(I+1))/(2.*DE**2.) + (P(IP) + T1(IT))/DE + NUM 179
1)\( P(IP)*(P(IP)+T1(IT))/(2*FN(I)) - 1^*/DG+B(IB)) - COLD(I+1)*((FN+DE**2.*}}
\(2(I) + FN(I+1))/(2*DE**2.*)) - (CBO+CBN)*(T1(IT)*XKP + P(IP)*(1^-*R1}}
\(2(I))*/DE + P(IP)/(2*FN(I)) - B(IB)*XM(IM)*SOLD(I)
\) GO TO 350
\) NUM 180
\) NUM 181
\) NUM 182
\) NUM 183
\) NUM 184
\) NUM 185
\) NUM 186
\) NUM 187
\) NUM 188
\) NUM 189
\) NUM 190
\) NUM 191
\) NUM 192
\) NUM 193
\) NUM 194
\) NUM 195
\) NUM 196
\) NUM 197
\) NUM 198
\) NUM 199
\) NUM 200
\) NUM 201
\) NUM 202
\) NUM 203
\) NUM 204
\) NUM 205
\) NUM 206
\) NUM 207
\) NUM 208
\) NUM 209
\) NUM 210
\) NUM 211
\) NUM 212
\) NUM 213
\) NUM 214
\) NUM 215

IF (I=MN) 468,470,470
468 AA(I) = (FN(I)+FN(I-1))/(4*DE**2.*} + P(IP)/(4*DE)
\) AB(I) = -1/25*(FN(I+1)+2*FN(I)+FN(I-1))/DE**2.* - 1*/DG
\) AC(I) = (FN(I+1) + FN(I))/(4*DE**2.*} - P(IP)/(4*DE)
\) AD(I) = -COLD(I-1)*((FN(I)+FN(I-1))/(4*DE**2.*} + P(IP)/(4*DE))
\(1 + COLD(I)*((FN(I+1)+2*FN(I)+FN(I-1))/(4*DE**2.*} - 1*/DG + 5*P(IP)/FN)
\) GO TO 350
\) NUM 466
\) NUM 468
\) NUM 470
\) NUM 475
\) NUM 479
\) NUM 480
\) NUM 484
\) NUM 500

GO TO 350

IF (LCON=1) 475,480,480
475 AA(I) = (FN(I)+FN(I-1))/(2*DE**2.*)
\) AB(I) = -(FN(I)+FN(I-1)*(2*DE**2.*} - (P(IP)*R2-T2(IT)-T1(IT))*0/5
\) AD(I) = -COLD(I-1)*((FN(I)+FN(I-1))/(2*DE**2.*} + P(IP)/(4*DE)
\) 1 FN(I-1))/(2*DE**2.*} + P(IP)*R2-T2(IT)-T1(IT))*0/5*P(IP)/FN)
\) GO TO 350
\) NUM 475
\) NUM 479
\) NUM 480
\) NUM 484
\) NUM 500

CALCULATE AMOUNT OF BOUND SOLUTE

SOLD(I+1) = DG*B(IB)*COLD(I+1) = XM(IM)*SOLD(I+1)) + SOLD(I+1)
COLD(I+1) = XKP2*CBO
SOLD(I+1) = COLD(I+1) + SOLD(I+1)

CONTINUE

XM(IM) = CCCC
B(IB) = BBBBB
I = 1

***************SOLVE MATRIX EQUATIONS TO DETERMINE SOLUTE DIMENSIONAL CONCENTRATION AT NEXT TIME STEP

CALL TRIDA (I,MN,AA,AB,AC,AD,RR)

***************REASSIGN SPATIAL DISTRIBUTION INTO COLD AND DETERMINE TOTAL SOLUTE IN THE WALL

DO 1100 I=1,MN
COLD(I) = RR(I)
TOLD(I) = COLD(I) + SOLD(I)
1100 CONTINUE

******PRINT RESULTS AT THE APPROPRIATE INTERVALS

LD = (ICNT/IFREQ)*IFREQ
IF (LD-ICNT) 1500,1150,1500

1150 K = 1
X(1) = 0.
X1 = MINC
X2 = M
XX = X1/X2
MMM = M + 1
DO 1200 I=1,MMM,MINC
C(K) = COLD(I)
S(K) = SOLD(I)
T(K) = TOLD(I)
K = K+1
X(K) = X(K-1) + XX
1200 CONTINUE
L = (M/MINC) + 1
IF (KL-1) 1250,1250,1400
1250 IF (LCON - 1 ) 1300,1280,1300
1280 T2(IT) = 0.
XX2 = 0.
1300 GO TO (1301,1302) * LLLL
1301 WRITE (MM,85000)
   GO TO 1305
1302 WRITE (MM,85500)
1305 GO TO (1310,1311,1312,1313,1314,1315), LKL
1310 WRITE (MM,85600)
   GO TO 1320
1311 WRITE (MM,85700)
1312 WRITE (MM,85800)
1313 WRITE (MM,85900)
1314 WRITE (MM,86000)
1315 WRITE (MM,86100)
1320 WRITE (MM,90000) T1(IT),T2(IT),P(IP),D*R2,TL(IT),XK1,XK2,J,B(IB),XJ
2(IM)*K2,R1,(X(K),K=1,L)
   KL = 2
1400 WRITE (MM,91000) T1,G,(C(K),K=1,L),S(K),K=1,L,T(K),K=1,L)

C PERIODICALLY CHANGE THE TIME INCREMENTS USED IN THE CALCULATION

C

NNNN = ICNT/IFREQ + 1
IF (NNNN=5) 1401,1401,4
1401 GO TO (1500,1,2,3,4), NNNN
1 DT = 0.2*IFREQ*DT
   DG = 0.2*IFREQ*DG
IFREQ = 10
ICNT = 10
GO TO 1500
2 DT = 5.0*DT
   DG = DG*5.0
GO TO 1500
3 DT = 1.1*DT
DG = 1.1*DG
GO TO 1500

4 JT = 0.5
DG = (DT*D/XL**2.)*3600.

1500 ICNT = ICNT + 1
IF (ICNT-1) 1700,1700,1600

1600 CBO = CBN
1700 G = G + DG
TI = TI + DT
IF (G-GMAX(ID)) 50,50,3000

3000 CONTINUE
6000 CONTINUE
4000 CONTINUE
5000 CONTINUE
7000 CONTINUE
8000 CONTINUE
8500 FORMAT(1',/T20,'UNIFORM DIFFUSIVITY')
8550 FORMAT(1',/T20,'VARIABLE DIFFUSIVITY')
8560 FORMAT(T20,'UNIFORM BINDING')
8570 FORMAT(T20,'EXPERIMENTAL DECAY BINDING')
8580 FORMAT(T20,'MIXED EXPONENTIAL BINDING 1.011442EXP(-6N) + 1.00906E')

1000 CONTINUE
4000 CONTINUE
5000 CONTINUE
6000 CONTINUE
7000 CONTINUE
8000 CONTINUE
8500 FORMAT(T20,'PARABOLIC BINDING -- 3.8(1.5-ETA)**2 + 0.05')
8550 FORMAT(T20,'HODARNA BINDING -- G(ETA) = 0.8798EXP(-2.4ETA) + 0.1202')
8610 FORMAT(T20,'EXPONENTIAL BINDING -- GN = 0.802EXP(-6*ETA) + 0.198')
9000 FORMAT(1',/E9.2,/E8.4,T10.3,F10.5,F10.5,F10.5)//)

STOP
END
FUNCTION FN(I)

IMPLICIT REAL*8(A-H,O-S),INTEGER(I-N)

DEFINITION OF VARIABLES

**************X2--NUMBER OF SPATIAL GRID INTERVALS

**************X--POSITION COUNTER RANGING FROM 0 TO X2

**************BETA AND GAMMA

X2 = 20.

Y = I - 1.

FN = 1.002236 - 0.902236*DEXP(-6.*X/X2)

RETURN

END

SUBROUTINE TRIDA (IP,L,A,B,C,D,V)

.............SEE CARNAHAN, ET AL. FOR DETAILED DESCRIPTION OF THIS

IMPLICIT REAL*8(A-H,O-S),INTEGER(I-N)

DIMENSION A(1),B(1),C(1),D(1),V(1),BETA(41),GAMMA(41)

***** COMPUTE INTERMEDIATE ARRAYS BETA AND GAMMA

BETA(IP) = B(IP)

GAMMA(IP) = D(IP)/BETA(IP)

IFP1 = IP + 1

DO 1 I=IFP1,L

BETA(I) = B(I) = A(I)*C(I-1)/BETA(I-1)

GAMMA(I) = (D(I) = A(I)*GAMMA(I-1))/BETA(I)

1 CONTINUE

***** COMPUTE FINAL SOLUTION VECTOR V

V(L) = GAMMA(L)

LAST = L - IP

DO 2 K=1,LAST

RETURN

END
I = L - K

2 V(I) = GAMMA(I) - C(I)*V(I+1)/BETA(I)
RETURN
END

/*
G. Data Tables

Included on the following pages are tables of data not included in the main section of this thesis. For a list of the tables in this part of the APPENDIX, the reader is referred to the list in the front of the thesis.
TABLE G.I

SUMMARY OF LDL PLASMA ISOTOPE DECAY

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Rabbit Weight (kg)</th>
<th>C_I (cpm/ml)</th>
<th>V_I (ml)</th>
<th>t (min)</th>
<th>C_p(t) (cpm/ml)</th>
<th>C_p(t)/C_p(0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>137 (67 hr)</td>
<td>3.60</td>
<td>440,055,000</td>
<td>4.0</td>
<td>0</td>
<td>(15,580,000)</td>
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<td>3978</td>
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<td>142 (24 hr)</td>
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<td>-</td>
<td>4.5</td>
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<td>Exp. No.</td>
<td>Rabbit Weight (kg)</td>
<td>Injectate</td>
<td>Plasma</td>
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<tr>
<td></td>
<td></td>
<td>$C_I$ (cpm/ml)</td>
<td>$V_I$ (ml)</td>
<td>$t$ (min)</td>
<td>$C_p$ (cpm/ml)</td>
<td>$C_p(t)/C_p(0)$</td>
</tr>
<tr>
<td>142</td>
<td>3.95</td>
<td>3.0</td>
<td></td>
<td>0</td>
<td>(11,805,600)</td>
<td>1.0</td>
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<tr>
<td>(4 hr)</td>
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<td>(4 hr)</td>
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<td>Exp. No.</td>
<td>Rabbit Weight (kg)</td>
<td>Injectate $C_I$ (cpm/ml)</td>
<td>$V_I$ (ml)</td>
<td>$t$ (min)</td>
<td>Plasma $C_p(t)$ (cpm/ml)</td>
<td>$C_p(t)/C_p(0)$</td>
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<td>147</td>
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<td>-</td>
<td>3.3</td>
<td>0</td>
<td>(7,272,400)</td>
<td>1.0</td>
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+ unscreened<sup>125</sup>I-LDL
++ screened<sup>131</sup>I-LDL
## TABLE G.II

**SUMMARY OF ALBUMIN PLASMA ISOTOPE DECAY**

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TABLE G.II (continued)

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<td>167</td>
<td>70</td>
<td>0.430</td>
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<td></td>
<td>71</td>
<td>0.621</td>
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<td>72</td>
<td>0.609</td>
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<td>73</td>
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<td></td>
<td>74</td>
<td>0.533</td>
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<td>75</td>
<td>0.488</td>
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<td></td>
<td>76</td>
<td>0.533</td>
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<td></td>
<td>77</td>
<td>0.572</td>
<td></td>
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<tr>
<td>168</td>
<td>100</td>
<td>0.649</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>102</td>
<td>0.601</td>
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</tr>
<tr>
<td></td>
<td>103</td>
<td>0.432</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>104</td>
<td>0.688</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>105</td>
<td>0.519</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>106</td>
<td>0.620</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>0.566</td>
<td></td>
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</tr>
</tbody>
</table>
### TABLE G.IV

**NORMAL RABBIT AORTA HYDRAULIC FLUX DATA**

<table>
<thead>
<tr>
<th>Time Interval (min)</th>
<th>Flux*</th>
<th>AP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left Flow Meters</td>
<td>Right Flow Meters</td>
</tr>
<tr>
<td></td>
<td>Upper</td>
<td>Lower</td>
</tr>
<tr>
<td>Exp. 107⁺: 25 - 46</td>
<td>0.0038</td>
<td>0.0034</td>
</tr>
<tr>
<td>Exp. 107⁺: 67 - 91</td>
<td>0.0048</td>
<td>0.0045</td>
</tr>
<tr>
<td>Exp. 107⁺: 109 - 139</td>
<td>0.0058</td>
<td>0.0052</td>
</tr>
<tr>
<td>Exp. 107⁺: 156 - 186</td>
<td>0.0061</td>
<td>0.0058</td>
</tr>
<tr>
<td>Exp. 107⁺: 205 - 230</td>
<td>0.0081</td>
<td>0.0074</td>
</tr>
<tr>
<td>Exp. 107⁺: 469 - 493</td>
<td>0.0031</td>
<td>0.0026</td>
</tr>
<tr>
<td>Exp. 107⁺: 537 - 561</td>
<td>0.0044</td>
<td>0.0039</td>
</tr>
<tr>
<td>Exp. 107⁺: 612 - 630</td>
<td>0.0072</td>
<td>0.0062</td>
</tr>
<tr>
<td>Exp. 121⁺: 66 - 103</td>
<td>0.0037</td>
<td>0.0027</td>
</tr>
<tr>
<td>Exp. 121⁺: 123 - 147</td>
<td>0.0059</td>
<td>0.0044</td>
</tr>
<tr>
<td>Exp. 121⁺: 167 - 191</td>
<td>0.0068</td>
<td>0.0057</td>
</tr>
<tr>
<td>Exp. 124⁺⁺: 138 - 363</td>
<td>0.0013</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

* ml/cm²-hr
+ Right side blocked with parafilm
++ Left side blocked with parafilm
**TABLE G.V**

HYDRAULIC PERMEABILITY CONTROL EXPERIMENT - BOTH CHAMBERS BLOCKED - Exp. 108

<table>
<thead>
<tr>
<th>Time Interval (min)</th>
<th>Left Flow Meters</th>
<th>Right Flow Meters</th>
<th>ΔP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>56 - 74</td>
<td>0.0007</td>
<td>0.0006</td>
<td>0.0012</td>
</tr>
<tr>
<td>106 - 124</td>
<td>0.0023</td>
<td>0.0018</td>
<td>0.0020</td>
</tr>
<tr>
<td>156 - 177</td>
<td>0.0028</td>
<td>0.0021</td>
<td>0.0029</td>
</tr>
<tr>
<td>215 - 233</td>
<td>0.0026</td>
<td>0.0019</td>
<td>0.0028</td>
</tr>
<tr>
<td>263 - 302</td>
<td>0.0028</td>
<td>0.0025</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

* ml/cm²-hr
<table>
<thead>
<tr>
<th>Time Interval (min)</th>
<th>Hydraulic Flux*</th>
<th>( \Delta P ) (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left Flow Meters</td>
<td>Right Flow Meters</td>
</tr>
<tr>
<td></td>
<td>Upper</td>
<td>Lower</td>
</tr>
<tr>
<td>Exp. 118⁺:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61 - 102</td>
<td>0.0039</td>
<td>0.0017</td>
</tr>
<tr>
<td>119 - 144</td>
<td>0.0044</td>
<td>0.0026</td>
</tr>
<tr>
<td>168 - 194</td>
<td>0.0051</td>
<td>0.0046</td>
</tr>
<tr>
<td>212 - 294</td>
<td>0.0052</td>
<td>0.0041</td>
</tr>
<tr>
<td>322 - 352</td>
<td>0.0060</td>
<td>0.0046</td>
</tr>
<tr>
<td>Exp. 120++:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 - 60</td>
<td>0.0035</td>
<td>0.0033</td>
</tr>
<tr>
<td>90 - 120</td>
<td>0.0050</td>
<td>0.0046</td>
</tr>
</tbody>
</table>

* ml/cm²·hr
⁺ Left side blocked
++ Right side blocked
TABLE G.VII
NORMAL RABBIT AORTA HYDRAULIC FLUX VARIATION OVER TIME

<table>
<thead>
<tr>
<th>Time+ (min)</th>
<th>Left Flow Meters</th>
<th>Right Flow Meters</th>
<th>ΔP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>132</td>
<td>0.0202</td>
<td>-</td>
<td>0.0178</td>
</tr>
<tr>
<td>138</td>
<td>0.0065</td>
<td>-</td>
<td>0.0067</td>
</tr>
<tr>
<td>150</td>
<td>0.0039</td>
<td>0.0031</td>
<td>0.0060</td>
</tr>
<tr>
<td>162</td>
<td>0.0026</td>
<td>0.0022</td>
<td>0.0053</td>
</tr>
<tr>
<td>174</td>
<td>0.0020</td>
<td>0.0012</td>
<td>0.0053</td>
</tr>
<tr>
<td>186</td>
<td>0.0020</td>
<td>0.0016</td>
<td>0.0051</td>
</tr>
<tr>
<td>198</td>
<td>0.0014</td>
<td>0.0010</td>
<td>0.0040</td>
</tr>
<tr>
<td>210</td>
<td>0.0012</td>
<td>0.0013</td>
<td>0.0054</td>
</tr>
<tr>
<td>222</td>
<td>0.0022</td>
<td>0.0014</td>
<td>0.0067</td>
</tr>
<tr>
<td>234</td>
<td>0.0012</td>
<td>0.0009</td>
<td>0.0051</td>
</tr>
<tr>
<td>246</td>
<td>0.0014</td>
<td>0.0010</td>
<td>0.0044</td>
</tr>
<tr>
<td>258</td>
<td>0.0010</td>
<td>0.0009</td>
<td>0.0049</td>
</tr>
<tr>
<td>282</td>
<td>0.0008</td>
<td>0.0008</td>
<td>0.0042</td>
</tr>
<tr>
<td>294</td>
<td>0.0013</td>
<td>0.0010</td>
<td>0.0068</td>
</tr>
<tr>
<td>306</td>
<td>0.0010</td>
<td>0.0005</td>
<td>0.0040</td>
</tr>
<tr>
<td>318</td>
<td>0.0009</td>
<td>0.0007</td>
<td>0.0049</td>
</tr>
<tr>
<td>330</td>
<td>0.0008</td>
<td>0.0009</td>
<td>0.0047</td>
</tr>
<tr>
<td>343</td>
<td>0.0006</td>
<td>0.0004</td>
<td>0.0041</td>
</tr>
<tr>
<td>363</td>
<td>0.0006</td>
<td>0.0005</td>
<td>0.0048</td>
</tr>
</tbody>
</table>

+ ΔP maintained at 60 mm Hg for 130 minutes and then step changed to 124 mm Hg

* ml/cm²-hr: calculated as the average flux between two consecutive readings
### H. Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a^- )</td>
<td>Internal radius of aorta, cm</td>
</tr>
<tr>
<td>( a^- )</td>
<td>Net counting rate, counts/min</td>
</tr>
<tr>
<td>( A^- )</td>
<td>Term defined by equation (6.31a)</td>
</tr>
<tr>
<td>( A^- )</td>
<td>Area of tissue slice, ( \text{cm}^2 )</td>
</tr>
<tr>
<td>( A_c^- )</td>
<td>Adventitial capillary effective surface area, ( \text{cm}^2 )</td>
</tr>
<tr>
<td>( A_m^- )</td>
<td>Aortic wall surface area, ( \text{cm}^2 )</td>
</tr>
<tr>
<td>( A_L^- )</td>
<td>Lymphatic channel effective surface area, ( \text{cm}^2 )</td>
</tr>
<tr>
<td>( b^- )</td>
<td>Outside radius of aorta, cm</td>
</tr>
<tr>
<td>( B^- )</td>
<td>Term defined by equation (6.31c)</td>
</tr>
<tr>
<td>( C^- )</td>
<td>Tissue radioactivity concentration, ( \text{cpm/cm}^3 )</td>
</tr>
<tr>
<td>( C_a^- )</td>
<td>Radioactivity concentration in adventitial extravascular space, ( \text{cpm/cm}^3 )</td>
</tr>
<tr>
<td>( C_a^- )</td>
<td>Mobile solute tissue concentration, ( \text{cpm/cm}^3 )</td>
</tr>
<tr>
<td>( C_b^- )</td>
<td>Concentration of binding substrate, ( \text{g/ml} )</td>
</tr>
<tr>
<td>( C_c^- )</td>
<td>Concentration of complexed solute radioactivity, ( \text{cpm/cm}^3 )</td>
</tr>
<tr>
<td>( C_L^- )</td>
<td>Concentration of radioactivity in lymphatic channel, ( \text{cpm/cm}^3 )</td>
</tr>
<tr>
<td>( C_p^- )</td>
<td>Plasma radioactivity concentration, ( \text{cpm/ml} )</td>
</tr>
<tr>
<td>( C_v^- )</td>
<td>Vesicle radioactivity concentration, ( \text{cpm/ml} )</td>
</tr>
<tr>
<td>( D^- )</td>
<td>Plastic sheet conversion factor, ( \text{cm}^2/\text{g} )</td>
</tr>
<tr>
<td>( D_{\text{eff}}^- )</td>
<td>Effective diffusion coefficient, ( \text{cm}^2/\text{sec} )</td>
</tr>
<tr>
<td>( f^- )</td>
<td>Tissue area correction factor</td>
</tr>
<tr>
<td>( F^- )</td>
<td>Fractional radioactivity due to blood contamination</td>
</tr>
<tr>
<td>( F_m^- )</td>
<td>Term defined by (6.31b)</td>
</tr>
<tr>
<td>( J_f^- )</td>
<td>Hydraulic flux, ( \text{ml/cm}^2\text{-hr} )</td>
</tr>
<tr>
<td>( J_v^- )</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>( J_c^- )</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>( k_1, k_2, k_3^- )</td>
<td>Reaction rate constants</td>
</tr>
<tr>
<td>( k_p, k_m^- )</td>
<td>Partition coefficients</td>
</tr>
<tr>
<td>( K_l^- )</td>
<td>Vesicular mass transport coefficient</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>( K'<em>{1}, K'</em>{2}, K'_{3} )</td>
<td>Constants defined by equation (6.28)</td>
</tr>
<tr>
<td>( K_{c} )</td>
<td>Capillary mass transfer coefficient, cm/sec</td>
</tr>
<tr>
<td>( K_{L} )</td>
<td>Lymphatic mass transfer coefficient, cm/sec</td>
</tr>
<tr>
<td>( L )</td>
<td>Thickness of tissue media plus intima, cm</td>
</tr>
<tr>
<td>( m )</td>
<td>Ratio of reverse to forward reaction rate constants, ( = \frac{k_{2}}{k_{1}C_{b}} )</td>
</tr>
<tr>
<td>( N )</td>
<td>Dimensionless concentration of complexed radioactivity, ( \frac{C_{c}}{C_{b}(0)} )</td>
</tr>
<tr>
<td>( N_{m} )</td>
<td>Flux of radioactivity, ( \text{cpm/cm}^{2} \cdot \text{sec} )</td>
</tr>
<tr>
<td>( N_{m} )</td>
<td>Number of tissue media 20( \mu ) slices</td>
</tr>
<tr>
<td>( p )</td>
<td>Ratio of ( ^{125} \text{I} ) radioactivity to ( ^{51} \text{Cr} ) radioactivity</td>
</tr>
<tr>
<td>( P )</td>
<td>Dimensionless Peclet number, ( = \frac{J_{f}L}{D_{\text{eff}}} )</td>
</tr>
<tr>
<td>( r )</td>
<td>Radial position, cm</td>
</tr>
<tr>
<td>( R )</td>
<td>Counting rate, counts/min</td>
</tr>
<tr>
<td>( R_{1} )</td>
<td>Reaction rate per unit volume</td>
</tr>
<tr>
<td>( R_{1} )</td>
<td>Solute rejection coefficient at endothelial surface</td>
</tr>
<tr>
<td>( R_{2} )</td>
<td>Solute rejection coefficient at media-adventitia interface</td>
</tr>
<tr>
<td>( R_{c} )</td>
<td>Solute rejection coefficient across capillary endothelium</td>
</tr>
<tr>
<td>( R_{v} )</td>
<td>Solute rejection coefficient at surface of venule</td>
</tr>
<tr>
<td>( Q_{m} )</td>
<td>Term defined by equation (6.31d)</td>
</tr>
<tr>
<td>( Q_{c} )</td>
<td>Average relative tissue concentration across the vessel wall</td>
</tr>
<tr>
<td>( S_{f} )</td>
<td>Term defined by equation (6.46)</td>
</tr>
<tr>
<td>( S_{v} )</td>
<td>Flux of vesicles across endothelial cells, vesicles/( \mu^{2} \cdot \text{sec} )</td>
</tr>
<tr>
<td>( t )</td>
<td>Time, hr</td>
</tr>
<tr>
<td>( T_{1} )</td>
<td>Endothelial Biot number, ( = \frac{K_{1}L}{D_{\text{eff}}} )</td>
</tr>
<tr>
<td>( T_{2} )</td>
<td>Adventitial capillary Biot number, ( = \frac{K_{A_{c}L}}{A_{D}} )</td>
</tr>
<tr>
<td>( T_{L} )</td>
<td>Lymphatic Biot number, ( = \frac{K_{A_{L}}L}{A_{D}} )</td>
</tr>
<tr>
<td>( V )</td>
<td>Volume, ml</td>
</tr>
<tr>
<td>( V_{v} )</td>
<td>Internal volume of a vesicle, cm(^{3} )</td>
</tr>
<tr>
<td>( W )</td>
<td>Weight of tissue outline, g</td>
</tr>
</tbody>
</table>
\( x \) - Distance, cm
\( Z_k \) - Term defined by equation (6.47)
\( \alpha \) - Pre-exponential coefficient
\( \beta \) - Plasma decay constant, hr\(^{-1}\)
\( e \) - Aortic media void volume
\( \varepsilon_a \) - Aortic adventitia void volumes
\( \xi \) - Total dimensionless tissue concentration
\( \eta \) - Dimensionless position, \( = x/L \)
\( \Theta \) - Thiele modulus, \( = k_1 C_b L^2 / D_{eff} \)
\( \kappa \) - Equilibrium constant
\( \lambda \) - Eigen value
\( \Pi \) - \( \Pi \)
\( \rho \) - Density, g/ml
\( \tau \) - Dimensionless time, \( = D_{eff} t / L^2 \)
\( \Psi \) - Dimensionless mobile tissue concentration, \( = C / C_p (0) \).
I. Literature Citations


J. Biographical Note

The author, Robert Lyman Bratzler, was born on May 1, 1946, in Lansing, Michigan, the third of four children. He attended elementary and secondary schools in Okemos, Michigan, graduating in 1964. In August 1968, he graduated cum laude from the University of Michigan with a B.S. in Chemical Engineering.

After graduation, the author worked for two years as a Product Research Engineer with the Procter and Gamble Company in Cincinnati, Ohio. In June 1969, he married the former Lori Marie Shingleton of East Lansing, Michigan.

In September 1970, the author enrolled in the Chemical Engineering Department of Massachusetts Institute of Technology. In 1971, he held a summer engineering position with the Arthur D. Little Corporation, Cambridge, Massachusetts.

The author is a member of Sigma Xi, American Institute of Chemical Engineers, the American Association for the Advancement of Science, Triangles Honorary, Phi Lambda Upsilon, and Tau Beta Pi. He is also a past president and treasurer of the Theta Xi social fraternity.

At the present time, he has accepted an appointment as Assistant Professor in the Chemical Engineering Department at Princeton University, Princeton, New Jersey, to begin in September 1974.