ANTITHROMBOGENIC DIALYSIS MEMBRANES FOR
THE ARTIFICIAL KIDNEY

by
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B.S., Purdue University (1962)
M.S., Massachusetts Institute of Technology (1963)

Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Science at the
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Accepted by:  
G.C. Williams, Chairman
Dept. Committee on Graduate Theses
DEDICATED TO

MY PARENTS
for their wise counsel and continual generosity

MY NEW BRIDE
for her understanding and patience
ANTITHROMBOGENIC DIALYSIS MEMBRANES
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Ben J. Lipps, Jr.

Submitted to the Department of Chemical Engineering on February 19, 1966, in partial fulfillment of the requirements for the degree of Doctor of Science at the Massachusetts Institute of Technology.

ABSTRACT

The purpose of this study was to establish certain fundamentals necessary for the eventual design of a compatible and efficient artificial kidney. Specifically, regenerated cellulose films and cellulose esters were treated to impart an antithrombogenic surface to them and studies of urea diffusion in human blood were performed to aid in such a design.

This new type of surface appears superior to existing materials in its versatility and its compatibility with blood. Heparin has been chemically bonded to these flexible and rigid materials after amination through graft polymerization with ethylenimine. Experiments employing radioactive heparin indicate that such chemically-adsorbed heparin surfaces are permanently coated even under conditions of high fluid shear, in contrast to previously reported processes by which heparin has been bonded to graphite substrates through physical adsorption.

In vitro coagulation tests have shown that such chemically-adsorbed heparin surfaces become "passivated" in contact with blood, and that they achieve a powerful antithrombogenic effect without loss of heparin from the surface to anticoagulate the contained blood by heparin in solution. This "passivation" appears to be the result of clotting factor adsorption onto the heparin surface. This adsorption has also been demonstrated by studies with radioactive plasma. In vivo implantation of intravascular prostheses prepared from such materials has resulted in extended patency in regions of low blood flow rate. Regenerated cellulose dialysis membranes, of the type currently used in the artificial kidney, were heparinized by this procedure.
and were observed to be at least the equal of the original membranes in their mechanical strength and permeability and far superior to the original materials in their compatibility with blood.

Thesis Supervisors: Edward W. Merrill
Professor of Chemical Engineering

Edwin R. Gilliland
Professor of Chemical Engineering

Edwin W. Salzman, M.D.
Massachusetts General Hospital
Professor William C. Greene  
Secretary of the Faculty  
Massachusetts Institute of Technology  
Cambridge, Massachusetts

Dear Professor Greene:

In accordance with the regulations of the Faculty, I hereby submit a thesis entitled "Antithrombogenic Dialysis Membranes for the Artificial Kidney," in partial fulfillment of the requirements for the degree of Doctor of Science in Chemical Engineering.

Respectfully submitted,

Signature redacted

Ben J. Lipps, Jr.
ACKNOWLEDGEMENTS

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## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I  SUMMARY</td>
<td>1</td>
</tr>
<tr>
<td>II INTRODUCTION</td>
<td>31</td>
</tr>
<tr>
<td>A. Purpose of the Study.</td>
<td>31</td>
</tr>
<tr>
<td>B. Physiology of Human Blood</td>
<td>31</td>
</tr>
<tr>
<td>1. Composition of Human Blood</td>
<td>33</td>
</tr>
<tr>
<td>2. The Red Blood Cell</td>
<td>33</td>
</tr>
<tr>
<td>3. The White Blood Cell</td>
<td>35</td>
</tr>
<tr>
<td>4. The Platelet</td>
<td>36</td>
</tr>
<tr>
<td>C. Human Renal Function</td>
<td>40</td>
</tr>
<tr>
<td>1. Renal Anatomy</td>
<td>40</td>
</tr>
<tr>
<td>2. Renal Functioning</td>
<td>41</td>
</tr>
<tr>
<td>a) Glomular Filtration</td>
<td>41</td>
</tr>
<tr>
<td>b) Tubular Reabsorption.</td>
<td>41</td>
</tr>
<tr>
<td>c) Tubular Secretion</td>
<td>45</td>
</tr>
<tr>
<td>D. Extracorporeal Hemodialysis</td>
<td>45</td>
</tr>
<tr>
<td>1. Extracorporeal Hemodialysis Operation</td>
<td>47</td>
</tr>
<tr>
<td>2. Dialyzer Comparisons</td>
<td>48</td>
</tr>
<tr>
<td>3. Dialyzer Mass Transfer Coefficients</td>
<td>52</td>
</tr>
<tr>
<td>4. Overall Urea Transfer Coefficients</td>
<td>54</td>
</tr>
<tr>
<td>5. Dialysate-side Mass Transfer Coefficient</td>
<td>57</td>
</tr>
<tr>
<td>6. Membrane Mass Transfer Coefficient</td>
<td>59</td>
</tr>
</tbody>
</table>
## TABLE OF CONTENTS (Cont)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Ion Related Artificial Kidneys</td>
<td>69</td>
</tr>
<tr>
<td>F. Human Blood Coagulation</td>
<td>72</td>
</tr>
<tr>
<td>1. Thrombin-Fibrinogen Reaction</td>
<td>77</td>
</tr>
<tr>
<td>a) Fibrinogen</td>
<td>77</td>
</tr>
<tr>
<td>b) Thrombin</td>
<td>78</td>
</tr>
<tr>
<td>c) Fibrin Formation</td>
<td>79</td>
</tr>
<tr>
<td>2. Prothrombin-Thrombin Reaction</td>
<td>80</td>
</tr>
<tr>
<td>3. Extrinsic Activation System</td>
<td>82</td>
</tr>
<tr>
<td>a) Factor V</td>
<td>83</td>
</tr>
<tr>
<td>b) Factor VII</td>
<td>83</td>
</tr>
<tr>
<td>c) Factor X</td>
<td>84</td>
</tr>
<tr>
<td>4. Intrinsic Prothrombin Activator</td>
<td>84</td>
</tr>
<tr>
<td>a) Factor XII and Factor XI</td>
<td>85</td>
</tr>
<tr>
<td>b) Factor IX</td>
<td>86</td>
</tr>
<tr>
<td>c) Factor VIII</td>
<td>87</td>
</tr>
<tr>
<td>G. Anticoagulant Heparin</td>
<td>89</td>
</tr>
<tr>
<td>1. Heparin Stability</td>
<td>93</td>
</tr>
<tr>
<td>2. Structure of Heparin</td>
<td>94</td>
</tr>
<tr>
<td>3. Reaction with Proteins</td>
<td>96</td>
</tr>
<tr>
<td>4. Biological Activity</td>
<td>99</td>
</tr>
<tr>
<td>5. Role of Surface Heparin</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>TABLE OF CONTENTS (Cont)</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td>APPARATUS AND PROCEDURE.</td>
</tr>
<tr>
<td>A</td>
<td>GBH Wall Shear Measurements.</td>
</tr>
<tr>
<td></td>
<td>1. Equipment</td>
</tr>
<tr>
<td></td>
<td>2. Experimental Procedure</td>
</tr>
<tr>
<td>B</td>
<td>Heparinization of Regenerated Cellulose Dialysis Tubing.</td>
</tr>
<tr>
<td></td>
<td>1. Surface Heparin Detection.</td>
</tr>
<tr>
<td></td>
<td>a) Radioactive Decay.</td>
</tr>
<tr>
<td></td>
<td>b) Beta Particle Detection.</td>
</tr>
<tr>
<td></td>
<td>c) Solid Scintillation Detection System</td>
</tr>
<tr>
<td></td>
<td>d) H$^3$ - Heparin Adsorption and Measurement Procedure</td>
</tr>
<tr>
<td></td>
<td>2. Regenerated Cellulose Surface Amination</td>
</tr>
<tr>
<td></td>
<td>a) Reaction Procedure and Equipment</td>
</tr>
<tr>
<td>C</td>
<td>In Vitro Testing of CIH Surfaces with Human Blood</td>
</tr>
<tr>
<td>D</td>
<td>Membrane Surface Microphotographs.</td>
</tr>
<tr>
<td>E</td>
<td>CIH Membrane Stress-Strain Measurements.</td>
</tr>
<tr>
<td>F</td>
<td>CIH Membrane Permeability Measurements</td>
</tr>
<tr>
<td>G</td>
<td>In Vivo Studies with Heparinized Surfaces.</td>
</tr>
<tr>
<td></td>
<td>1. Description of Testing Procedure</td>
</tr>
<tr>
<td>H</td>
<td>Urea Diffusion Through Human Blood</td>
</tr>
<tr>
<td></td>
<td>1. Diffusion Capillary and Instrumentation</td>
</tr>
</tbody>
</table>
### TABLE OF CONTENTS (Cont)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Procedure.</td>
<td>146</td>
</tr>
<tr>
<td>IV RESULTS AND DISCUSSION</td>
<td>151</td>
</tr>
<tr>
<td>A. Tritiated Heparin</td>
<td>151</td>
</tr>
<tr>
<td>B. GBH - Wall Shear Measurements.</td>
<td>154</td>
</tr>
<tr>
<td>C. Heparinization of Regenerated Cellulose Film (CIH Membranes)</td>
<td>160</td>
</tr>
<tr>
<td>D. <em>In Vitro</em> Compatibility Studies with Human Blood.</td>
<td>193</td>
</tr>
<tr>
<td>E. CIH Membrane Surface Microphotograph</td>
<td>206</td>
</tr>
<tr>
<td>F. Proposed Interaction Between Surface Heparin and Human Blood.</td>
<td>219</td>
</tr>
<tr>
<td>G. Preliminary <em>In Vivo</em> Compatibility Studies in Canine Vein</td>
<td>235</td>
</tr>
<tr>
<td>H. CIH Membrane Stress-Strain Measurements</td>
<td>256</td>
</tr>
<tr>
<td>I. CIH Membrane Permeability Measurements</td>
<td>269</td>
</tr>
<tr>
<td>J. Urea Diffusion in Whole Human Blood</td>
<td>274</td>
</tr>
<tr>
<td>K. Design Considerations for Compatible Artificial Kidney</td>
<td>287</td>
</tr>
<tr>
<td>V CONCLUSIONS.</td>
<td>290</td>
</tr>
<tr>
<td>VI RECOMMENDATIONS.</td>
<td>294</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>295</td>
</tr>
<tr>
<td>A. Derivation of Relationship Between Overall Membrane Mass Transfer Coefficient and Dialysance</td>
<td>295</td>
</tr>
<tr>
<td>B. Conversion of Urea to Ionic Products</td>
<td>297</td>
</tr>
<tr>
<td>C. <em>In Vitro</em> Blood Coagulation Test Procedures</td>
<td>298</td>
</tr>
</tbody>
</table>

viii
TABLE OF CONTENTS (Cont)

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.</td>
<td>Couette Wall Shear Cell.</td>
<td>301</td>
</tr>
<tr>
<td>E.</td>
<td>Calculations Pertaining to H&lt;sup&gt;3&lt;/sup&gt;-Heparin Detection.</td>
<td>304</td>
</tr>
<tr>
<td>F.</td>
<td>Ethylenimine Health Hazards</td>
<td>305</td>
</tr>
<tr>
<td>G.</td>
<td>Heparin Monolayer Calculations</td>
<td>307</td>
</tr>
<tr>
<td>H.</td>
<td>Membrane Diffusion Calculations</td>
<td>308</td>
</tr>
<tr>
<td>I.</td>
<td>Urea Diffusion in Human Blood</td>
<td>309</td>
</tr>
<tr>
<td>J.</td>
<td>Literature Citations</td>
<td>311</td>
</tr>
<tr>
<td>K.</td>
<td>Nomenclature and Abbreviations</td>
<td>321</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>Typical Solute Concentrations of Plasma, Urine, and Artificial Kidney Dialysate.</td>
<td>296</td>
</tr>
<tr>
<td>2-1</td>
<td>Summary of Artificial Kidney Characteristics and Performance.</td>
<td>49</td>
</tr>
<tr>
<td>2-2</td>
<td>Roman Numeral Nomenclature for Human Blood Coagulation Factors</td>
<td>74</td>
</tr>
<tr>
<td>4-1</td>
<td>Calculated Surface Heparin(Monolayer)-GBH Surface</td>
<td>184</td>
</tr>
<tr>
<td>4-2</td>
<td>Urea Diffusion Through Membranes and Solutions</td>
<td>273</td>
</tr>
</tbody>
</table>

## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Illustration of Kidney Nephron.</td>
<td>43</td>
</tr>
<tr>
<td>2-2</td>
<td>Schematic Diagram of Kidney Nephron</td>
<td>44</td>
</tr>
<tr>
<td>2-3</td>
<td>Schematic of Artificial Kidney-Patient System</td>
<td>50</td>
</tr>
<tr>
<td>2-4</td>
<td>Modified Wilson Plot.</td>
<td>58</td>
</tr>
<tr>
<td>2-5</td>
<td>Fringed Micelle Model</td>
<td>64</td>
</tr>
<tr>
<td>2-6</td>
<td>Effect of Increasing Membrane Permeability with No Reduction in Blood-side Resistance</td>
<td>75</td>
</tr>
<tr>
<td>2-7</td>
<td>Human Blood Coagulation Mechanism</td>
<td>76</td>
</tr>
<tr>
<td>2-8</td>
<td>Proposed Heparin Molecule</td>
<td>97</td>
</tr>
<tr>
<td>2-9</td>
<td>Proposed Crosslink in Heparin Molecule</td>
<td>97</td>
</tr>
<tr>
<td>3-1</td>
<td>Couette Surface Shear Cell</td>
<td>107</td>
</tr>
<tr>
<td>3-2</td>
<td>Schematic Beta Scintillation System</td>
<td>114</td>
</tr>
<tr>
<td>3-3</td>
<td>H³-Heparin Solid Scintillation Detector</td>
<td>117</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3-4</td>
<td>Scintillation Detector with Amplification Instrumentation.</td>
<td>118</td>
</tr>
<tr>
<td>3-5</td>
<td>Typical H(^3) - Heparin Membrane Measurement.</td>
<td>118</td>
</tr>
<tr>
<td>3-6</td>
<td>Resin-Kettle Amination Reactor</td>
<td>123</td>
</tr>
<tr>
<td>3-7</td>
<td>Membrane Diffusion Cell.</td>
<td>131</td>
</tr>
<tr>
<td>3-8</td>
<td>Membrane Permeability Measuring System</td>
<td>132</td>
</tr>
<tr>
<td>3-9</td>
<td>Microphotograph of CAIH Surface, I.</td>
<td>139</td>
</tr>
<tr>
<td>3-10</td>
<td>Schematic of Canine Inferior Vena Cava</td>
<td>140</td>
</tr>
<tr>
<td>3-11</td>
<td>Importance of Second + Terms in the Capillary Cell Series Expansion.</td>
<td>147</td>
</tr>
<tr>
<td>3-12</td>
<td>Human Erythrocyte Sedimentation Rate as a Function of Hematocrit</td>
<td>148</td>
</tr>
<tr>
<td>3-13</td>
<td>Urea Diffusivity Measuring Equipment</td>
<td>149</td>
</tr>
<tr>
<td>4-1</td>
<td>Anticoagulant Activity of Tritiated Heparin</td>
<td>155</td>
</tr>
<tr>
<td>4-2</td>
<td>Transfer of Tritium From Heparin to Water</td>
<td>156</td>
</tr>
<tr>
<td>4-3</td>
<td>Typical Surface Adsorption Curve with H(^3) - Heparin</td>
<td>157</td>
</tr>
<tr>
<td>4-4</td>
<td>Effect of Wall Shear on GBH Surface Heparin</td>
<td>158</td>
</tr>
<tr>
<td>4-5</td>
<td>Effect of Various Ethylenimine and RCF Reaction Schemes on the Quantity of Adsorbed Heparin</td>
<td>181</td>
</tr>
<tr>
<td>4-6</td>
<td>Effect of Catalyst Loading and Ethylenimine Reaction Time on the Quantity of Adsorbed Heparin</td>
<td>182</td>
</tr>
<tr>
<td>4-7</td>
<td>Comparison of Surface Heparin - GBH vs. CIH Procedures</td>
<td>183</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4-8</td>
<td>Amination of RCF Using Ethylenimine with Various Catalysts.</td>
<td>187</td>
</tr>
<tr>
<td>4-9</td>
<td>Distribution of Amino Nitrogen with Various Reaction Schemes.</td>
<td>188</td>
</tr>
<tr>
<td>4-10</td>
<td>Distribution of Amino Nitrogen with Ethylene Oxide Pretreatment.</td>
<td>191</td>
</tr>
<tr>
<td>4-11</td>
<td>Theoretical Substitution of Cellulose as a Function of Nitrogen Content.</td>
<td>192</td>
</tr>
<tr>
<td>4-12</td>
<td>Effect of Wall Shear on Surface Heparin of CIH Membranes.</td>
<td>199</td>
</tr>
<tr>
<td>4-13</td>
<td>Effect of Ethylenimine and Methanol on Human Plasma.</td>
<td>200</td>
</tr>
<tr>
<td>4-14</td>
<td>Effect of Polyethylenimine and Cationic Surfactants on Human Plasma.</td>
<td>201</td>
</tr>
<tr>
<td>4-15</td>
<td>Effect of Polyethylenimine on Human Plasma.</td>
<td>202</td>
</tr>
<tr>
<td>4-16</td>
<td>Compatibility of Heparinized Cellulose Dialysis Tubing (CIH) With Human Blood</td>
<td>209</td>
</tr>
<tr>
<td>4-17</td>
<td>Microphotographs of Regenerated Cellulose Membrane Surface.</td>
<td>210</td>
</tr>
<tr>
<td>4-18</td>
<td>Microphotographs of CIH Membrane Surfaces, I.</td>
<td>211</td>
</tr>
<tr>
<td>4-19</td>
<td>Microphotographs of CIH Membrane Surfaces, II.</td>
<td>212</td>
</tr>
<tr>
<td>4-20</td>
<td>Microphotographs of CIH Membrane Surfaces, III.</td>
<td>213</td>
</tr>
<tr>
<td>4-21</td>
<td>Microphotographs of CIH Membrane Surfaces, IV.</td>
<td>214</td>
</tr>
<tr>
<td>4-22</td>
<td>Microphotographs of CIH Membrane Surfaces, V.</td>
<td>215</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4-23</td>
<td>Effect of CIH Tubes on Fresh Whole Human Blood</td>
<td>223</td>
</tr>
<tr>
<td>4-24</td>
<td>Interaction of CIH Tubes With Fresh Human Plasma - 20h Washing Prior to Heparinization</td>
<td>224</td>
</tr>
<tr>
<td>4-25</td>
<td>Interaction of CIH Tubes With Fresh Human Plasma - 120h Washing Prior to Heparinization</td>
<td>225</td>
</tr>
<tr>
<td>4-26</td>
<td>Interaction of CIH Tubes with Fresh Human Plasma - 360h Washing Prior to Heparinization.</td>
<td>226</td>
</tr>
<tr>
<td>4-27</td>
<td>Adsorption of Plasma Components Onto Heparinized Surfaces</td>
<td>231</td>
</tr>
<tr>
<td>4-28</td>
<td>Surface Heparinization of Cellulose Acetate</td>
<td>241</td>
</tr>
<tr>
<td>4-29</td>
<td>Effect of Reaction Impurities on Prothrombin Time of Plasma Incubated in CAIH Tubes</td>
<td>242</td>
</tr>
<tr>
<td>4-30</td>
<td>Effect of Surface Deacetylation On Whole Blood Clotting Time</td>
<td>247</td>
</tr>
<tr>
<td>4-31</td>
<td>Effect of Extended Prothrombin Time On Whole Blood Clotting Time</td>
<td>248</td>
</tr>
<tr>
<td>4-32</td>
<td>Microphotographs of CAIH Surfaces, II.</td>
<td>249</td>
</tr>
<tr>
<td>4-33</td>
<td>In Vivo Clotting Times of CIH Prostheses Placed in the Abdominal IVC Below the Renals.</td>
<td>253</td>
</tr>
<tr>
<td>4-34</td>
<td>In Vivo Clotting Times of CAIH Prostheses Placed in the Canine Abdominal IVC Below the Renals</td>
<td>254</td>
</tr>
<tr>
<td>4-35</td>
<td>Force-Strain Curves for CIH Membranes-H_{2}O Catalyzed</td>
<td>259</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4-36</td>
<td>Force-Strain Curves for CIH Membranes - .04N HCl Catalyzed</td>
<td>260</td>
</tr>
<tr>
<td>4-37</td>
<td>Force-Strain Curves for CIH Membranes - .2N HCl Catalyzed</td>
<td>261</td>
</tr>
<tr>
<td>4-38</td>
<td>Force-Strain Curves for CIH Membranes - .4N HCl Catalyzed</td>
<td>262</td>
</tr>
<tr>
<td>4-39</td>
<td>Effect of Reaction Conditions on Membrane Breaking Force</td>
<td>265</td>
</tr>
<tr>
<td>4-40</td>
<td>Effect of Reaction Conditions on Membrane Thickness</td>
<td>266</td>
</tr>
<tr>
<td>4-41</td>
<td>Stress-Strain Curves for Various Reaction Conditions</td>
<td>267</td>
</tr>
<tr>
<td>4-42</td>
<td>Effect of Reaction Conditions on Membrane Breaking Stress</td>
<td>268</td>
</tr>
<tr>
<td>4-43</td>
<td>Total Liquid Side Resistance for Membrane Diffusion Cell</td>
<td>273</td>
</tr>
<tr>
<td>4-44</td>
<td>Urea Membrane Diffusion Results</td>
<td>274</td>
</tr>
<tr>
<td>4-45</td>
<td>Diffusivity of Urea Through Saline and Distilled Water</td>
<td>279</td>
</tr>
<tr>
<td>4-46</td>
<td>Diffusivity of Urea Through Human Plasma</td>
<td>280</td>
</tr>
<tr>
<td>4-47</td>
<td>Diffusivity of Urea Through Citrated Human Blood - Hematocrit of 50</td>
<td>280</td>
</tr>
<tr>
<td>4-48</td>
<td>Diffusivity of Urea Through Citrated Human Blood - Hematocrit of 65</td>
<td>281</td>
</tr>
<tr>
<td>4-49</td>
<td>Diffusivity of Urea Through Heparinized Human Blood - Hematocrit of 70</td>
<td>281</td>
</tr>
<tr>
<td>4-50</td>
<td>Diffusivity of Urea Through Human Red Cell - Saline Suspension - Hematocrit of 70</td>
<td>282</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4-51</td>
<td>Diffusivity of Urea Through Citrated Human Blood - Hematocrit of 78.</td>
<td>282</td>
</tr>
<tr>
<td>4-52</td>
<td>Urea Diffusion Coefficients for Human Blood</td>
<td>283</td>
</tr>
<tr>
<td>4-53</td>
<td>Comparison of Theoretically Predicted and Experimentally Measured Urea Diffu-</td>
<td>284</td>
</tr>
<tr>
<td></td>
<td>sion Coefficients for Human Blood</td>
<td></td>
</tr>
</tbody>
</table>
I. SUMMARY

Synopsis

The purpose of this study was to establish certain fundamentals necessary for the eventual design of an efficient and compatible artificial kidney. By compatible is meant a material whose surface is sufficiently passive toward human blood to avoid completely, or minimize the activation of clotting factor during blood-surface contact. Specifically regenerated cellulose films, henceforth abbreviated RCF, were treated to impart an antithrombogenic property to them. The strength and permeability characteristics of the treated films were compared to the original RCF. The interaction of this film with human blood was studied and an hypothesis developed for the cause of the surface passivation. This hypothesis, confirmed partially with two independent tests, is believed to be one of primary importance in design of compatible artificial organs. Studies of urea diffusivities in human blood were also performed to aid in such a design.

Introduction

In the healthy human waste metabolites are removed in the form of urine, which is the product resulting from the action of the kidney nephrons on the blood. A schematic of a nephron is presented in Figure 2-2. Normally blood is "filtered" by the glomerulus producing a cell and protein concen-
trate on one side of the glomerular membrane and a solution of small molecules and ions on the other side including the waste product. The two streams from the glomerulus pass cocurrently, separated by the tubular membrane. This membrane has the unique property of actively transporting $\text{Na}^+$ and other necessary products back into the concentrated blood stream. The water and electrolyte content of the blood are adjusted by this membrane to the desired level and the remaining ultrafiltrate becomes urine. About 99% of the ultrafiltrate is reabsorbed into the blood stream.

Extracorporeal hemodialysis is substituted for human kidney functioning in the case of uremia. Currently this artificial kidney takes over only for the function of the glomerulus and therefore the removal of impurities from the blood requires careful balancing of the dialysis fluid against blood. Common clinical designs consist of either a long dialysis tubing coil through which blood is pumped, with the dialysis bath outside, or a parallel design where alternating blood and dialysate channels are stacked together to form a laminate. In both designs RCF is used as the dialysis membrane. A schematic of the "coil" artificial kidney in relation to the patient is shown in Figure 2-3.

During hemodialysis using "regional heparinization" blood is drawn from the arterial cannula and is anticoagulated with
heparin, for otherwise it would rapidly clot in its progress through the artificial kidney as a result of surface activation of some of the innate clotting factors. Subsequently the anticoagulated blood is pumped through the dialyzer and returned to the patient. Prior to entering the patient, the blood has its clotting mechanism reinstated by neutralizing the heparin with protamine. Regional heparinization, although it produces large quantities of the heparin-protamine complex in the patient, is preferred over total heparinization of the patient's blood during hemodialysis because of the complications of internal bleeding and bone brittleness commonly associated with patient heparinization. (94).

Although the exact cause of the uremic syndrome is not as yet clear, unmistakably the artificial kidney must remove more waste metabolite urea than all the other solutes combined. This results from the body's 20 gm per day production rate for urea. Water may be removed by ultrafiltration through the RCF membranes to correct any excess of fluid in the patient. Therefore it is appropriate to consider urea removal as the basis for an assessment of the efficiency of a given unit. An efficient artificial kidney should have a high urea removal rate with a minimum of membrane area or blood volume. A compilation of performance characteristics of the current "coil" and "parallel" kidneys is given in Table 2-1.
Human blood is a complex suspension of three general types of solid particles in a continuous medium, known as plasma. The solid particles are red cells (erythrocytes), white cells (leukocytes) and platelets. Plasma in turn, is an exceedingly complex aqueous solution of organic and inorganic salts, proteins and other organic macromolecules. The red cells, which account for the majority of blood solids, are biconcave disks $8.5\mu$ in diameter with a volume of about $90\mu^3$. They are largely comprised of water and hemoglobin, the latter accounting for 90% of the solid matter. Water and many solutes pass rapidly across the cell membrane but some ions such as sodium and potassium are transported against concentration gradients by a mechanism which requires energy from metabolism. These red cells themselves are not mobile, but rhythmical movements of the surface, which cause rapid fluctuations in the thickness of the cell, have been observed with phase-contrast microscopy (119).

The interaction between human blood and foreign surfaces, usually manifested by rapid coagulation of the blood, is believed to be related to the globulin fraction of the plasma proteins (124). Although the exact nature of the coagulation mechanism is still unknown, a cascade mechanism, which is initiated by foreign surface contact or tissue injury, has received much support in recent years and is important in the
interpretation of the results of this thesis as will be seen below. (71,9) The proposed mechanism is shown in Figure 2-7. Following tissue injury, coagulation can proceed through two parallel paths, the intrinsic clotting system or the extrinsic clotting system, to the prothrombin activation step. With the formation of either the intrinsic or extrinsic prothrombin activator, known as intrinsic or extrinsic thromboplastin, prothrombin is converted to thrombin, which in turn, converts fibrinogen to fibrin. Fibrin, a rapidly produced polymer of fibrinogen, forms three dimensional water insoluble networks which can be observed as the blood or plasma clot. All clotting factors required for coagulation are present in normal blood and are needed during the intrinsic clotting route. For the extrinsic route tissue extract is substituted for the phospholipids of the platelets. Few of the clotting factors of the extrinsic or intrinsic system have been isolated or rigorously identified and are presumed to exist only by the absence or presence of their clotting activity. For the sake of clarity the clotting factors have been given the Roman numeral designations widely adopted in current hematology.

Foreign surfaces normally produce blood coagulation via the intrinsic clotting system. Factors XII and XI are thought to initiate this coagulation by becoming activated at the foreign surface. This activated Factor XII-XI complex in
turn activates other clotting factors, and so on through a series of consecutive activations until fibrin is formed.

Three types of coagulation tests were used during this study to determine the potency of the intrinsic clotting system, extrinsic clotting system, and the fibrinogen to fibrin step. These tests are respectively the kaolin-cephalin test, the prothrombin test and the thrombin test.\(^1\)

Because of the importance of heparin in blood coagulation and hemodialysis, as well as its most recent utilization in thrombus resistant surfaces, a brief discussion of its properties will be given. Heparin is a mucopolysaccharide found in the liver and many tissues of the body. It is a highly sulfonated polyanion which has been found to react with many proteins and complex bases. (20, Chapt. 3, 23) This reaction with proteins is considered to be a simple salt formation in which reversible dissociation of the salt occurs according to

\[ \text{1. With the kaolin-cephalin test, the time required for coagulation of platelet-poor plasma in the presence of all clotting factors and kaolin at } 37^\circ\text{C is known as the "kaolin-cephalin time." In this test the brain extract, cephalin, is substituted for platelets to give standardized results and kaolin supplies the surface for activation. The "prothrombin time" resulting from the prothrombin test is the time required for coagulation of platelet-poor plasma at } 37^\circ\text{C when tissue extract has been added. The "thrombin time," which refers to the thrombin test, is the time required to form fibrin from fibrinogen in the presence of thrombin.} \]
the mass action law.

Heparin is known to interfere with coagulation in the intrinsic clotting system by inhibiting the formation of intrinsic thromboplastin. In the presence of a plasma cofactor, "thrombin inhibitor," heparin also interferes with the fibrinogen-thrombin reaction. The former anticoagulant effect is thought to be the more important of the two. O'Brien (80,81) postulates that heparin reversibly complexes with Factor IX of the intrinsic system, resulting in sufficient activity reduction to produce anticoagulation. Because of the highly cationic nature of protamine sulfate, this heparin neutralant is thought to have a higher affinity for heparin than the clotting factors. Hence the clotting factor in the heparin complex is probably replaced by protamine reinstating the coagulation activity.

The interference of heparin with the fibrinogen-thrombin reaction, in the presence of a plasma factor, was used in this study as an indication of heparin's presence. Extended "thrombin times" were a definite evidence of heparin activity.²

The molecular weight of heparin is thought to be around 15,000 to 16,000 (4). The most probable structure for the basic tetrasaccharide repeating unit of heparin can be seen

2. See footnote 1.
in Figure 2-8. Heparin is known to contain $\alpha(1 \rightarrow 4)$ linkages and hence a helical structure similar to that for amylase has been proposed. (23,107,122,123). It has been further suggested that a certain amount of rigidity might be conferred on such a structure by means of a small number of intramolecular sulfate bridges of the type shown in Figure 2-9. Considering this, the heparin molecule might be expected to be a collapsed helical structure with sulfate sites present around its perimeter. In this configuration, heparin might be adsorbed to a solid substrate and still maintain anticoagulant activity since a portion of heparin's sulfate and amino sulfate groups will continue to be exposed to the solution contacting the substrate. It seems likely that the degree and distribution of sulfate groups and the molecular shape of heparin contribute much towards its anticoagulant activity (11).

Studies by Whiffen, Gott, and Young (33,34,35,113,114,115,116,117,118) indicate that the adsorption of heparin onto their graphite-benzalkonium surfaces gives extended in vitro whole blood clotting times, hereafter abbreviated WBC, as well as extended in vivo patency, when heparinized prostheses were placed in the canine circulatory system near the heart. However, fresh whole blood when contacted with the graphite-benzalkonium-heparin surface, hereafter abbreviated GBH, was found in this study to be anticoagulated by heparin desorbed
from the surface. Thus the cause of long term *in vivo* patency is not immediately obvious. Possibly an irreversibly adsorbed heparin surface might be passive enough toward human blood to permit the design of compatible extracorporeal circuits which require no heparinization of the patient's blood.

**Apparatus and Procedure**

To gain a better understanding of the GBH surface, it was subjected to well defined fluid shears using fresh plasma and saline. The wall shear rates corresponded to those normally existing in the cannulas used in present heart-lung and hemodialysis circuits. The fluid shear was produced in the equivalent of a Couette viscometer in which the inner cylinder surface was coated with graphite-benzalkonium chloride-heparin. The heparin migrating from the surface into the solution as a result of shear was analyzed by means of a modified thrombin test. The total heparin appearing in solution was compared to the original heparin on the inner cylinder before shear, measured with tritiated heparin (radioactive counting).

This tritiated heparin was produced by exposure of heparin to tritium gas, commonly known as the Wilsbach technique (120) and was used to determine the quantity of adsorbed surface heparin. The anticoagulant activity of the tritiated heparin closely resembled that of the non radioactive heparin. Tritiated heparin was selected rather than $^{35}$-heparin, which has been
produced by canine ingestion of Na\textsuperscript{35}O\textsubscript{4} followed by heparin extraction of the dog's liver, because of the short half life of S\textsuperscript{35} and the ideal range of tritium emission. With tritium one could measure only the surface heparin present, even on thin (1 mil) membranes.

The adsorbed tritiated heparin was detected and measured with a solid scintillator counter assembly similar to the schematic shown in Figure 3-2. A photograph of the actual photomultiplier tube assembly and preamplifier is presented in Figure 3-3.

RCF of the type currently used in hemodialysis was selected as the substrate for irreversible heparinization because of its immediate and well founded biological potential. The general reaction scheme for producing this heparinized RCF consists of, 1) surface amination to attach cationic and basic groups to the RCF and 2) heparinization of the aminated film by immersion in an aqueous sodium heparin solution, 5 to 100 Mg/ml, for 10 to 15 minutes. Thus, the surface heparin was chemically bonded to the substrate through the amino linkage. Various amination techniques were studied and evaluated by measuring the quantity of adsorbed surface heparin. Normally a balance between membrane strength and surface heparin dictated the usefulness of the amination technique.

Only RCF amination techniques employing ethylenimine as
the aminating agent gave adsorbed heparin quantities com-
parable to those of the GBH surface with a retention of mem-
brane strength. Generally, amination with ethylenimine took
place in the presence of a catalyst, such as water or acid,
with ethylenimine vapor at temperatures between 70 and 90°C.
The ethylenimine was believed to graft polymerize onto the
RCF in the following manner.

\[
\text{Cel - OH} + \begin{array}{c}
\text{H} \\
\text{H}_2\text{C} - \text{CH}_2
\end{array} \rightarrow \text{Cel - O - CH}_2\text{CH}_2\text{NH}_2
\]

\[
\text{Cel - O - CH}_2\text{CH}_2\text{NH}_2 + \begin{array}{c}
\text{H} \\
\text{H}_2\text{C} - \text{CH}_2
\end{array} \rightarrow \text{Cel-O-[CH}_2\text{CH}_2\text{NH}]_n\text{-CH}_2\text{CH}_2\text{NH}_2 \quad n
\]

With or without protonation of the RCF - grafted polyethyl-
enimine surface a high affinity for heparin exists, giving far
more adsorbed heparin than with the GBH procedure, but still
maintaining adequate membrane strength.

To aid in the design of more efficient artificial kidneys,
be they compatible or not, other studies were undertaken to
determine experimentally the diffusion coefficient at 20°C of
\(^{14}\text{C}\) labelled urea through human blood, in a capillary diffusion
cell. The same scintillation detection instrumentation as that
used for the tritiated heparin detection was employed to follow
the diffusion of the \(^{14}\text{C}\)-urea from a capillary, fabricated from
a solid scintillator, into a reservoir. A photograph of the
capillary, the reservoir, and the detection instrumentation is
Results and Discussion

1) **Fluid Shear Experiments with Couette Geometry-Graphite-Benzalkonium-Heparin Surface**

As much as 20% of the original 2 $\mu$ gm/cm$^2$ surface heparin on the Lucite-GBH surface can migrate into fresh plasma in five hours when the surface and plasma are in contact but with no fluid shear. In the presence of a wall shear of 9.5 dynes/cm$^2$, comparable to the maximum shear in the heart-lung machine, approximately 80% of the original heparin on the inner cylindrical surface migrates from the surface into the fresh plasma within five hours. With a wall shear comparable to the maximum shear in the artificial kidney, about 40% of the original GBH heparin is lost to the plasma in five hours.

In contrast to the fluid shear results with plasma, virtually no GBH surface heparin loss from the inner cylindrical surface observed when the shearing solution was saline, even with a wall shear of 9.5 dynes/cm$^2$ for five hours.

This wall shear data indicates that a definite interaction exists between the GBH surface heparin and fresh plasma but appears to be absent between saline and the surface heparin. The data also points to a limitation on the utility of GBH surfaces in regions of high shear but indicate that sufficient surface heparin might remain in regions of low flow to give
2) Heparinization of Regenerated Cellulose Film

Amination procedures involving the action of chloroalkylamines or aminoalkyl hydrogen sulfate with alkali cellulose were studied in an attempt to surface aminate RCF. These procedures were greatly limited by the sodium hydroxide treatment of the RCF to produce alkali cellulose. A vigorous sodium hydroxide treatment is necessary to produce amination but results in destruction of the RCF structure. Maximum heparin adsorption with an acceptable membrane structure was of the order of 0.3 $\mu$ gm/cm$^2$, well below that of the GBH surface.

Four different reaction schemes using ethylenimine were studied: 1) ethylene oxide pretreated RCF (air dry) + ethylenimine vapor at $85^\circ$C, 2) initially water swollen RCF + ethylenimine vapor at $70^\circ$C and $85^\circ$C, 3) RCF + NH$_4$Cl and ethylenimine in toluene, reflux $80^\circ$C, and 4) initially acid swollen RCF + ethylenimine vapor at $85^\circ$C.

Ethylenimine is known to react with groups containing active hydrogen atoms, such as the hydroxyl groups on the cellulose molecule, if the proper catalyst is present. Assuming that this reaction resembles homopolymerization of ethylenimine, the first step in the grafting reaction is probably the production of the ethylenimmonium ion according to
RX + NH $\rightarrow$ RNH $^+$ + X$^-$ where

\[ \text{H}_2\text{C-CH}_2 \quad \text{H}_2\text{C-CH}_2 \]

R may be H also. This ion is then believed to react with the RCF according to

\[ \text{Cel-OH} + \text{RNH}^+ \rightarrow \text{Cel-O-CH}_2\text{CH}_2\text{NH}_2 + \text{H}^+ \]

\[ \text{H}_2\text{C-CH}_2 \]

The grafted polyethylenimine successfully competes for the H$^+$ ion and eventually limits the polymerization by depleting the acid present. (2,53,54) Branching of the polyethylenimine is possible through the interaction of the immonium ion and secondary nitrogens of the polymer but no proof of its existence has yet appeared.

The ethylene imine grafting procedures of this study used water, HCl, and NH$_4$Cl as catalysts. The purpose of the ethylene oxide pretreatment was to produce a more uniform surface amination by converting the secondary cellulose hydroxyls to primary hydroxyls prior to amination through etherification.

The influence of these various reaction procedures on the quantity of adsorbed surface heparin can be seen in Figure 4-5. The ethylene oxide pretreatment gave the highest values of adsorbed surface heparin. Additional water beyond
that contained in the initially swollen membrane was required
to produce further amination after 50 hours of contact with
ethylenimine vapor at one atmosphere and 70°C. With a reac-
tion temperature of 85°C and an initially water swollen mem-
brane, the quantity of absorbed heparin appeared to level at
50 hours also, but at significantly higher levels. Both pro-
cedures are probably terminated by evaporation of the original
water from the membrane, for dry cellulose will not react with
ethylenimine.

With the toluene phase amination procedure, the grafting
of ethylene imine did not appear to level off with time indi-
cating the possibility of unlimited and perhaps excessive sur-
face polymerization. The procedure using acid catalysis showed
the highest tendency toward leveling off of the subsequently
adsorbed heparin as a function of ethylenimine exposure time.
This equilibrium level was found to be inversely related to
the initial acid loading of the swollen membrane. This indi-
cates that the acid must play a part in the termination of
the graft polymerization. The acid probably protonates the
grafted polyethylenimine at an early stage causing termination,
since protonated nitrogens and immonium ions have very little
tendency to react. A consequence of this termination might
be a more uniform amination, since after protonation of the
grafted polyethylenimine the immonium ions have only cellulose
Nitrogen analyses and the data of Figure 4-5 indicate that those membranes which were aminated in the presence of HCl have the deepest and possibly most uniform amination. The toluene amination procedure tends to produce surface amination. The ethylene oxide pretreatment probably results in a uniform but not necessarily as penetrating an amination as the acid catalyzed procedure. With water as the catalyst a penetration intermediate between the acid catalyst and ethylene oxide process is expected.

WBC tests with fresh human blood were used to determine the compatibility of the various heparinized surfaces. The results of these studies are presented in Figure 4-16. The data of Fig. 4-16 showing the extended whole blood clotting times obtained with these surfaces are definitely the result of the transformed nature of the surface and not artifacts such as elutable heparin or elutable reaction impurities. The absence of heparin in the blood, that could have come from elution or leaching of the membranes, is indicated by the normal value of the "thrombin time" on aliquots of plasma incubated in the heparinized regenerated dialysis tubes. Additional evidence of an irreversibly adsorbed heparin surface is afforded by the results of fluid shear measurements.

Heparinized regenerated cellulose membranes, hereafter called...
CIH, sheared with fresh plasma at 9.5 dynes/cm² for 15 hours showed no loss of heparin from the membrane.

Another potential artifact other than elutable heparin is ethylenimine or its homopolymer formed during the amination. Concentrations of greater than 30 ppm of monomer or polyethylene imine produce an extended "prothrombin time." In fact, the prothrombin time serves as a semi-quantitative indicator of these impurities. Since these impurities are highly soluble in water, all aminated membranes were washed for a sufficient period of time with water to give a normal "prothrombin time" with aliquots of citrated plasma incubated in the CIH tubes.

The data of Figure 4-16 indicate that the cellulose membrane surfaces prepared via the water-ethylenimine vapor or liquid phase amination schemes are incompletely covered in the subsequent heparinization. A more concentrated heparinizing solution produced considerably more surface heparin on the water catalyzed membranes but only a slight improvement in compatibility was observed. The sensitivity of surface bound heparin, as measured by tritium emission, to the heparinizing solution is the aqueous sodium heparin solution described on page 10. The higher concentration referred to here is 100 Mg of heparin per ml of water.

3. The heparinizing solution is the aqueous sodium heparin solution described on page 10. The higher concentration referred to here is 100 Mg of heparin per ml of water.
ized solution concentration indicates a possible multiple attachment between the heparin and the grafted ethylenimine. Such bonding could render useless much of the surface heparin by, in effect, completely sequestering it. Since a proportionate increase in compatibility was not observed with this increase in surface heparin, one tentatively concludes that non-heparinized regions of membranes exist.

Microphotographs of the heparinized membrane surfaces and surfaces of the original RCF show surface crystallites of from 20 to 50\(\mu\) in size. Due to the hydrogen bonding between hydroxyls in the cellulose crystallites, these crystalline regions are notoriously less reactive than the amorphous regions. (82,83) Consequently, these surface crystalline regions might well be the non-heparinized portions of the surface.

The CIH surfaces with the ethylene oxide pretreatment and the lowest acid catalyst concentration, .04N HCl, have improved compatibility but the most compatible surfaces do produce a clot in 90 to 110 minutes. Surface crystals were observed with these surfaces also but to a lesser extent than with the water catalyzed schemes.

Surfaces showing whole blood clotting times well in excess of 110 minutes are obtained consistently only with an acid catalyzed amination achieved by prior swelling of the
membrane in a solution of hydrochloric acid having a concentration of 0.2 N or greater. The time of exposure to ethylenimine vapor at 85°C in the presence of such acid catalysts need only be about two hours for successful amination. The maximum amination time and catalyst loading is usually governed by considerations of membrane strength, since the acid is known to hydrolyze the glucosidic linkages of cellulose in the presence of heat.

Microphotographs of these acid catalyzed CIH surfaces show a complete absence of surface crystallites. Since both intra- and intercrystalline swelling of cellulose has been noted with acids (83), the hydrochloric acid, when present in sufficient concentrations, apparently penetrates the surface crystalline regions causing amination in the presence of ethylenimine and disruption of the crystallites.

Additional in vitro experimentation with the most promising acid catalyzed CIH membranes indicates that an irreversibly heparinized surface, when totally heparinized, prevents blood coagulation by: 1) actively adsorbing certain clotting factors thus rendering the blood indefinitely anticoagulated, or 2) becoming passivated through the adsorption of the blood clotting factors, hence in the presence of sufficient clotting factor activity the blood-like layer adsorbed to the heparin surface has a much reduced tendency toward
contact activation of the clotting mechanism.

When fresh blood is incubated at 35°C in CIH tubes for lengthening periods of time, the clotting time of the incubated 'blood' when it is removed and incubated in a glass test tube becomes progressively longer in proportion to its CIH incubation time. However, after a CIH incubation time of 70 to 90 minutes the fresh blood is permanently coagulated.

Incubations of fresh citrated plasma in CIH tubes show prolonged "kaolin-cephalin times" and normal "prothrombin" and "thrombin" times. The extended "kaolin-cephalin times" were corrected to normal with a 1 to 1 part dilution of the incubated plasma with normal plasma. This indicates that probably the prolonged "kaolin-cephalin time" was a result of clotting factor deficiencies in the intrinsic clotting system. These data do not completely rule out clotting factor deficiencies in the extrinsic system but do indicate that their level is sufficient, after incubation with the CIH membrane, to give a normal "prothrombin time."

Since heparin is known to inhibit the formation of intrinsic thromboplastin when used as an anticoagulant in solution (13,9,23) the in vitro data of this study apparently indi-

4. This clotting time is exclusive of the CIH incubation time.
cate a similar anticoagulant ability for heparin irreversibly adsorbed to surfaces.

Considering O'Brien's hypothesis (81, 82) that heparin in solution reversibly complexes with Factor IX of the intrinsic clotting system, one might, on the basis of the above data, extend O'Brien's theory to include heparin irreversibly bonded to solid substrates, in such a way as to have a portion of the active sites of heparin exposed to the contacting solution. From this point of view, the adsorbed heparin layer is thought to be active and adsorb at least one or more intrinsic clotting factors resulting in anticoagulation of the initial aliquot of blood placed in the heparinized tube. With complete saturation of the heparin surface a passive outer surface presumably consisting of plasma constituents such as proteins, clotting factors, etc. is expected. This heparin-blood clotting factor complex is thought to result in little or no denaturing of the clotting factors as in the case of heparin in solution. This protein-like outer surface may behave as a permanent antithrombogenic surface when not denatured by the atmosphere or other solutions, thus producing a far superior surface for in vitro or in vivo blood contacting devices.

Additional evidence of heparinized surface activity is presented in Figure 4-27. Tritiated human plasma was observed to strongly adsorb onto heparinized surfaces in much larger
quantities than those observed for silicone rubber and cellulose. This represents independent evidence of the activity of a heparinized surface. The quantity of plasma components adsorbed onto the surface bound heparin is seen to reach a saturated level of about an equal weight of components per weight of surface heparin.

The effectiveness of the heparinized surface can be reduced considerably by a concentrated protamine sulfate rinse, 10 Mg/ml. This has been demonstrated both in vitro and to some extent with the radioactive plasma. With the tritiated plasma one cannot distinguish between surface protamine replacement by the plasma components and plasma component adsorption on the protamine surface itself.

3) In Vivo Compatibility Studies

The limit of compatibility of the CIH surfaces can best be determined by controlled in vivo experimentation where the heparinized surface can become saturated with no exposure to alien environments. Since thin CIH membranes do not lend themselves to prostheses fabrication without some type of adhering support holder, prostheses for blood vessel replacement were fabricated from cellulose acetate. The sur-

5. These studies were performed at the Massachusetts General Hospital in Dr. G. Austin's Laboratory.
faces of these cellulose acetate prostheses were deacetylated with a methanolic solution of 1;3% sodium methylate. Amination and heparinization procedures resembled those for the regenerated cellulose films.

An erratically wide range of in vivo clotting times were observed for 3 cm. cellulose acetate prosthesis implanted into the canine thoracic inferior vena cava, the vein which returns blood to the heart. Therefore, the heparinized prostheses of this study were substituted for sections of the canine inferior vena cava below where the renal veins enter the vena cava. In this region of low blood flow, controls of silicone rubber and cellulose acetate were observed to occlude totally in 1 to 1.5 hours after the implantation. The venous pressure was monitored to determine the occurrence of clotting. Irreversibly heparinized cellulose acetate prostheses have remained patent for at least 8 1/2 to 9 hours, at which time the dogs were sacrificed to determine the degree of patency. This extended patency in such a severe thrombus producing situation is clear indication that an irreversibly heparinized surface is certainly antithrombogenic, probably because of its tendency to adsorb plasma components.

The results of these preliminary in vivo studies should in no way be construed as the limit of compatibility for the heparinized surface, since the actual prosthesis design and
fabrication procedure needs to be improved markedly. With these experimental heparinized cellulose acetate prostheses the surface was quite rough and the walls thick, .07 to .1 cm to prevent stress cracking. Even though the entrance and exit regions were bevelled, stagnation points existed producing slow vortex circulation and trauma. Each tend to produce coagulation in in vivo systems.

Membrane Strength Measurements

Stress-strain measurements were performed on swollen CIH membranes at 37°C to determine the effect of the acid catalyzed amination on membrane strength. None of the membranes measured, including the regenerated cellulose films, showed a yield stress under these stressing conditions. The force-strain curves for the amination procedures using the minimum acid catalyst are given in Figure 4-37. With amination times of up to 12 hours no more than a 15% decrease in breaking force is observed. With longer reaction times or higher initial acid concentrations more degradation in CIH membrane strength is observed. The influence of amination conditions on the breaking strength of CIH membranes can be observed from Figure 4-39. Reaction conditions which produce simultaneously both acceptable surface compatibility and sufficient membrane strength can be selected from this figure.
However, due to the membrane swelling which occurs during the amination as a result of the hydrochloric acid and ethylenimine treatment, the actual breaking stress and Young's modulus for the CIH membranes are somewhat lower than those for regenerated cellulose. These values are given in Figure 4-41.

**Diffusion Studies**

Measurements of membrane permeability to urea, using $^{14}$C labelled urea, indicate that RCF and CIH membranes, the latter having been saturated with plasma, have comparable urea transport coefficients. However, since the CIH membrane swells during amination to about twice the thickness of the original RCF, the permeability of the CIH membrane (per unit area and compared at equal thicknesses) is about twice that of RCF. Nevertheless, the urea clearance of an artificial kidney, using a CIH membrane should be comparable to that of current hemodialyzers, since the increased permeability and membrane swelling will have cancelling effects.

Urea diffusion coefficients measured for human blood are presented in Figure 4-53 as well as those predicted by the Prager (85,86) model for suspensions and the inverse viscosity rule. The experimentally measured values lie well above the predicted values. This is probably a result of assuming total impermeability of the red cell by the Prager model or a result
of the red cell membrane flickering which has been described previously. The effect of the red cells becomes noticeable above a hematocrit of 50 which might very well indicate a damping out of the membrane flickering effect because of more pronounced red cell-red cell interaction at the high hematocrits. For the purposes of mass transfer correlations and engineering designs a value of $0.75 \times 10^{-5}$ cm$^2$/sec seems appropriate based on present data, for the urea diffusivity through normal human blood at 20°C.
Conclusions

A) Artificial Kidney Performance

1. The current artificial kidneys appear to be operating at 20-30% of their membrane urea removal capacity.

2. These efficiencies apply equally well to extracorporeal hemodialysis units dialyzing actual patient blood or saline as a blood simulant. Thus an excessive increase in membrane resistant due to the adsorption of blood components onto the membrane must be ruled out.

3. The major resistance to urea transport appears to exist in the blood.

B) Graphite-Benzalkonium-Heparin Surface

1. About \(2 \mu \text{gm/cm}^2\) of adsorbed heparin is present on the Lucite-GBH surface. This value is of the same order of magnitude as that calculated for a monolayer of heparin assuming a collapsed helical coil configuration for heparin.

2. Surface heparin can be desorbed from the GBH surface by fresh plasma with little or no fluid shear at the surface, but not with saline even at wall shears of 9.5 dyne/cm\(^2\), suggesting strong complexing power of the plasma proteins.

3. The heparin removal rate from the GBH surface is a function of fluid shear at the surface when the shearing solution is plasma.

4. The weak bond in the GBH surface is probably the
physical bond between the graphite particle and the benzalkonium organic tail.

C) Heparinization of Regenerated Cellulose Films

1. Heparin can be irreversibly bonded to regenerated cellulose dialysis tubing following amination with ethylenimine.

2. An acid catalyst must be present during the amination to give effective surface amination if surface crystalline regions are present on the substrate. The total adsorbed surface heparin ranges from 9 to $12 \mu \text{gm/cm}^2$ depending on the acid concentration of the solution initially used to swell the cellulose.

3. Such heparinized surfaces give WBC times in excess of 100 minutes whereas the controls are normally 20 minutes.

4. The surface heparin, when adsorbed in the preferred manner, interacts with plasma and whole blood. This interaction appears to be a result of complexing between the surface heparin and one or more intrinsic clotting factors.

5. The adsorption of clotting factors onto the heparinized surface is, in many cases, sufficient to anticoagulant the contacting aliquot of blood. Of course, the degree of saturation of the heparin surface dictates the surface adsorption power.
6. Tritiated plasma adsorption studies with heparinized surfaces indicate that the heparin surface can be saturated with plasma components.

7. The heparin surface appears to adsorb about its own weight in plasma components whereas medical grade silicone rubber and RCF adsorb far less, but a definite amount of plasma components.

8. Treatment of a heparinized surface with a protamine sulfate solution renders the antithrombogenic nature of the surface much less effective.

9. In vivo experiments with heparinized cellulose acetate prostheses placed in veins with low blood flow in dogs indicate that an irreversibly adsorbed heparin surface, when saturated with plasma components, possesses superior antithrombogenic properties.

10. The structural properties of the CIH membranes are comparable to those of regenerated cellulose if the acid catalyst concentration or amination times is not excessive.

11. The breaking force and breaking strains can be maintained with 10 to 15% of those for regenerated cellulose while the compatibility of the CIH surface is far superior to that for RCF.

12. Because of the membrane swelling during amination the breaking stress and Young's modulus are about 50% lower
than those for regenerated cellulose.

13. The urea membrane transfer coefficients for CIH membranes saturated with fresh plasma are comparable to those of regenerated cellulose membranes.

14. As a result of amination and swelling of the CIH membranes, the CIH membrane diffusivity for urea is about twice that for regenerated cellulose or $0.5 \times 10^{-5}$ cm$^2$/sec.

15. For the purposes of mass transfer correlations or engineering designs the diffusivity of urea through human blood was experimentally determined to be $0.75 \times 10^{-5}$ cm$^2$/sec at $20^\circ$C.
II. INTRODUCTION

A. Purpose of the Study

The purpose of this study was to develop the fundamentals necessary for the eventual design of a compatible and more efficient artificial kidney. In particular, a dialysis membrane comparable to regenerated cellulose but considerably more compatible with human blood was developed. Regenerated cellulose was selected as the standard because of its proven effectiveness as the dialysis membrane for the existing clinical artificial kidneys. A theory describing the interaction of this novel membrane (CIH) with human blood was proposed and substantiated by in vitro and in vivo testing. The physical strength and permeability of this antithrombogenic membrane were compared to those of regenerated cellulose.

Concerning the problem of artificial kidney efficiency, a fundamental study of solute transfer through human blood was conducted to help elucidate the nature of the blood side mass transfer resistance. This latter resistance has in the past received virtually no attention, but in most cases has an impressive effect on hemodialyzer performance.

B. Physiology of Human Blood

The main function of blood is to act as the vehicle through which tissues of the body receive their nourishment and into which they discharge their wastes. To qualify as
this vehicle the blood must, of course, circulate in the proximity of the tissues. The heart and circulatory system of the body, consisting of arteries, veins, veinules, capillaries, etc., provide the means by which the tissues are continuously contacted with fresh blood. Since the blood has certain tolerable limits of solute concentrations, it must also be circulated, if not totally at least in part, through organs, such as lungs, liver, kidneys, etc. These organs have the responsibility of replenishing the blood stream with the desired nutrients, as well as removing the metabolic wastes. Of course, there will also be natural degradation of the blood components themselves and they must be replaced to maintain blood functionality.

To prevent the loss of this vital fluid via leakage due to injury or capillary rupture, the circulatory system and blood itself have an additional built-in safeguard, usually called hemostasis. Whenever blood vessels are severed or ruptured, the adjacent portions of the damaged vessel automatically contract. This is called vasoconstriction. This constriction reduces the blood leakage somewhat, but it usually is not sufficient to stop bleeding entirely. The ultimate stoppage of blood is generally accomplished by blood coagulation. As much as the basic problem of blood coagulation has been studied, because of its importance to normal body func-
tioning, the precise mechanism by which blood coagulates is still unknown. This is by no means a reflection on the hematologists and the medical profession but a testimonial to the exceedingly complex nature of the human blood.

1. Composition of Human Blood

Human blood is a suspension of three types of particles in a complex suspending medium. The three types of particles are erythrocytes, commonly known as red cells, leukocytes, known as white cells, and platelets. The suspending medium, known as plasma, consists of numerous inorganic and organic salts, proteins, organic macromolecules, and water. Since these components will be referred to frequently in the latter portions of this report, it seems advisable to discuss them in more detail at this time.

2. The Red Blood Cell

The primary function of the red blood cell is to transport oxygen from the lungs to the tissues. Oxygen combines reversibly with hemoglobin, a high molecular weight material present in the red cell, at the lung and is carried to the tissue. There it is readily released in the tissue capillaries where the oxygen tension is much lower than in the lungs. The oxygen does not become ionic oxygen but is carried as molecular oxygen to the tissues. (38, pg. 151)

Besides their function of supplying oxygen to the tissue, the red cells also play an important part in the re-
moval of the metabolic waste carbon dioxide from the tissue. For instance, the erthrocytes contain a large quantity of

\[ \text{carbonic anhydrase, which catalyzes the reaction between carbon dioxide and water, increasing the rate of reaction by 250 times. This increased reaction rate makes it possible for blood to react with large quantities of carbon dioxide and thereby transfer it from the tissues to the lungs.} \]

Finally the red cells, because of their large quantities of electrytes, account for approximately 70% of the buffering power of whole blood. (38, pg. 540)

The blood of a normal man usually contains about 47 volume percent red cells while that of a female contains about 42 volume percent or a "hematocrit" of 42.\textsuperscript{1} The red cell is normally a biconcave disk having a mean diameter of approximately 8 microns and a thickness of 2 microns at the thickest point and a center thickness of 1 micron or less. The average volume of the red cell is about 87 cubic microns and it contains about 70 percent water.

The outer portion of the red cell consists of a semi-permeable membrane, 10-20 millimicrons thick, and a backup support known as stroma. Together the membrane and stroma make up 2 to 5 percent of the total red cell weight. Fifty

\textsuperscript{1} Hematocrit refers to the volume percent of red cells in any suspending medium.
percent of this laminate is protein. Large macromolecules such as hemoglobin, $\text{Mw } 66800$, do not penetrate the red cell membrane and are contained within the cells. Small molecules, such as urea, and certain electrolytes, such as chloride and bicarbonate ions, readily pass through the red cell membrane by passive diffusion: half times for such molecules range from .001 to .1 second at $37^\text{0} \text{C}$. (¶19) All materials, however, do not pass through the red cell membrane by simple passive diffusion. For instance, at $37^\text{0}$ potassium is actively transferred into the red cell to a concentration thirty times that in plasma, while the sodium ion concentration in the red cell is about one tenth that in plasma. (¶19)

3. The White Cell

The white cells belong to a group of tissue and cells known as the reticuloendothelial system which constantly combat any infectious agents that try to invade the body. The leukocytes are transported by the blood to areas of infection or inflammation where the white cells ingest foreign organisms or foreign particulate matter of any type. These are not the only cells in the reticuloendothelial system which exhibit the property of phagocytosis, ability to ingest foreign particulate matter, for there are other phagocytic cells that wander through tissue and phagocytic cells of bone marrow, spleen, liver and lymph nodes.
There are five types of white blood cells, the polymorphonuclear eosinophils, polymorphonuclear basophils, the monocytes and the lymphocytes. The total white cell count in the healthy adult numbers about 1/1000 that of the red cells. The average diameter of these easily deformable spheres is 8 to 15 microns. (38, pg. 164)

The white cell membrane has permeabilities similar to those of the red cells for water and ionic species, but the overall concentration within the white cell represents the plasma more so than the red cell. The white cell membrane is also more elastic than that of the red cell and can expand considerably with the ingestion of foreign materials. The white cell membrane is more hydrophilic than that of the red cells, which appears to be hydrophobic, and will adhere to wettable surface. This property is considered important in phagocytosis, since the foreign material must be attracted to the white cell prior to ingestion. (95)

4. The Platelets

Platelets are the smallest of the formed elements in blood and are classically pictured as small disks 1 to 3 microns in diameter. There are about 1/10 as many platelets in human blood as red cells. It is also believed that platelets are fragments of megakaryocytes, which are produced by the bone marrow. Theory is that these megakaryocytes are the pre-
cursors to white blood cells.

It is commonly agreed that platelets have a key role in the initial phases of both haemostasis and thrombus formation. When a small vessel is injured, the platelets start to adhere to each other and to the injured vessel. This adhesion forms a platelet plug which covers the lesion and is at first permeable to the blood but soon becomes impermeable and thus bleeding stops. The platelet plug is thought to undergo irreversible morphological alternations which possibly initiates or accelerates coagulation by causing a release of lipids from the platelets. As a result new platelets are entangled in and adhere to the forming blood clot. They disintegrate under the influence of a plasma factor and thrombin, a natural coagulant of fibrinogen which appears in the blood following activation of the clotting mechanism. This renewed disintegration of platelets releases more lipids and thus a vicious circle is established which resembles autocatalysis, causing a rapid enlargement of the blood clot or thrombus. (46)

5. The Plasma

The continuous medium of blood, termed plasma, can be separated by centrifugation from the formed elements. It has a straw-amber color, a PH of 7.46, and a density of 1.03. (95) Human plasma consists primarily of water, (92-93%), proteins (6-7%) and dissolved organic and inorganic salts. Past
researchers have been able to isolate and identify the most abundant of the protein constituents but many clotting factors, allegedly proteins, can only be identified by their presence or absence of biological activity. Due to the net electronegativity of plasma proteins at physiological pH, electrophoretic measurements are used to identify the protein fractions. There are six easily separated protein components in blood plasma, namely, albumin(3.5 Wt.%), Alpha 1 and Alpha 2 globulins(0.83 Wt.%), beta globulins(0.89 Wt.%), gamma globulins(0.70 Wt.%), and fibrinogen(0.49 Wt.%). One of the important functions of the plasma albumin(Mw 69000) is the regulation of osmotic pressure in the organism. If the total protein level is diminished below 5.5 weight percent in the plasma or if the albumin is below 3 wt. percent for a sufficient length of time, excessive accumulation of water in the tissue, known as edema, results.

The globulins are much more heterogenous chemically than the albumins. For instance, the \( \gamma \) - and B- globulins contain lipo and glycoproteins while the \( \gamma \)-globulin's heterogeneity results from the incorporation of large numbers of antibodies. The biological significance of the plasma proteins is primarily related to the globulins. It is believed that many of the proteins active in blood coagulation are members of this family. Even though only a few of these coagulation factors
have been isolated and identified, the globulins have been
electrophoretically typed into three general classes, namely
alpha-globulins (MW 75000), beta-globulins (MW 93000), and
gamma-globulins (MW 160,000). (A24, pg. 35)

Fibrinogen has been isolated in rather pure form but
care must be taken to prevent denaturing of this protein by mi-
nor chemical or physical influences. Fibrinogen (MW 300,000) is
usually characterized as a double dumbbell with a length of
approximately 500 Å and dumbbell diameters of 50 to 70 Å.(A24,
pg. 38). The fibrinogen molecule is believed to be composed of
many amino acid residues but the number and percent composition
can only be estimated. Fibrinogen is of great biological signi-
ficance because of its transformation from soluble fibrinogen
to insoluble fibrin during the blood coagulation process. This
conversion, usually observed as the final insoluble blood clot,
is considered to be a polymerization of fibrinogen into a three-
dimensional network which rapidly forms an elastic gel. Although
the exact coagulation mechanism is presently unknown, much has
been inferred concerning coagulation and will be discussed in
considerable detail in a later section.

Besides water and proteins the human plasma contains nu-
merous other organic species, such as metabolic by products:
namely urea, uric acid, creatinine, etc., and numerous elec-
trolytes. A list of the more abundant substances present
in human blood is presented in Table 2-1 of reference
while a complete listing can be found in reference (38). For a more detailed discussion of the plasma protein chemistry one is referred to reference (124).

C. Human Renal Function

An understanding of the physiologic anatomy and functioning of the human kidney is a prerequisite for the understanding of the performance expected of and the constraints placed on the artificial kidney.

1. Renal Anatomy

The human kidneys perform two major functions: namely the secretion of end-products of metabolism and the control of the concentrations of most of the constituents of the body fluids. The kidney is an aggregation of about 1,000,000 nephrons, each of which is capable of producing urine. In a typical nephron, which is illustrated in Figure 2-1, blood enters the glomerulus through the afferent arteriole and leaves the glomerulus through the efferent arteriole. The glomerulus is a filter compound of 50 or so parallel capillaries encased in Bowman's capsule, in which the glomerular filtrate is collected. Most of the filtered blood passing from the efferent arteriole flows through the peritubular capillary network surrounding the tubules through which the glomerular filtrate passes. Most of the water and some of the solutes of the glomerular filtrate are then reabsorbed into the peritubular capillaries.
with the water and solutes not reabsorbed becoming urine. The reconstituted blood from the peritubular capillaries finally flows back into the veins along with the blood shunted from the efferent arteriole directly to the veins.

2. Renal Functioning

The formation of urine, through which the kidneys perform their desired functions, can be divided into three major processes: 1) glomular filtration, (2) tubular reabsorption, (3) tubular secretion.

a) Glomular Filtration

About 10 volume percent of 1200 cc/min. passing through the kidneys is filtered through the glomerulus into Bowman's capsule to become glomerular filtrate, GF. This ultrafiltrate contains no formed elements of the blood, about 1/200 of the protein in the plasma, and solute concentrations similar to those of the plasma. Although the normal driving force for this filtration is about 18 mm Hg (glomerular pressure minus the sum of glomerular colloid osmotic pressure and the Bowman's capsular pressure), the body is capable of regulating the GF rate by altering the glomerulus pressure and thus the urine formation rate.

b) Tubular Reabsorption
Approximately 99 percent of the glomerulate filtrate, which passes from Bowman's capsule into the tubules, see Figure 2-2 for a schematic diagram of the nephron, is reabsorbed in the tubules. Both active and passive absorption have been observed in the tubules. Passive reabsorption or diffusive transfer usually occurs secondary to active reabsorption. For instance, approximately 1200 grams of sodium pass into the GF each day and all but a few grams are actively reabsorbed into the peritubular capillaries. Thus the increased osmotic pressure and the requisite of electrical neutrality force most of the water and counter ions to diffuse back from the GF into the blood stream. Not only sodium but other important substance necessary for the metabolic functioning of the cells are actively reabsorbed. Some of these substances are: glucose, other related monosaccharides, all amino acids, all aceto-acetic acid and all proteins. Another important characteristic of the tubules in their poor reabsorption of end products of metabolism. The tubules are only slightly permeable to urea and uric acid -- 40% of the urea is reabsorbed leaving nearly 25 grams per day to pass into the urine. Creatinine is not reabsorbed at all by the tubules allowing about 5gms/day to accumulate in the urine.
FIGURE 2-1 ILLUSTRATION OF KIDNEY NEPHRON
FIGURE 2-2 SCHEMATIC DIAGRAM OF KIDNEY NEPHRON
c) **Tubular Secretion**

Besides active reabsorption the tubules are capable of active secretion. The main components secreted by the tubules are hydrogen ions and potassium ions. Because of the large amounts of acids produced from metabolism and the narrow PH tolerance, the kidney contributes to the acid-base balance of the body by actively secreting the hydrogen ions in the tubules. Potassium must also be secreted by the distal tubules as a counter measure to the non selective reabsorption of potassium along with the water and other substances in the proximal tubules. An idea of the tubules' ability to concentrate certain metabolic end products in the urine while conserving in the blood those metabolically important substances can be seen in Table A-1. (38, Chapter 8, 5)

D. **Extra Corporeal Hemodialysis**

With chronic renal insufficiency a kidney replacement or artificial kidney must remove the end products of metabolism along with certain salts, such as Na$^+$ and K$^+$, and water. This, of course, must be accomplished with a minimum of loss or damage to blood formed elements as well as vital proteins. Dialysis seemed to be a natural for this operation since small toxic molecules could be allowed to pass through a selective
membrane into a dialysate tank while the large vital proteins and formed elements would be retained in the blood. Those small molecules vital to body functions could also be retained in the blood by balancing their blood concentrations in the dialysate solution. Table A-1 in Appendix A lists the typical dialysate composition.

With the development of regenerated cellulose dialysis tubing it has been theoretically possible to sustain the lives of uremic patients by hemodialysis, but early attempts to extend these lives were unsuccessful because of limited cannulation sites. With the development of permanent cannulation in 1960 periodic hemodialysis became a practical therapy for uremia. In many cases the patient led an active life even with total absence of renal function. Permanent cannulation as developed by Quinton, et al (88), allows the patient's blood to be shunted from the arterial cannula to the venous cannula between dialyses. This continuous circulation through the cannulas and shunt reduces the clotting tendency in these conduits. Silicone rubber cannulas are the most thrombus resistant, but even these with circulation have an average life of only 4 to 5 months. Of course, this tri-yearly cannulation represents a considerable improvement over the bi-weekly cannulation prior to each hemodialysis.
1. **Extra Corporeal Hemodialysis Operation**

   The clinical artificial kidney has changed very little since its conception 20 years ago although many alternate techniques and designs have been studied. This attests to both the solidarity of Kolff's original design and the overwhelming breadth between this first technical plateau and the next.

   Currently two basic dialyzer designs are popular, the coil and the stack artificial kidneys. Both use regenerated cellulose as the dialysis membrane and their major difference lies in the blood flow channel configuration. The coil kidney has a long, narrow, flat channel (10 m x 7 cm x .5 to .75 cm) constructed with regenerated cellulose tubing. Whereas, the stack kidney is composed of alternating blood channels (75 cm long x 25 cm wide x .3 to .5 cm thick) and dialysate channels arranged in a stack. The characteristics and performance of two such clinical artificial kidneys, the modified "chronic" coil kidney and the six layer Skeggo-Leonards kidney, are summarized in Table 2-1. A schematic of the patient-artificial kidney arrangement during hemodialysis is presented in Figure 2-3.

   Anticoagulant heparin is administered during hemodialysis to prevent coagulation of the patient's blood as it contacts the foreign surfaces of the dialyzer. Deleterious side effects of anticoagulation, such as internal bleeding
through porous capillaries, requires that the heparin be neutralized as soon as possible after hemodialysis. Protamine sulfate is the clinically administered heparin neutralant.\(^{(125)}\)

Prior to the development of regional heparinization the patient's entire blood volume was anticoagulated during hemodialysis. This procedure created a potential bleeder for the duration of the dialysis and in many instances internal bleeding was a definite problem. Regional heparinization, however, allows for the anticoagulation of the patient's blood while in the dialyzer but not the internal blood volume. A sodium heparinate solution is injected continuously into the blood egressing from the body and neutralized with protamine sulfate solution before the blood returns to the body. This method nevertheless has two disadvantages, 1) continuous monitoring of the patient's blood is required and 2) with a blood flow rate of 200 to 250 ml/min., a total of eight to ten times more heparin is injected into the patient than with total heparinization. Even though this heparin is neutralized with protamine sulfate a reversal of the heparin-protamine complex can easily produce major complications. Instances of this reversal have been witnessed up to 24 hours postdialysis.

2. **Dialyzer Comparisons**

It is generally believed that the artificial kidney must be efficient in the removal of nitrogenous and toxic prod-
<table>
<thead>
<tr>
<th>Kidney Type</th>
<th>Membrane Area</th>
<th>Priming Volume</th>
<th>Blood Flow Rate</th>
<th>Optimum Dialysate Flow Rate</th>
<th>Overall Urea Transfer Coef. cm/sec 10^4</th>
<th>Urea Membrane Transfer Coef. cm/sec 10^4</th>
<th>Kov Km</th>
<th>RL</th>
<th>Rov</th>
<th>Fraction Resistance in blood phase</th>
<th>Solvent</th>
<th>Reference</th>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Par.&quot; MN-C</td>
<td>1.0</td>
<td>__</td>
<td>200</td>
<td>3.5</td>
<td>2.0</td>
<td>7.4</td>
<td>.27</td>
<td>.73</td>
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<td>Saline Urea</td>
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<tr>
<td>&quot;Par.&quot; MN-C</td>
<td>1.0</td>
<td>__</td>
<td>200</td>
<td>3.5</td>
<td>1.5</td>
<td>7.4</td>
<td>.20</td>
<td>.80</td>
<td></td>
<td>Blood in vivo</td>
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<td>(73)</td>
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<td>200</td>
<td>4.0</td>
<td>3.3</td>
<td>10.9</td>
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<td>.70</td>
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<td>Saline &amp; Urea</td>
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<td>(60)</td>
</tr>
<tr>
<td>&quot;Par.&quot; Kiil</td>
<td>2.1</td>
<td>425</td>
<td>200</td>
<td>4.0</td>
<td>2.0</td>
<td>7.4</td>
<td>.27</td>
<td>.73</td>
<td></td>
<td>Blood in vitro</td>
<td></td>
<td>(27)</td>
</tr>
<tr>
<td>&quot;Par.&quot; Kiil</td>
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<td>280</td>
<td>200</td>
<td>2.5</td>
<td>2.1</td>
<td>7.4</td>
<td>.28</td>
<td>.72</td>
<td></td>
<td>Blood in vivo</td>
<td></td>
<td>(27)</td>
</tr>
<tr>
<td>&quot;Coil&quot; Travenol</td>
<td>1.90</td>
<td>1000</td>
<td>200</td>
<td>9.0</td>
<td>1.6</td>
<td>7.4</td>
<td>.22</td>
<td>.78</td>
<td></td>
<td>Blood in vitro</td>
<td></td>
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<tr>
<td>&quot;Coil&quot; Travenol</td>
<td>1.90</td>
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<td>200</td>
<td>9.0</td>
<td>.7</td>
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<tr>
<td>&quot;Coil&quot; Travenol</td>
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<td>1000</td>
<td>300</td>
<td>9.0</td>
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<td>.30</td>
<td>.70</td>
<td></td>
<td>Blood in vivo</td>
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<td>(27)</td>
</tr>
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</table>

**TABLE 2-1 SUMMARY OF ARTIFICIAL KIDNEY CHARACTERISTICS AND PERFORMANCE**

49
FIGURE 2-3 SCHEMATIC OF ARTIFICIAL KIDNEY-PATIENT SYSTEM
ucts of metabolism. Efficient removal in this case does not necessarily mean rapid removal of these metabolites from the blood stream. With rapid removal of solutes, notably urea, from the blood stream a disequilibrium syndrome appears. It is characterized by headache, nausea, vomiting, blood pressure changes and mental disturbances. Researches on dogs and humans indicate this disequilibrium syndrome is related to slow diffusion of urea out of the central nervous system.

If urea is removed too rapidly from the blood stream an osmotic gradient is formed across the blood brain barrier, resulting in cerebral edema, accumulation of water in the brain. This disequilibrium effect is temporarily improved by raising the blood osmotic pressure with intravenous injections of fructose or mannitol or the addition of urea or glucose to the dialysate bath.

It now becomes apparent that the artificial kidney has an upper bound on the allowable total solute removal rate. Performance estimates must then consider both the dialyzer volume and the total membrane area required to achieve this upper bound. The dialyzer volume must enter into this consideration because of its clinical importance. With priming volumes of 500 ml or more a potentially hazardous blood transfusion to the patient-dialyzer system is required prior to dialysis. Therefore, artificial kidneys with equal solute
clearance rates and membrane areas cannot be considered as equally efficient if their priming volumes are considerably diverse.

Generally, however, when considering similar dialysis designs the priming volume and membrane area are closely related. With this in mind the efficiency can be defined in terms of actual to theoretical solute permeability. For dissimilar artificial kidneys then the relative efficiencies must reflect not only the permeability ratio but also the priming volume and total membrane area necessary for a given solute removal rate. Although it can be argued that the above approach to artificial kidney efficiency is too simplified, it does, however, allow one to quantify dialyzer performances and compare them on some common basis.

In the final evaluation of any artificial kidney, consideration must be given to the above described efficiency as well as characteristics necessary for practical clinical application, namely, 1) negligible permeability to albumin and higher molecular weight proteins, 2) reliability and safety, 3) presterilizability, 4) ease of assembly, 5) manufacturer's cost and 6) clinical attention required during dialysis.

3. Dialyzer Mass Transfer Coefficients

Although the artificial kidney must remove salts and water as well as metabolites from the urenic's blood, most of
the literature deals with urea transport because of the need to remove large quantities, 25 gm/day, of this solute. Considerable discussion exists concerning the exact nature of the uremic syndrome and which solutes need be removed from the blood. Undeniably, however, the artificial kidney is called upon to remove far more urea from the blood stream than all other solutes combined. Until the exact nature of the uremic syndrome is understood, it seems acceptable to calculate dialyzer performance in terms of urea permeability.

In the past artificial kidneys have usually been compared in terms of their dialysance. The concept of dialysance was introduced by Wolf et al. and is defined as:

\[
Dy = \frac{Q_b (C_l - C_o)}{(C_l - C_b)}
\]

where:

\[Dy = \text{Dialysance of a specific solute, i.e. urea}\]
\[Q_b = \text{Blood flow rate through dialyzer}\]
\[C_l = \text{Concentration of solute in dialyzer inflow blood stream}\]
\[C_o = \text{Concentration of solute in dialyzer outflow blood stream}\]
\[C_b = \text{Concentration of solute in dialyzer bath}\]

This concept has received wide spread acceptance but represents a questionable approach to the description of the mass transfer characteristics of the artificial kidney. As one can see from a comparison of dialysance with the actual analytical description of this solute transfer process,
namely

\[ \text{Kov} = \frac{2Qb}{A} \frac{(C_l - C_o)}{(C_l + C_o)} \]

where:

\[ (2) \]

Kov = Overall mass transfer coefficient for a specific solute
A = Dialyzer membrane area, dialysance is a linear function
of area and is based on a specialized driving force condition,
i.e. \( C_l \approx C_o \). These two considerations prohibit the use of di-
alignedance itself as a measure of the mass transfer efficiency
of clinical dialyzers. It can be used accurately only as an
expression of the total removal capacity of a particular mach-
ine at a particular set of operating conditions.

4. Overall Urea Transport Coefficient

A more fundamental approach to artificial kidney per-
formance is required to understand the nature of the dialyzer
resistance to solute removal. It is helpful to consider the
actual path the solute must travel as it is removed from the
blood. The solute, i.e. urea, must 1st) diffuse through the
blood to the membrane, 2nd) diffuse through the membrane to
the dialysate and 3rd) diffuse from the membrane edge to the
dialysate bulk. In all cases the flux is inversely propor-
tioned to the mass transfer resistance and directly propor-
tional to the driving force, i.e.

2. This assumes \( C_b \ll C_l \) which is actually the case because
the dialyzer bath volume \( \gg \) body blood volume especially
with flow-through dialysate or bath changes.
Flux = Driving Force/Mass Transfer Resistance.

The total resistance encountered by the urea molecule diffusing from blood to dialysate is equal to the sum of the individual resistance along the path. It is therefore convenient to consider the overall mass transfer resistance as a composite of three individual resistances, namely, the blood-side resistance, the membrane resistance, and the dialysate-side resistance. The mass transfer resistance is usually represented as the reciprocal of the mass transfer conductance, which is defined as the mass transfer coefficient times the area for transfer.

Since the diffusional driving force is solute concentration, the flux can then be described as: $N = K \ A \Delta C$ (3)

where: $N =$ Flux, mass/unit time

$K =$ Mass transfer coefficient

$A =$ area for transfer

$C =$ concentration gradient.

In the case of the artificial kidney the urea flux from the blood to the dialysate can be obtained by rearranging equation (2), i.e.

$$N = Qb(C_1 - C_0) = Kov \ A \left(\frac{C_1 + C_0}{2}\right)$$ (4)

The overall coefficient can also be expressed in terms of the individual coefficients as follows:

$$\frac{1}{Kov} = \frac{1}{Ko} + \frac{1}{Km} + \frac{1}{Kb1}$$ where (5)
Equation (5) provides the means to analyze the overall performance of the artificial kidney and ascertain the contribution of each resistance to the overall. Since these resistances are in series, it is obvious that the overall mass transfer coefficient must be lower than any individual coefficient. Thus very little overall improvement is obtained by greatly increasing the non limiting coefficient but not the limiting one. To ascertain the contributions to the overall coefficient and hence the bottleneck, one must first experimentally obtain this overall coefficient. Equation (6) provides the link between the existing dialysance data and the overall mass transfer coefficient for the current artificial kidneys.

\[ \text{Kov} = -\frac{Q_b}{A} \left[ \ln \left( 1 - \frac{D_v}{Q_b} \right) \right]. \quad (6) \]

The assumptions contained in the derivation, as well as the derivation, can be found in Appendix A.

Overall coefficients for popular clinical dialyzers at their normal operating conditions, i.e. blood flow, dialysate
flow, temperature etc., are listed in Table 2-1 along with the dialyzer's characteristics. A discussion of these coefficients and their significance will be delayed until the nature of the individual resistances have been discussed.

5. Dialysate-side Mass Transfer Coefficient

The dialysate-side coefficient depends upon the flow pattern, flow regime and fluid velocity and cannot be accurately estimated unless these parameters are known. In all conventional flow patterns this dialysate film-side coefficient varies in a direct nature with fluid velocity. Whereas it is difficult to analytically estimate this coefficient, it can be readily measured experimentally with a modified Wilson type plot, see Figure 2-4. The total mass transfer resistance for the dialyzer, \( \frac{1}{K_{ov}} \), is plotted versus the reciprocal of the blood flow rate to the 0.8 power. The intercept represents an infinite blood flow rate and hence all resistance can be divided between dialysate film and membrane. An estimation of the membrane resistance gives the dialysate side mass transfer resistance and, of course, the coefficient.
This method is seldom used to estimate the dialysate side coefficient, since the dialysate flow rate is usually above the optimum rate and the resistance obtained from the Modified Wilson Plot is of the same order of magnitude as the experimental error. The optimum dialysate flow rate for each dialyzer has been determined by increasing the dialysate flow rate, at the maximum blood flow rate, until the overall urea transport coefficient is no longer a function of dialysate flow rate. Contrary to the blood side, it is then possible to conduct hemodialysis with a dialysate flow rate above the optimum. This is the normal clinical procedure. Although the dialysate film mass transfer resistance is currently of small
consequence in hemodialysis, improvements in the dialysate channel design can and should be made.

Presently a dialysate flow rate of from 5 to 9 liters per minute is required. As the blood side and membrane resistances are reduced, the need will arise to either increase the dialysate flow rate to an even higher value or improve the design. The former is limited by the venous pressure since it is desirable to operate counter- or cross-currently with a relative positive pressure in the blood channel. This also reduces the possibility of infection if a membrane leak should occur. Therefore, the latter seems to be the only alternative worthy of future consideration.

6. **Membrane Mass Transfer Resistance**

A membrane is a thin barrier separating two fluids which prevents all hydrodynamic flow so that transport through the membrane is by diffusion. Most commercial membranes are composed of a high polymer or a mixture of high polymers and are considered to correspond to a model consisting of a network of linear or branched chains of atoms, crosslinked at various points, and filled with molecules of solvent and of mobile solute. Each linear segment may flex and coil if it is attached to only one crosslink point or vibrate and distend if attached at two crosslink points. In a sense the membrane may be said to be partially dissolved in the im-
imbibed medium. Complete solution is prevented by the inhibiting forces of the crosslinkage which bind together polymer chains. (62) Although this adequate model describes a wide range of high polymer membranes, it certainly is not the complete picture. Possibly a porous network might coexist with the partial solution situation. The typical pore could be quite tortuous and irregular in diameter but nevertheless exist as an open channel filled with imbibed solvent.

The probability of the existence of such a porous membrane structure could possibly be related to the size of the crosslinkage junction and the number of linear segments attached at both ends or at two crosslinkages. As the size of the junction and the degree of double attachment increase, the possibility of forming pore volumes between the randomly oriented lamella, crosslinkages and linear segments, is envisioned to increase considerably.

Thus two basic theories of membrane transport, the capillary and the dissolution theory, have arisen from previous membrane structure considerations. Both theories show that the rate of solute diffusion through a membrane may be expressed by equation (3) where the concentration gradient is now across the membrane and the overall coefficient refers to the membrane. This coefficient is always less than that of an equal thickness of free liquid.
With the capillary theory the solute transport through a membrane is postulated to occur by diffusion through the liquid in the capillary pores. The solute membrane diffusion coefficient, $D_m$, is less than the diffusivity through the solvent as a result of three facts: 1) the solid structure decreases the area available for diffusion, 2) the path through the membrane is no doubt tortuous, and 3) there is drag on the diffusing molecule because of the proximity of the solid capillary walls. Lane and Riggle (65) postulated an equation to calculate membrane diffusivities for small molecules which take the above three effects into account. The equation is:

$$D_m = \frac{D F V'}{z}$$  \hspace{2cm} (7)$$

where: $D =$ Solute diffusivity in pure solvent  
$V' =$ Relative volume occupied by membrane pores  
$z =$ Tortuosity factor  
$F =$ Drag factor on diffusing molecules owing to the proximity of the capillary walls.

The relative volume and tortuosity factor must be determined experimentally while the drag factor can be calculated with a knowledge of the average pore diameter and effective solute diameter. The relative volume is determined by comparison of the dry to wet membrane weights. The tortuosity is usually determined with a small solute, i.e. NaCl, so the drag factor will approximate one.
The experimental membrane diffusion coefficient for urea diffusing through regenerated cellulose, whose structure seems to be conducive to capillary type pores, agrees fairly well with that calculated from Equation 7. For larger molecules the picture becomes much more complicated and generally a larger reduction in the diffusion rates exist than can be predicted with this simple drag factor treatment. A distribution of pore size rather than a single pore diameter adds much to complicate the drag factor calculations.

In the case of the dissolution theory, where the membrane permeability for certain substances depends on the solubility of this substance in membrane, one is forced quickly to rely on experimental measurements. Although it is possible to, a priori, predict the relative solubilities of certain solutes in polymeric membranes, membrane diffusion theory has not been advanced sufficiently to predict accurately the coefficients from a basic knowledge of characteristics of the solute and the membrane.

As far as hemodialysis is concerned an ideal membrane would permit instantaneous passage of every toxic molecule and complete rejection of vital molecules, such as proteins, etc. In practice this is currently impossible, although new synthetic copolymer membranes, such as those described by Lyman (70) seem to be the first step in this direction. Lyman indicates that
his copolymer membranes based on polyoxyethylene glycol and polyoxyethylene terephthalate have the ability to separate urea from similar sized molecules by absorption and solution mechanism. These membranes have, at least, a factor of two selectivity but require considerably more research prior to being classed as ideal.

In practice regenerated cellulose has been used as the hemodialysis membrane since its incorporation in the first artificial kidney designed by Kolff. This membrane has a structure similar to the high polymer model previously described but relies solely on crystalline regions as the cross-linking junctions. Because of the rigidity of the cellulose molecule and the abundance of free hydroxyls, this material is easily crystalized. The molecules can orient close to each other and hydrogen bond through the hydroxyls.

Sharply defined crystallites are not believed to be present as the basic structure unit of the cellulose fiber. If they were present the relatively firm cohesion of fibers swollen in water could not be explained in light of the observations that water merely entered the structure between micelles or crystalline regions and did not penetrate them. Later when Wyssling (125) postulated that a single cellulose molecule could take part in more than one crystalline region, the fringe micellar theory gradually emerged from the work of many
According to this theory, the micelles are considered as statistically distributed regions of latticed order in a mass of substance consisting of approximately parallel chain molecules as illustrated in Figure 2-5. Generally the "crystalline" regions alternate with less well ordered "amorphous" regions, and within broad limits there is no connection between the length of the crystalline regions and the molecular chain length. There will be no sharply defined crystalline limits but gradual transitions along the molecules from regions of high lattice order to regions of low lattice order. The complete structure will be reticular in nature with primary valence chains anchored at various points by the high cohesive forces in the ordered regions holding the structure together in a coherent three-dimensional network. It should also be noted that variations will range from a rigid crystal network in which the amorphous areas are only lattice faults to an extremely flexible chain network in which the crystals are so small that they approach equivalence to a chemical cross-link.
The network structure has a great influence on the course of most cellulose reactions and definitely on the permeability of regenerated cellulose films. It is generally agreed that the amorphous material is always attacked more rapidly than the crystalline regions. For instance, during dialysis of aqueous solutions water absorbs into the film primarily in the amorphous regions filling any voids or capillaries and breaking hydrogen bonds formed between the flexible segments while in the dry state. Normally the soaked film contains 50% water by weight. (83)

The capillary diffusion model seems to adequately describe the regenerated cellulose hemodialysis films and would be expected in light of crystallinity effects previously discussed. Segregation of solutes by this membrane appears to be related strongly to the solute size. Thus small toxic solutes, i.e. urea, uric acid, creatinine, etc., are allowed to pass from the blood to the dialysate while higher molecular weight albumin is retained almost quantitatively during the dialysis. Regenerated cellulose is also not ideal but has performed remarkably in practice because most metabolic by-products have low molecular weights while the vital components are of considerably higher molecular weights. All is not so simple, since certain cellulose permeable small molecules, such as ions and glucose, are required by the blood and must be re-
tained by balancing their concentration driving force across the membrane to prevent their removal from the blood. A typical dialysate and plasma composition is listed in Table A-2.

The urea transport coefficient through commercially available regenerated cellulose, defined as \( K_m = \frac{D_m}{L} \) where \( L \) refers to the wet membrane thickness, ranges from \( 5.5 \times 10^{-4} \) cm/sec at \( 22^\circ C \) to \( 7.4 \times 10^{-4} \) cm/sec at \( 35^\circ C \). These values correspond to a 1.5 mil thick wet membrane and membrane diffusivities of \( 0.2 \times 10^{-5} \) cm\(^2\)/sec at \( 22^\circ C \) to \( 0.27 \times 10^{-5} \) cm\(^2\)/sec at \( 35^\circ C \).

7. **Blood Side Urea Transfer Coefficient**

In hemodialysis the blood side urea transport coefficient can be estimated analytically if the blood channel configuration is known or measured experimentally. Theoretically the blood side coefficient is infinite at the inlet of the dialyzer and quickly drops to an asymptotic value. Clark and Kays (15) derived this asymptotic value for heat conduction to a fluid flowing between flat plates but it applies equally well to mass transfer. They found that \( \frac{hD_b}{K_b} \) approaches 7.6 asymptotically with increasing transfer length, if a constant wall concentration and parabolic flow were assumed. In this expression \( h \) is the channel thickness and \( D_b \) the diffusivity of urea in blood. In order to estimate \( K_b \) one must know \( D_b \), which to date has not been measured for blood. Therefore a
more reliable estimate of the blood side coefficient can probably be obtained from the previous hemodialysis research. This coefficient can be estimated experimentally by two methods, 1) a Modified Wilson Plot with the reciprocal of the dialysate flow rate plotted against the reciprocal of the overall mass transfer coefficient as described earlier, and 2) a direct estimation from Equation (5), since the dialysate side resistance can be assumed negligible and the membrane resistance can be estimated accurately.

This latter approach has been used to estimate the percentage of the overall resistance contributed by the blood side resistance. These percentages are listed in Table 2-1.

The current clinical artificial kidneys appear to be operating at about 30% of their membrane capacity. The cause for this low efficiency lies primarily with the blood side resistance, which is estimated to account for 60-70% of the overall resistance. These dialyzers maintain approximately the same efficiency regardless of the solvent system, i.e. urea-saline or urea-blood. This precludes the idea of an additional layer absorbed from the blood onto membrane of such significance as to alter the membrane coefficient.

Another potential inefficiency might be channeling of the blood or dialysate so that not all of the membrane surface is available for dialysis. This effect is probably of minor
importance in the dialyzers of Table 2-2 because the dialy-
sance showed no irregularities as either the blood or the di-
alsate flow was increased well beyond the operating conditions.

One method of increasing the efficiency of these dialy-
zers is to increase the blood flow rate. Although this sounds
reasonable, it is quite difficult in practice for 200 ml/min
represents an optimum blood flow rate with respect to dialy-
zer performance, blood damage due to pumping, cannula flow
rate, artery flow rate and vein flow rate. With the maximum
blood flow rate determined the only alternative for increas-
ing efficiency is to design a better dialyzer. A clue to a
better design can be found in Figure 2-6. It can be seen that
with a blood side resistance of 60% increasing the membrane
permeability by a factor of ten will only increase the over-
all transfer coefficient by 56%.

In summary, two areas of hemodialysis need attention if
the artificial kidney is to fill the void created by the in-
creasing rise of renal failures. The first and most diffi-
cult area deals with compatibility. A compatible artificial
kidney capable of operating for short periods without the use
of anticoagulants would greatly simplify the dialysis proce-
dure and lend itself readily to use in the home by the patient.
The second need concerns dialyzer design. It is necessary to
design a more efficient dialyzer which will have a low prim-
ing volume and be inexpensive. One step in the right direction would be to reduce the blood-side resistance.

E. Ion Related Artificial Kidneys

Alternatives to hemodialysis have been proposed and evaluated on a preclinical basis. Two approaches, hemoelectrodialysis and ion exchange, appear to have potential as a specific means of quickly removing certain ions from the blood stream. In both cases neutral molecules, such as urea, glucose, creatinine, are not removed from the blood as efficiently as in passive dialysis and these approaches for that reason alone will not challenge hemodialysis for the continued treatment of chronic uremia. A brief discussion of each process will be included here because additional complications exist and must be considered prior to clinical application if the urea removal efficiency is ever increased.

Berkowitz and Bluemle (5) found that the transport rates of cations from saline were from 5 to 15 times higher with electrodialysis than with passive dialysis. The saline in this electrodialysis cell was bounded by anion permeable membranes.

Further investigations by these authors (6) with human blood uncovered a host of complications. With charged membranes bounding the blood, significant hemolysis occurred. The hemoglobin content rose by 250 Mg% per hour. Other deleteri-
ous effects on the blood were observed, the platelet and leukocyte counts dropped by 30% per hour. The latter were plated out on the anion permeable membrane near the anode. Replacement of the charged membranes with regenerated cellulose eliminated the hemolysis as well as the platelet and leukocyte losses at the previous current density. It also reduced the electrolyte transport rate, necessitating a higher current density to show significant improvement over passive dialysis.

Smith, Berkowitz and Bluemle (104) later demonstrated that electrodialysis with relatively high current densities, $36 \text{ ma/cm}^2$, for a duration of clinical significance led to severe hemolysis and hyperkalemia, excess of potassium in bloodstream. Hemolysis was found to be a function of both time and current density. The hyperkalemia was probably a result of the rupture of the red cells releasing the stored potassium into the plasma.

Certainly new electrodialysis designs, where the easily fouled anion permeable membrane is replaced with a cation permeable membrane can be conceived which would allow the effective use of hemoelectrodialysis for the specific removal of excess poisons or electrolytes at low current densities.

However continued use of electrodialysis, even with an improved design, can not be recommended. Thus, as it exists
today, hemoelectrodialysis has very little therapeutic value for the treatment of chronic uremia.

The use of ion exchange resins as artificial kidneys again suffers from its low urea absorbing or removing capacity. Aside from this, the previous work with ion exchange kidneys have been encouraging.

Kissark, et al (61) developed ion specific resins which were capable of: 1) exchanging sodium for potassium while not influencing the blood calcium concentration, 2) exchanging sodium for ammonia without altering the blood calcium or potassium concentration and 3) removing salicylic acid with a slight rise in bicarbonate and pH with a minimal phosphate and chloride change. These specific resins were prepared by slurrying the non specific resins with equilibrium concentrations of the ions which are to remain unaltered in the blood. While the clinical efficacy of the ammonia specific resin is unknown, about 1300 gm of the potassium specific resin would lower a 70 kg. man's potassium level from 6.4 meg/l to 1 meg/l. Approximately the same quantity of salicylic acid specific resin would remove 30 gm of salicylic acid from the blood. For reference, an artificial kidney holding 1400 gm of exchange resin would have a priming volume of about 700 ml. of fluid.

The use of ion exchange resins for efficient removal of specific blood electrolytes appears to have promising poten-
tial. Ion exchange artificial kidneys, as they now exist, are of little value in the treatment of chronic uremia because of their low urea removal capacity. If the urea could be efficiently converted to an ionic form in the blood stream, the ionic urea might then be selectively absorbed onto an ion exchange resin. Such an artificial kidney, if quite small and easily used, would be a valuable adjunct to the current hemodialysis procedure. Intermittent treatment with this kidney would lengthen the time permitted between hemodialyses. A method capable of converting urea to ionic products can be found in Appendix B.

F. Human Blood Coagulation

Earlier it was stated that the exact mechanism of blood coagulation still remains a mystery. The investigation of blood coagulation is not easy, as it deceptively appears at first sight, but is extremely difficult. The blood contains not only the clotting factors which initiate, accelerate and limit coagulation as the situation requires but also safety devices to prevent the blood from coagulating within the vessels and for dissolving unwanted fibrin. To make matters worse, the investigation of blood coagulation is restricted to the observation of the final stage or fibrin formation, which is the sole indicator available to the experimenter. With the exception of platelet agglutination, no other observable change,
physical or chemical, takes place during coagulation. Therefore, everything that has been learned about blood coagulation has been inferred from the occurrence of fibrin formation, which is the end result of a long series of reactions.

Very few of the coagulation factors to be discussed have been isolated. Their presence or absence is inferred from their activity or influence on fibrin formation. Certain clotting factors have been isolated and are known to be free of other recognized factors but very little can be said concerning the absence of currently unrecognized factors or the purity of the isolated factor. The reader is referred to Table 2-2 which lists the Roman numeral designations for the blood coagulation factors and the estimated quantity present per ml of plasma. Although hematologists differ among themselves as to the exact number or sequence of reactions involved in blood coagulation, the mechanism is generally considered to consist of at least 3 and sometimes as many as 12 separate reactions. A four step reaction scheme which allows one to both understand the rudiments of coagulation and interpret the results of existing coagulation tests is presented in Figure 2-7.

Following a tissue injury coagulation is usually initiated by two separate mechanisms, the extrinsic system or the intrinsic system, while contact with a foreign synthetic sur-
<table>
<thead>
<tr>
<th>Factor</th>
<th>Synonyms</th>
<th>Mg/l.0 ml Normal Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
<td>4.9 Mg</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
<td>.15 Mg</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
<td>.10</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin, labile factor</td>
<td>&gt;.02 Mg</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin, serum prothrombin conversion accelerator</td>
<td>?</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihaemophilic factor (AHF), platelet co-factor I</td>
<td>.002 Mg</td>
</tr>
<tr>
<td>IX</td>
<td>Plasma thromboplastin component (PTC), Christmas factor</td>
<td>&lt;.002 Mg</td>
</tr>
<tr>
<td>X</td>
<td>Stuart - Prower factor</td>
<td>.02 Mg</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma Thromboplastin Antecedent (PTA)</td>
<td>&lt;.002 Mg</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
<td>&lt;.002 Mg</td>
</tr>
</tbody>
</table>
Resistance Unchanged

Blood Side Resistance Unchanged

FIGURE 2-6 EFFECT OF INCREASING MEMBRANE PERMEABILITY WITH NO REDUCTION IN BLOOD SIDE RESISTANCE
FIGURE 2-7 HUMAN BLOOD COAGULATION MECHANISM

- **TISSUE INJURY**
  - FOREIGN SURFACE CONTACT

- **INTRINSIC SYSTEM**
  - XII, XI, IX, VIII
  - PLATELETS, Ca²⁺, X, V

- **EXTRINSIC SYSTEM**
  - TISSUE FACTOR, VII, Ca²⁺²
  - X, V

- **EXTRINSIC PROTHROMBIN ACTIVATOR**

- **INTRINSIC PROTHROMBIN ACTIVATOR**

- **PROTHROMBIN**

- **THROMBIN**

- **FIBRINOGEN**

- **FIBRIN**
face usually initiates coagulation through the intrinsic system. It is believed that these two systems are independent although they have some constituents in common. The extrinsic system is thought to derive its activator from the tissue while the intrinsic system draws from the blood constituents. The extrinsic prothrombin activator has also been called extrinsic thromboplastin or prothrombinase. The intrinsic prothrombin activator is also referred to as the intrinsic thromboplastin or plasma thromboplastin.

Since the initial phases leading to coagulation can be studied only by observing their effect on fibrin formation, the coagulation mechanism will be discussed in reverse order.

1. **Thrombin - Fibrinogen Reaction**
   a) **Fibrinogen**

   Fibrinogen is defined as the plasma protein clotted by thrombin and is normally present in concentrations of 300 to 500 Mg per 100 ml of plasma. It is denatured at about 47°C and can be precipitated from plasma by 25 percent saturated solution of ammonium sulphate or 50 percent saturated solution of sodium chloride. Caspary and Kekwich(12) estimated its molecular weight to be 340,000 for both human and bovine fibrinogen. Electron photomicrographs by Hall and Slayter (41) suggest that the fibrinogen molecule consists of a rod about 475 Å long, with two terminal nodules about 65 Å
in diameter, and a central nodule about 50 Å in diameter.

Tristram (106) lists nineteen amino acids found in fibro-
gen and their relative proportions. Bailey et al., (1) and
Lorand and Middlebrook (69) suggested that the terminal amino
groups of bovine fibrinogen are tyrosine and glutamic acid,
and the stability of fibrinogen solutions depends on the nega-
tive charges conferred by the latter acidic group.

b) Thrombin

And now for a little circular reasoning; throm-
bin is usually defined as the natural coagulant of fibrinogen
which appears in the blood following activation of the clot-
ting mechanism. Its activity is usually measured in terms of
thrombin units. One unit causes coagulation in 15 seconds of
1 cc of a .1 per cent human fibrinogen solution at 37°C with a
PH of 7.3 and a sodium chloride concentration of .154 M.
Thrombin is usually derived from purified prothrombin by the
action of tissue thromboplasatin on prothrombin, which will be
discussed in more detail later. The molecular weight of this
thrombin is approximately 31,000(100). Miller et al. (77) have
identified twenty amino acids in the thrombin molecule. It is
regarded functionally as a highly specific proteolytic enzyme
whose activity is destroyed by strong acids and alkalis or by
heating to 60°C. The best preparations have an activity of
about 3800 units per mg.
c) **Fibrin Formation**

Fibrin refers to the strands of insoluble, protein whose formation causes coagulation of the whole blood or plasma. Fresh fibrin adheres strongly to certain surfaces, particularly body tissues, metals and some glass surfaces (78). *In vitro* it can be seen to form a three-dimensional network of branching fibers which have a diameter of .1\(\mu\).

The enzymatic action of thrombin on fibrinogen to form fibrin has been largely clarified. The first action of thrombin on fibrinogen seems to be proteolysis and is probably due to the disruption of arginyl-glycine bonds in the fibrinogen molecule. A fibrinopeptide portion of it then separates. This results in the loss of negative charges from the remaining molecules, which are now termed fibrin monomers. Polymerization of these monomers now takes place without the need for additional thrombin, possibly by electrostatic attraction (68) or by hydrogen bonding of groups unmasked by the removal of the peptide. Polymerization does, however, depend on the PH and ionic strength. Fibrin molecules apparently orientate end-to-end to form fibrils which later become arranged side-to-side to form coarser strands of fibrin as seen by the light microscope.

All *in vitro* blood clotting tests are performed with plasma, for it is necessary to standardize the reaction conditions for the thrombin-fibrinogen reaction. Plasma was
chosen because in plasma many of the factors which influence this reaction are controlled automatically. The salt concentration and PH do not vary greatly from one sample of plasma to another and the fibrinogen level is fairly constant in normal plasma. Usually it is greater than the optimum for the conversion.

Thrombin times, the time required after the addition of thrombin for coagulation of plasma, are reproducible to within 1 to 2 seconds with the standard procedure listed in Appendix C regardless of the donor, if healthy. Thrombin times, of course, vary with the quantity of thrombin added.

2. Prothrombin - Thrombin Reaction

Since thrombin is not detected in normal circulating blood, it was postulated that an active precursor must exist in the plasma and this precursor was called prothrombin. Seegers and his collaborators (101) isolated a purified prothrombin which was practically homogeneous in the ultracentrifuge and electrophoretically as well as almost quantitatively converted into thrombin by various means. This dispelled any doubts as to the existence of a single substance from which thrombin is wholly derived. The prothrombin level in normal plasma is about 12 to 15 mg. per 100 ml of plasma. Purified prothrombin behaves electrophoretically, like an alpha 2 globulin and has an isoelectric point of PH 4.2. Its molecu-
lar weight is estimated to be around 68000 (64) and the pro-

thrombin molecule is thought to be ellipsoidal in shape, about
120 Å long and 35 Å in diameter. Prothrombin is also thought
to be composed of eighteen amino acids with glutamic and as-
partic acid and arginine predominating.

The activation of prothrombin in whole blood or plasma
during the clotting process involves many factors and a ser-
ies of reactions which are not well defined. It is therefore
difficult or almost impossible to currently detect physical
or chemical changes during this plasma clotting phenomenon.
It is known, however, that prothrombin in plasma can be acti-
mated in a few seconds to thrombin by the addition of tissue
extracts, a reaction which requires the presence of factors
VII, X, and V as well as ionized calcium. These latter reac-
tants are usually present in normal plasma. Prothrombin can
also be activated by components contained within the blood it-
self, following contact with foreign surfaces. This "intrin-
sic" or "contact" system is thought to require the presence of
platelets, factors VIII, IX, X and V and calcium. Since both
the extrinsic and intrinsic system require factors X and V, it
is thought there is a final common path for both of these sys-
tems. The end product of both of these systems, the extrinsic
prothrombin activator and the intrinsic prothrombin activator,
which may be one and the same, is a specific prothrombin acti-
vator capable of bringing about prothrombin activation in seconds. Prothrombin is easily measured by a one-stage method called the "prothrombin clotting test." In the one-stage method the solution containing prothrombin and factors VII and X is mixed with factor V and tissue extract. Fibrinogen and calcium chloride are added to the mixture and the clotting time is observed from the addition of the calcium. The clotting time is usually termed the "prothrombin time."

In this one-stage prothrombin test the final clotting time is dependent on the level of the different clotting factors. Since these factors, as well as fibrinogen, are present in fresh normal plasma at levels above their optimum, prothrombin times are conventionally determined on fresh plasma. Reproducible prothrombin times of from 13 to 15 seconds are obtained with fresh normal plasma irrespective of the plasma donor, if healthy. For abnormal plasma an extended prothrombin time gives an indication of a plasma factor deficiency or absence of calcium, this assumes the fibrinogen level is optimal which can be checked with the thrombin time. (9)

3. **Extrinsic Activation System**

For many years it has been known that tissue extracts accelerate blood coagulation, but in recent years a more clarified picture of the formation of the extrinsic prothrombin activator has evolved. The substance central to this for-
mation seems to be factor X which is quantitatively converted to a product which requires phospholipids and factor V for full prothrombin activity. Factor X conversion depends on tissue extract being activated by factor VII. Calcium is required for one or both of these reactions.

Although the factors discussed above have not been isolated in purified forms, certain properties are known and will be discussed because of their relevance to the interpretation of later in vitro clotting results.

a) Factor V

Quick was first to note that normal plasma contains a "labile factor" necessary for rapid prothrombin conversion in the presence of tissue extract. This "labile factor" was later called factor V and is believed to be an Ac - globulin. Ware and Seegers (110), through great care, have been able to precipitate a 50% pure factor V substance. Factor V deteriorates rapidly on storage and is destroyed by heating to 50°C for 30 minutes. It is also consumed during coagulation.

b) Factor VII

Factor VII is a protein found in the beta-globulin fraction of normal plasma or serum, plasma without prothrombin and the intermediate products of clotting.
It is adsorbed from normal plasma and serum by inorganic precipitates such as BaSO₄ and Al(OH)₃. It is heat labile and destroyed by pH3. Factor VII activity is difficult to extract from plasma because factor VII and prothrombin are precipitated or adsorbed together during most fractionation methods. Recently Koller (62) has developed a chromatographic method to separate prothrombin from factor VII.

c) **Factor X**

Factor X resembles alpha-globulin electrophoretically and is unaffected by pH changes between 6 and 9. It can be adsorbed by Ba(SO₄) and Al(OH)₃ and is eluted by .14 M sodium citrate. It is destroyed by heating to 56°C and is stable at 4°C for two months. The clotting of blood in the presence of excess tissue extract leads to the disappearance of factor X activity. (9, Pg. 79)

4. **Intrinsic Prothrombin Activator**

The exact nature of the interactions of the plasma protein factors in the intrinsic system is as yet not well understood, but there appears to be general agreement as to the factors which participate in this system. Consensus indicates that clotting is initiated when factors XII and XI contact a foreign surface. These proteins are believed acti-
vated by the surface and to form an activated complex which
interacts with the precursor of factor IX to form factor IX.
Factor IX through a series of reactions with factor VIII, fac-
tor X, phospholipids from the platelets, and calcium forms
another active complex which interacts with factor V in the
presence of calcium to form the intrinsic prothrombin activa-
tor. As with the extrinsic system, these proteins have not
been isolated but certain of their properties are known and
will be discussed briefly because of their importance in under-
standing this "contact activation" by foreign surfaces.

a) Factor XII and Factor XI

The structure and nature of the "contact system"
participants, noteably factors XII and XI, have not
as yet even reached the conjectural stage. The only
data available thus far is related to their heat sen-
sitivity and their gross interactions with certain
surfaces. Factor XII, the Hageman factor, is de-
stroyed by heating to 60°C while factor XI, the PTA
factor, has been observed to be partially resistant
to heating to 56°C.

As for the surface contact most of the evidence
suggests that initially factor XII is adsorbed onto
glass or other activating surfaces. In the adsorbed
state it then reacts with and activates factor XI.
This can be a surface reaction if factor XI is also adsorbed or factor XII can be desorbed and react with factor XI in the plasma. The consensus of opinion seems to favor a double adsorption. The contact activation product of factors XII and XI is then thought to activate the factor IX precursor. This latter activation probably takes place on the surface and in solution, for the complex can be eluded from glass in its active state by saline and then reacted with the factor IX precursor.

b) **Factor IX**

Plasma samples have been observed to contain less factor IX than the serum produced after clotting. Factor IX activity in plasma is also increased by contact with glass. These, and other observations too numerous to mention, indicate factor IX is normally circulating in the blood stream in an inactive form and its activation is depended, in some way, on the contact with foreign surfaces.

The factor IX potential in normal plasma is considered to be less than .2 mg per 100 ml of plasma. It can be adsorbed by Al(OH)₃,Ba SO₄ and Ca₃ (PO₄)₂. It can be precipitated with 50 percent saturated (NH₄)₂ SO₄ and by dilution and acidification. Elec-
trophoretically it resembles a beta-globulin. Factor IX is relatively stable on storage at low temperatures but is destroyed by heat. Its interaction with the anticoagulant heparin will be discussed later. (9, pg. 79)

c) Factor VIII

Factor VIII is the substance generally lacking in haemophilic blood and is necessary for normal haemostasis and wound healing. Attempts to prepare factor VIII have been thus far unsuccessful. Most fractionation methods separate factor VIII and fibrinogen together. The task of separating these two is formidable but progress is being made; for example, Simonetti et al (103) have separated factor VIII from fibrinogen to produce small amounts of factor VIII.

The factor VIII level in normal plasma is approximately 50-200 mg per 100 ml plasma. It is very unstable and in fact, certain researchers feel that much activity is lost during the time taken to separate plasma from fresh blood. In addition, factor VIII has been shown to be unstable at -4°C. After a week of storage at -4°C the factor VIII activity has been observed to be 30% or less of the original. (84) Usually microcoagulation initiated during blood collection is cited as the cause for such extreme instability.
In summary, the proposed blood coagulation scheme discussed above represents the current state of the art. There is no question as to the existence of the mentioned factor activities, however, the physical and chemical nature of the factors themselves have yet to be resolved. As far as this study is concerned, the current scheme will give sufficiently accurate information to evaluate relative surface compatibilities.

Many *in vitro* clotting tests based on the above theory are available to test the functioning of various portions of the scheme. Two of interest have been discussed previously, namely, the thrombin clotting test and the prothrombin clotting test. The former tests the fibrinogen-fibrin reaction while the latter tests the functioning of the extrinsic system. Two other *in vitro* tests have been designed specifically for the intrinsic system and of course, the whole blood clotting test also serves as an indicator of intrinsic system functioning.

Those specific to the intrinsic system are the cephalin clotting test and the kaolin-cephalin clotting test. The cephalin test is a surface sensitive test with more reproducibility than the whole blood clotting test, WBC. The cephalin test is a better indicator of surface compatibility than the WBC test because the platelets of the WBC test are replaced by a standard brain extract rich in phospholipids. This re-
moves the variation from donor to donor caused by platelet count fluctuations. The major drawback of the cephalin test concerns its utility with surfaces of a more compatible nature than the collection vessel. Because the factor XII-XI complex is initiated and produced by the collection vessel surface, regardless of the anticoagulant, in accord with its compatibility, only those surfaces with a degree of compatibility less than the collection vessel can be tested.

The kaolin-cephalin clotting test serves as an excellent indication of a deficiency in the intrinsic system. Cephalin is again added as a platelet substitute and kaolin is added to completely activate factors XII and XI. The clotting time then depends solely on the activities of the necessary clotting factors in the intrinsic system assuming the fibrinogen and prothrombin levels are normal. Thus, this test is independent of surface characteristics.

The exact clinical procedures for these in vitro coagulation tests can be found in Appendix C.

G. Anticoagulant Heparin

Heparin, a unique highly sulfonated mucopolysaccharide found in mammalian circulatory tissue, possesses important biological properties, notably as an anticoagulant and an antilipaemic agent. Although heparin has received much attention since its discovery in 1916, it has so far withstood complete
chemical characterization. A similar situation exists concerning its anticoagulant activity, which is to be expected considering the unknowns in the clotting mechanism.

The extraction and purification of heparin from tissues requires drastic measures since it is firmly bounded to the tissue. The alteration of the heparin molecule during these procedures add greatly to the confusion concerning the heparin structure. Most extraction procedures currently used follow the methods of Charles and Scott with the exception of latter purification steps. In the Charles and Scott procedure the autolyzed tissue is extracted with alkaline ammonium sulfate solution, the extract precipitated with acid, but removed by solvent extraction and protein removed by tryptic digestion (14). This gives a crude heparin which, when prepared from tissues reasonably rich in heparin, will contain about 5-15% of the actual heparin.

Purification depends upon the particular tissue and species being investigated. Purification procedures commonly used are treatment with: 1) ammonium carbonate, 2) charcoal, 3) benzidine or 4) precipitation as the barium salt.

The most significant recent developments have been the use of amines and ion exchange techniques for purification of the initial tissue extracts. Heparin, being a polyanion, is precipitated from aqueous solutions at suitable concentrations
of cations, including amines (99). The polysaccharide is recovered by dissolution of the complex in 33% sodium chloride solution and followed by cation or amine removal through dialysis. Regenerated cellulose is used because of its impermeability to heparin. The heparin is then solidified by freeze drying. Similar processes using ion exchange resins are described in the literature (98,36).

No heparin can be extracted from normal blood with the method of Charles and Scott although added heparin is readily recovered(14). In considering alternative methods of isolation, Jaques and Bell(52) concluded that convincing results have been obtained only with injected heparin or with endogenous heparin liberated during anaphylaxis in the dog. Therefore, the question of endogenous heparin in the blood stream is still unanswered but indications are that if present its level must be quite low.

Isotope studies with $^{35}S$ heparin indicate that exogenous heparin is removed from the blood through various pathways, and is not destroyed by blood itself since it can be incubated with blood in vitro for many hours with little loss of potency. Following the intravenous injection of $^{35}S$ labeled dog liver heparin into dogs, it was found that the urinary excretion accounted for 20% of the injected isotope at a time when anticoagulant activity had disappeared from the plasma. All the or-
gans and peritoneal mast cells contained the labeled heparin, indicating diffusion into the tissue and possible storage(67). Other experiments with $^{35}$S heparin indicated that small exogenous heparin amounts are completely desulfated while larger quantities were partially excreted as unchanged or partially desulfated heparin. (17,19)

Heparin is not a simple medication but is a powerful tool and must be administered accordingly. A review by Zinn (125) of the side effects of heparin therapy indicates that these effects can be classed as either allergic manifestations or abnormal bleeding and clotting. Sensitivity to purified heparin is rare but local histamine release effects are common. At the site of injection a red slightly raised 2 to 6 cm stinging area appears. With continued heparin treatment these areas usually disappear in several weeks. The major problem in heparin usage is bleeding especially when administered to subjects with blood dyscrasias or abnormalities. Even though instances of this heparin toxicity are rare, the results can be anticipated only if foreknowledge of the dyscrasias exists. Another annoying side effect occurring during heparin therapy is the development of large ecchymoses, extravasation of the blood into the surrounding tissue. The patient's susceptibility to ecchymoses depends on the heparin load to a large extent and on the blood vessel health to a lesser extent. By
avoiding subcutaneous and intravenous injections during peak heparin loads, the chances of painful ecchymosis can be decreased.

Another heparin side effect, abnormal clotting or embolism, results from human error for the most part. Care must be taken to observe the patient's clotting time during heparin treatment since the actual heparin dose might be cleared from the blood prior to the next heparin injection. During this normal blood clotting time fibrin formations will build up on the infarction causing a complete occlusion of the vessel or the fibrin might be shed as an emboli, which will block other vessels of smaller diameter.

Abnormal clotting becomes a problem quite often subsequent to bleeding. The heparin therapy is reduced or stopped to eliminate the bleeding, but the patient's clotting times are not observed and fibrin deposition takes place if anticoagulant therapy is not resumed before the blood is returned to normal. This injudicious interruption in heparin therapy is most frequently fatal.

1. **Heparin Stability**

There are apparently no substantiated examples of animal tissue showing heparinase activity. It is generally assumed that proteolytic enzymes are without effect on heparin (99) and there is no evidence that heparin is inactivated dur-
ing autolysis of the tissue (23,55). Furthermore Jorpes (15) reported that isolated heparin was not inactivated by digestion with trypsin. The consensus appears to be that no enzymatic activity for heparin is produced by the body.

The exact nature of changes in heparin caused by alkali treatment are not known but alkaline treatment at either high temperature, 80-100°C, or high alkali concentration, $\approx 10.0 \text{N}$, does cause quick loss of activity. Jorpes (56) reported that dissolution in 1.0 N sodium hydroxide at 100°C caused a 75% loss of activity in 5 minutes.

Heparin is also significantly inactivated by warm acidic solutions (32). This sensitivity to acids has been shown to be the result of hydrolysis of the N sulfate groups in the heparin molecule. Jorpes (56) and Foster et al (24) showed that treatment with 0.04 N hydrochloric acid at 95-100°C for two hours resulted in loss of all activity as well as all of the N- and some of the O- sulfate groups. This is in contradiction to the results reported by Hejzard (45) showing that pure aqueous heparin solutions did not decrease in activity when heated to 100-200°C for 15 to 60 min or on subsequent storage at from 4 to 40°C for up to 40 months.

2. **Structure of Heparin**

The best representation of the basic unit of heparin is present in Figure 2-8. The basic repeating unit of heparin
is envisioned to be a tetrasaccharide in which all the glucosidic linkages have the \( \alpha(1 \to 4) \) configuration. The possibility of some of the glucosidic linkages in the polysaccharide having \( \alpha(1 \to 6) \) structure and/or the \( \beta \) configuration cannot as yet be definitely ruled out. It should also be recognized that different heparin preparations sometimes have varied sulphate contents. This may be due to a degree of de-N-sulphation during purification or a random variation in the heparin synthesis.

The values recorded for heparin's molecular weight range from 12,000 to 18,000. The determinations by physical methods are complicated by the extreme polyelectrolyte nature of heparin. Hence, molecular weight data have found to be dependent on the ionic strength of the test solution.

Barlow and his collaborators (4) used light scattering and salt solutions of 0.05 to 1.0 M and obtained a molecular weight of 16,000. One possible explanation for the molecular weight scatter might be that it is a direct result of purity deviations, since a wide variety of purification steps are currently being used.

Concerning heparin's molecular shape, an important factor in controlling the molecular shape of polysaccharides is the nature of the glucosidic linkage. Amylose, which is an unbranched \( \alpha(1 \to 4) \) linked poly-D-glucopyranose has a hexical
structure while cellulose which possesses a β-linkage is a linear polymer. The presence of α(1→4) linkages in heparin leads one to conjecture that heparin has a hexical structure. If this be the case, active sulfate and amino sulfate sites would be present around the perimeter of the rod shaped molecule. It has also been suggested (23) that a certain amount of rigidity might be conferred onto the molecular structure if a small number of intramolecular sulfate bridges of the type shown in Figure 2-9 were present. These bridges would be hard to detect chemically but the presence of a few bridges per molecule would certainly dictate the configuration and one would expect a collapsed hexical type structure.

3. Reaction with Proteins

In 1937 Chargoff and Olsen (13) found that protamine effectively neutralized the anticoagulant activity of heparin both in vitro and in vivo. The effect is now recognized as the result of salt formation between heparin and protamine. Salt formation is the general reaction between heparin and proteins and is a function of the strong anionic character of heparin. Under certain conditions, heparin forms insoluble salts with albumin, and protamine (4), but if the salt is soluble its formation can be noted by observing the changes in the biological activity of the protein or the reduction in anticoagulant activity of the heparin. Heparin also reacts
FIGURE 2-8  PROPOSED HEPARIN MOLECULE

\[ \text{C}_2\text{H} - \text{NH} - \text{SO}_2 - \text{O} - \text{C}_2\text{H} \]

FIGURE 2-9  PROPOSED CROSSLINK IN HEPARIN MOLECULE
in a similar manner with complex organic bases such as benzidine, histamine or amines (52).

Considering this reaction between heparin and basic substances, two characteristics must be kept in mind: 1) the relative proportion of acidic and basic groups in the heparin and base will determine the amount of heparin in the resulting salt complex; and 2) the dissociation constant for the complex will determine the quantities of free heparin and free protein present in the solution. The latter also greatly determines the amount of heparin needed to remove all the protein from the solution. Proteins such as protamine or clupein, which are strongly cationic at physiological conditions, have a relative low dissociation constant and are effective heparin neutralizers. Other plasma and serum proteins are now listed in order of their increasing affinity for heparin: B-lipoproteins < thrombin clotting system < factor IV < platelet protein < protamine sulfate (80, 81). A shift in PH to the alkaline side increases dissociation of the heparin-protein complex, but complex formation can be demonstrated up to PH 10. A strong anion such as trichloroacetic causes dissociation of the heparin-protein compound even at extremely acid PH values. Inorganic salts increase dissociation and this effect increases with the valency of the ions, i.e. Ca$^{++}$ is more effective than Na$^+$. 
4. Biological Activity

The biological activity of heparin is normally divided into two areas: 1) anticoagulant activity and 2) fat-clearing activity. Considerable effort has been devoted to the understanding of heparin's biological activity with most of the work concentrating on the structural features of the polysaccharide. Although a full understanding is not yet possible, two principle contributing features have emerged, 1) degree of sulphation and distribution of the sulphate groups and 2) molecular shape and size. It is unlikely that these factors contribute equally to heparin's anticoagulant and antilipaemic activities.

Concerning heparin's anticoagulant activity, it functions primarily by inhibiting formation of the prothrombin activator and as a backup, by inhibiting the action of thrombin on fibrinogen in the presence of a plasma cofactor. In the presence of heparin, clotting factors V and VIII which are normally used up during formation of thromboplatin formation were not utilized (9). O'Brien (81) has reported that heparin combines with and inactivates factor IX and that at moderate heparin concentrations, apart from its effect on thrombin, heparin does not inactivate any other coagulation factors. Thus, O'Brien envisioned heparin anticoagulating blood by complexing with factor IX with subsequent release of the intact
factor IX when protamine sulfate is added to neutralize heparin. This theory seems conceivable, in part, for heparin does appear to have a higher affinity for protamine than for factor IV. Biggs and MacFarlane consider this theory doubtful because they have been able to prepare highly active therapeutic samples of factor IX which contain heparin (8).

However, the increased factor IX activity of the Briggs and MacFarlane preparation was possibly in excess of that required to complex the heparin added during their factor IX purification. Hence one would expect factor IX activity, even in the presence of heparin.

5. **Role of Surface Heparin**

Besides the major activities of heparin previously described, there is another area of heparin activity which is very much in the conceptual stage but of immense potential importance. There is evidence that heparin functions in the repair of injured vascular and peritoneal endothelium. McGovern
(74) has shown that, following experimental injury to blood vessels in the rat, the reformation of the intercellular cement and the surface endothelial films involves a heparin-like component. The negative electrical potential of normal intima, which becomes positive after injury, may be related to the strong negative charge of heparin. Injected heparin also has an affinity for the intercellular cement (95,21). These findings give strong indication that heparin might normally function as part of the vascular endothelium, which is the epitome of a compatible surface.

This concept of a heparinized endothelium is substantiated somewhat by the recent work of Whiffen and Gott (114,115,116,117). These authors adsorbed heparin onto graphite coated plastic surfaces by means of a cationic surfactant, usually benzalkonium chloride. This laminate was prepared by: 1) imbedding colloidal graphite particles, .1 to 5 μ in diameter, into the surface of the plastic, by soaking the plastic in a suspension of graphite particles in a weak solvent for the plastic and removing the solvent by evaporation, 2) adsorbing the hydrophobic tail of the surfactant onto the graphite surface and 3) adsorbing heparin to the cationic portion of the surfactant.

Whiffen and Gott found that in vitro whole blood clotting times, in test tubes heparinized by their procedure and rinsed thoroughly with saline, were greater than 10 hours while the
normal for glass is about 8 minutes. After 10 hours in contact with the heparinized surface, the blood was anticoagulated and would not clot when placed in glass without the addition of protamine sulfate. This indicates that some of the heparin passed from the wall into the blood and probably explains the extremely long whole blood clotting time.

Further work by Whiffen and Gott (35) indicate graphite-benzalkonium-heparin prostheses, when placed in the canine vena cava near the heart or in the canine heart, would remain patent for periods up to two years. These authors also claimed that prosthetic conduits of plain plastic or silicone coated surfaces will become completely occluded or contain large amounts of thrombus within 2 hours after placement in the canine vena cava near the heart. A recent publication by Whiffen and Gott, (117) seems to contradict these earlier statements concerning the occlusion of the controls. Seven of ten teflon controls remained patent for two months when placed in the thoracic inferior vena cava near the heart. Similar controls constructed partially of polycarbonate were found to clot within four hours and 9 out of 10 graphite-benzalkonium-heparin (GBH) surfaces were found to be patent after 2 months in the same position. The authors also noted the inability to maintain long-term patency in GBH coated intravascular anastomosis rings in vessels 4 mm or less in diameter.
Young, Gott and Rowe (107) reported on the use of GBH coated hinged mitral and aortic valves in human patients. Although insufficient time has elapsed to evaluate the design advantages of these valves over the popular ball and cage valve, certain conclusions can be drawn concerning their thrombus-resistance properties. Two of the ten patients have thus far died, less than six months after valve placement, from valve clotting and the authors recommend that anticoagulant therapy should not be abandoned. Currently the mortality rate following mitral ball-valve replacement remains in the neighborhood of 13% whereas similar procedures on the aortic valve have a 4% mortality rate.

Whiffen and Gott (116) postulate that the benzalkonium chloride remains adhered to the graphite because of preliminary studies with C14 benzalkonium chloride. They feel that the initial heparin is removed into the blood stream and that other substances such as heparin-like mucopolysaccharides or negatively charged proteins or phospholipids might, in time, be adsorbed onto the graphite-benzalkonium surface preventing thrombus formation in the long term observations.

In summary, the existing evidence on heparinized surfaces does not allow one to form a consistent theory concerning the effect of an irreversibly adsorbed heparin surface. Additional research under more controlled conditions on GBH sur-
faces is required to understand their mode of action.

An extension of this GBH heparinization technique to semi-permeable membranes has not as yet been performed and is not recommended because of the graphite barrier to mass transport and the rigidity of the GBH surface.

Heparin must be irreversibly adsorbed onto a surface with the necessary degree of coverage before any conclusions concerning the thrombus-resistant qualities of a heparinized surface can be determined. If the substrate happens to be a semi-permeable membrane of clinical importance, so much the better.
III. APPARATUS AND PROCEDURE

A. GBH Wall Shear Measurements

Previous researchers with GBH surfaces hinted that heparin might transfer from the surface into the blood. The conditions under which this transfer does occur, if it does, and the rate of transfer must be known if the utility of the GBH surface is to be evaluated. Such findings will also shed light on the current uninterpretable in vivo data.

The GBH surface was subjected to a range of wall shears for five hour durations while the heparin transferring from the surface was detected and measured by means of the standard thrombin clotting test. Two shear values corresponded to the cannula or maximum shear witnessed in the artificial kidney and the heart lung machine. Both platelet poor plasma and saline were used as the shearing solution.

1. Equipment

The shear cell used for these measurements resembled a Couette type viscometer with the inner spindle rotating at a constant speed. An expanded view of the spindle arrangement can be seen in Figure 3-1A while the assembled cell and constant temperature bath are shown in Figure 3-B. The spindle diameter and annulus thickness were designed to give the desired wall shear at the available motor speeds for both plasma and saline at 20°C. The calibration curve and design equa-
tions for this shear cell can be found in Appendix D.

2. Experimental Procedure

The heparin removal rate from the spindle surface is determined by measuring the heparin present in aliquots of shearing solution as a function of time. Since the total shearing solution volume and aliquot sample volumes are known, the total amount of heparin in solution is determined easily from the aliquot heparin concentration. The thrombin clotting test was used to determine the quantity of heparin present in the aliquot. The raw data and calculations can be found in Appendix D.

The detailed procedure for the shear runs was as follows:

a) the GBH surface was prepared onto the Lucite spindle and a flat control according to the procedure outlined in (33).

b) The amount of surface heparin on the flat control was measured by means of radioactive heparin. This step will be discussed in more detail later.

c) The GBH spindle was subjected to the predetermined fluid shear with sampling of the shearing solution during the five hour shear time. The total shearing solution at the start was 10 ml and the sample aliquots were about .10 ml.
FIGURE A
Expanded View
Motor-Coupling-Spindle Arrangement

FIGURE B
Assembled Shear Cell and Constant Temperature Bath

FIGURE 3-1 COUETTE SURFACE SHEAR CELL
d) The sample aliquots were diluted 1 to 3 with normal saline and standard thrombin times were determined on fresh plasma with .10 ml of the diluted aliquot added to the thrombin time plasma aliquot prior to the addition of thrombin.
e) The thrombin times were then converted to actual heparin present in the shearing cell with the help of a thrombin time calibration curve, and the dilution factors. The total heparin in the shearing solution was then compared to the initial radioactivity measured heparin as a function of shearing time.

B. Heparinization of Regenerated Cellulose

1. Surface Heparin Detection-Dialysis Tubing Substrate

Because of regenerated cellulose's impressive performance thus far in the artificial kidney and its semipermeability characteristics, it retains large protein molecules, albumin and above, and has a urea membrane diffusivity of .2-.3 x 10^{-5} cm^2/sec, it was selected as the substrate to be surface heparinized. Conventional heparin determination methods based on its anticoagulant activity or metachromatic activity were not sufficiently sensitive to characterize a heparinized surface. Such a characterization was critically needed to evaluate alternate heparinization processes.

The only recourse seemed to be radioactive heparin.
Eibar and his co-workers (19) prepared $S^{35}$ labeled heparin by incorporating the $^{35}SO_4$ into canine liver heparin through intraperitoneal injection of $Na_2^{35}SO_4$. Maximum incorporation occurred at 28 hrs and the heparin was isolated as the barium acid salt, usually $50 \text{Mg} / l \text{kg}$ of liver. Because of the preparational expense, $S^{35}$'s short half life, 87 days, and the $S^{35}$ range, heparin in the membrane and on both sides could be detected with $S^{35}$, routine surface heparin measurement with this isotope left something to be desired. A far superior tritium gas labeling and measuring technique was developed. Tritiated heparin was used to measure the actual heparin present in the upper $2.5/\mu$ of a $25/\mu$ thick membrane.

New England Nuclear Corp. of Boston, Mass. prepared the tritiated heparin by means of a gas exposure technique proposed by Wilsbach in 1957 (119). With this technique a random labeling of the heparin with Tritium, $H^3$, was obtained because of the exchange of hydrogen induced by tritium radiation when organic compounds are exposed to tritium gas. To perform such an exchange bulk heparin, purchased from Organon Inc. of West Orange, N.J., was sealed in an ampoule with carrier-free tritium gas for three weeks. To avoid radiation decomposition low tritium activities - long exposure times were used, i.e. $500 \text{Mg}$ of bulk heparin was exposed to three curies for three weeks at $27^\circ \text{C}$ and $250 \text{mm Hg}$. After three weeks of exposure
the labile tritium was removed by solution in water followed by lyophilization. Specific activities of about .2 millicurie per milligram of heparin were consistently obtained.

Prior to the discussion of the actual \( H^3 \)-heparin detection equipment and measuring procedure a brief review of scintillation detection theory will be presented.

a) Radioactive Decay

A radioactive atom such as tritium or carbon-14 contains a nucleus which is unstable, i.e. the nucleus contains an extra neutron and extra energy not found in a normal, ground state, stable atom. The nucleus, therefore, is inclined to release this extra energy and change the number of neutrons in its nucleus. The process by which it does this for tritium and carbon 14 is known as beta particle decay. In beta particle decay an electron is ejected from the nucleus along with a neutral massless (essentially) particle known as a neutrino. The two ejected particles share the energy of decay of the atom, i.e., the excess energy that the unstable atom possessed. The fact that the two particles share the energy in random proportions accounts for the fact that a beta source does not emit particles of one energy but of a spectrum of energies. The maximum energy of the beta particles emitted by a given radioactive nuclide is characteristic
of that nuclide and may be used as a means of identification. For carbon-14 and tritium the maximum beta energy is 0.154 and 0.018 million electron volts (MEV), respectively.

b) **Beta Particle Detection** (87)

The neutrino being an electrically neutral massless particle is virtually impossible to detect. The thickness of mass necessary to stop a neutrino would be measured in light years. The beta particle, on the other hand, possessing both a negative electrical charge and a finite mass (1837 times lighter than a proton) is easily detected by several means such as ionization chambers, electroscopes, Geiger-Mueller counters or scintillation counters.

A beta scintillation counter can be constructed using either an organic fluorescent solute dissolved in an organic liquid solvent or an organic fluorescent solute dissolved in an organic solid solvent. The principle of operation is the same in both cases. When a beta particle travels through the organic solvent it causes electrons in the shells of the solvent molecules to be excited to higher energy states. The excited electrons quickly decay to their original states with the subsequent emission of a photon of energy equal to the energy
of excitation. This photon is absorbed by a fluorescent solute molecule which emits a photon of light upon decaying to the ground state. The original energy of excitation and the subsequent energy of the light photon are proportional to the energy of the exciting beta particle. In order for the scintillator to be applicable to scintillation counting it must be transparent to its own emitted light.

In a scintillation counter the light from the scintillator is fed either directly or by use of a light pipe into a photomultiplier (PM) tube. A photon of light entering a PM tube strikes a photosensitive surface with the subsequent emission of an electron. Since the PM tube has a high voltage applied across it the emitted electron or photoelectron is accelerated and smashes into another photosensitive plate called a dynode. This releases several electrons which are again accelerated and again strike a dynode. The process continues through several dynode stages eventually producing a sizeable electrical pulse which is amplified and counted by an electronic or mechanical counter.

Depending on the data required from the scintillation counter the pulses from the PM tube pre-amplifier may be fed into a counting rate meter or a scaler. Both
of these instruments are usually supplied with circuits to provide the high voltage for the PM tube and the power for the pre-amplifier. In either instrument the pulse is further amplified and shaped before being displayed. The difference between the ratemeter and the scaler is in the manner in which the pulse is used. In a ratemeter the pulses are fed to a tank capacitor shunted with a resistor. The voltage across the capacitor is displayed on a calibrated linear voltmeter and is proportional to the counting rate at any instant of time. In addition the ratemeter may have an output for a recorder such that the counting rate at a function of time may be displayed. In the scaler, on the other hand, the amplified pulses are fed to an integrating counting circuit. Each pulse causes another neon bulb to light on the display panel the tenth pulse causing a bulb in a new decade to light, etc. The counts on this type of instrument may be summed for any chosen length of time within the capacity of the display unit.

A schematic representation of a beta scintillation system is given in Figure 3-2.

c) Solid Scintillation Detection System

The actual solid scintillator assembly and photomultiplier tube used in this study are displayed in Fig-
FIGURE 3-2 SCHEMATIC BETA SCINTILLATION SYSTEM

- Beta Source
- Scintillation Crystal
- Photomultiplier Tube
- Pre-amplifier
- SCALAR
- COUNT RATEMETER
- RECORDER
The solid scintillator was purchased from Pilot Chemical, Inc. of Watertown, Mass. and the template is a 10 mil thick Tenite Acetate sheet with a one inch diameter center hole. The weight was necessary to keep the membrane flush on the template. The typical standard deviation for this detector system with a tritium source was measured to be about 2.5% of the mean count.

The assembled scintillation detector can be seen in Figure 3-3B. This entire unit mounts inside of a lead cylinder, which in turn is placed in a light tight refrigerator, see Figure 3-4. The purpose of the refrigerator is two fold, 1) since the PM is sensitive to light, the refrigerator served as a light tight compartment and 2) since the circuit noise level is a function of preamplifier temperature, the refrigerator, with a relay and temperature indicator, removed the preamplifier heat and maintained constant temperature. The detection instrumentation was mounted on top of the refrigerator. In this setup the Baird-Atomic Ratemeter Model 432 supplied the high voltage to the preamp and converted the signal from the preamp to an average count-per-minute. The count rate was then recorded on the Bauch-Lomb VOM5 Recorder. A typical membrane measurement is presented in
Figure 3-5. The initial over-shoot is caused by weak light exposure while placing the membrane on the template and the high voltage over-shoot as the high voltage is raised from 300 to 1200 volts, the measurement voltage. The Baird-Atomic Scaler Model 131 also shown in Figure 3-4 was used to obtain accurate counts for a given period of time. The actual measurements were taken on the scaler after an equilibrium count rate was indicated by the ratemeter.

d) $^3$H-Heparin Adsorption and Measurement Procedure

1. Adsorption

In developing a procedure to adsorb radioactive heparin onto membranes one must be cognizant of three restraints: namely, 1) the total heparin adsorbed might be a function of degree and time of agitation, 2) there is a distinct possibility of tritium transfer from the heparin to the solvent, water, and 3) tritiated heparin costs approximately $120 for 500 mg and should be conserved. The routine $^3$H-heparin adsorption procedure adopted for this study took these three considerations into account and was as follows:

a) $^3$H-Heparin Storage - the bulk $^3$H-heparin was stored as a solid in 25 mg packages and used as required. This maintained the specific activity
FIGURE A
Photomultiplier Tube and Solid Scintillator Assembly

(L-R)
Std. Weight, Membrane Template, Solid Scintillator, PM Tube

FIGURE B
Assembled Preamplifier, Photomultiplier Tube, and Solid Scintillator Assembly

FIGURE 3-3 $^3$H - HEPARIN SOLID SCINTILLATION DETECTOR
FIGURE 3-4 SCINTILLATION DETECTOR WITH AMPLIFICATION INSTRUMENTATION

FIGURE 3-5 TYPICAL H³-HEPARIN MEMBRANE MEASUREMENT
of the heparin during storage.

b) **H₃^-Heparin Soaking Solution** - Fresh H₃^-heparin solutions were prepared shortly before the membranes were to be soaked. All solutions contained .75 mg H₃^-heparin per ml of distilled water. The life of the solutions was considered to be no more than three hours.

c) **Radioactivity Calibration** - The radioactivity of the H₃^-heparin solution was checked by measuring the activity in a certain sized aliquot with a calibrated liquid scintillation counter. These checks were performed by the Occupational Medicine Department of MIT. Planchets of known activity were then prepared from the H₃^-heparin solution and used to check the efficiency of the solid scintillation detection system previously described. The efficiency of this system for tritium was found to be .5 ± .03%. Liquid scintillation systems generally achieve about 3% efficiency with tritium.

d) **Membrane Soaking Technique** - Five membranes are attached to 15 x 125 mm test tubes with rubber bands and placed in a 100 ml beaker. Fifty ml of H₃^-heparin solution is added to the beaker, sealed
with parafilm and placed on a Yankee agitator for 1 1/2 hours. Following agitation the membranes are rinsed in distilled water for 15 minutes, wiped with a damp cloth and the surface blotted dry. The membranes are then allowed to air dry while being compressed between cloths to give a uniform surface for heparin measurement.

2. $^3$H-Heparin Measurement

Prior to scintillation counting the PM tube must be dark adapted for 20 hours with a voltage of at least 300 V to the grid. Care must be exercised not to allow direct light to contact the PM tube during membrane loading of the assembly, which is performed in a darkened room. The membrane can be placed safely onto the template with a high voltage to the preamp (HV) of 300 volts. This reduces the intensity of the initial overshoot and allows an accurate measurement to be taken within about 15 minutes after the HV is increased to 1200 volts. The general level of the count rate is followed on the recorder from the ratemeter but the actual measurement is read on the more accurate scaler after the count rate has leveled off as evidenced by the tracing on the recorder. See Figure 3-5 for a typical measurement.
2. **Regenerated Cellulose Surface Amination**

Reingertz and Reichard (92) demonstrated that heparin could be adsorbed tightly onto aminated cellulose ion exchange resins. These basic ion exchange resins could be prepared by any of the following methods: 1) the reaction of 2 aminoethyl sulfuric acid and cellulose in the presence of sodium hydroxide, 2) the reaction of \( \text{CH}_{2}\text{Cl}-\text{CH}_{2}\text{N} \text{EtN} \text{Et} \) amine with cellulose in the presence of sodium hydroxide and 3) the reaction of epichlorohydrin and triethanolamine with cellulose (47,51,37,91). An attempt to adapt these reactions to the surface amination of regenerated cellulose met with limited success. The main difficulty with these reaction procedures was insufficient surface amination at the point of film degradation.

A highly successful alternate surface amination process was developed which capitalized on the instability of the ethylenimine molecule and its reactivity toward active hydrogens. In this process regenerated cellulose film is reacted with ethylenimine with subsequent protonation by an acid, usually hydrochloric acid. This aminated surface has a high affinity for heparin because of its abundance of basic nitrogen groups appearing on the surface due to the cationic polymerization of ethylenimine to polyethylenimine. The basic
chemical reactions for this procedure can be expressed as:

\[
\begin{align*}
\text{Cell} \quad -\text{OH} & \quad + \quad \left\{ \begin{array}{c}
H \\
HC - CH \\
\text{N} \\
H
\end{array} \right\} \quad H_3O^+ \\
\text{(Regenerated Cellulose)}
\end{align*}
\]

\[
\begin{align*}
\text{Cell} \quad -O- \left( \text{CH}_2\text{CH}_2\text{NH} \right)_{M-1}^+ \quad \text{CH}_2\text{CH}_2\text{NH}_3^+ \\
\text{(Aminated regenerated cellulose)}
\end{align*}
\]

a) **Reaction Procedure and Equipment**

Seamless regenerated cellulose manufactured by Union Carbide Corp. of Chicago, Illinois, the current dialysis membrane used in coil artificial kidneys, was selected as the membrane to be surface amination and heparinized. This 1 mil thick tubing is plasticized with glycerine prior to delivery and must be boiled or soaked in water to remove the glycerine. In this study #20 dialysis tubing was boiled in distilled water for one hour before amination. The boiled tubing was aminated in a resin-kettle reactor while being supported by test tubes. See Figures 3-6A and 3-6B. The outside of the membrane tube was preferentially aminated, although a small amount of inside surface amination was observed.

The detailed procedure for the amination portion of the process is as follows:

1) the boiled membranes were slipped over the test tubes and placed in the holder,
FIGURE A
Disassembled Reactor

FIGURE B
Assembled Reactor
(Max. capacity - 12 tubes)

FIGURE 3-6  RESIN-KETTLE AMINATION REACTOR
123
2) the holder and membranes were soaked one hour in aqueous catalyst solution
3) the holder and the membranes were blotted dry, placed in the resin-kettle, and the kettle was evacuated to 29-30" Hg vacuum with an aspirator. (Vacuum line was attached to one L connector and vacuum gage to the other.)
4) the kettle was placed in a heating bath at a temperature of from 85 to 100°C
5) ethylenimine is added dropwise into the reactor from the separatory funnel until the pressure of the imine gas inside the resin-kettle was one atmosphere.
6) additional ethylenimine is required as the amination proceeds to maintain one atmosphere pressure in the kettle. It is imperative that oxygen be absent because of the degrading effect oxygen and the catalyst have on the cellulose film.
7) the reaction time varied from 2 to 60 hours during this study depending on the amination conditions.
8) the membranes were washed in distilled water for at least 150 hours prior to heparinization.

A word of caution concerning the hazards of ethylenimine is in order at this time. Ethylenimine is a highly toxic material and special precautions must be taken when handling
or using it. Some of the more pertinent precautionary measures and appropriate first aid treatments are listed in Appendix F. It should also be noted that these safety measures pertain only to ethylenimine and have little or no relevance to polyethylenimine or the cellulose-imine-heparin (CIH) membrane.

C. In Vitro Testing of CIH Surfaces with Human Blood

The development of the CIH membrane with its irreversibly adsorbed surface heparin provided the necessary tool to study the interaction of human blood with a heparinized surface. The analytical tests used to characterize this interaction were the standard in vitro blood clotting tests. These tests were referred to in the Introduction but will be briefly repeated here along with their significance. The tests are listed in order of increasing sensitivity:

1. **Whole blood clotting test** - this test acts as an initial screen for the interaction of foreign surfaces with human blood. Surfaces providing an extended whole blood clotting time might do so for various reasons, but consistent normal whole blood clotting times with the test surface indicate the surface represents a negligible improvement in compatibility.

2. **Thrombin Time** - This clotting test serves as an indicator of heparin activity. Aliquots of citrated plasma are placed in CIH tubes, removed and tested for heparin activity.
An extended thrombin time, over that of the control, indicates the presence of heparin.

3. **Prothrombin Time** - A normal prothrombin time indicates the functioning of the extrinsic clotting system. A sizable deficiency in one or more of the clotting factors of this system will cause an extended prothrombin time. This test is not as sensitive to heparin as the thrombin test.

4. **Kaolin-Cephalin Time** - A normal kaolin-cephalin time indicates intrinsic system activity, as well as prothrombin and fibrinogen activity. This clotting test in conjunction with the prothrombin time test can provide invaluable information concerning the presence of clotting factors in the intrinsic and extrinsic system.

The typical procedure consisted of, first, determining the overall surface compatibility by performing a WBC test and, secondly, studying the interaction between promising surfaces and citrated plasma with the above clotting tests. Certain factors, such as factors V and VIII, are unstable which necessitated the use of fresh plasma and the continual comparison with controls.

The CIH tubes were formed by inverting the dialysis tubing as it came from the amination reactor and tying a knot in
one end. These tubes were placed in capped Lucite sleeves and incubated in a water bath, at approximately 39 to 40°C, allowing the plasma to incubate at 35-37°C.

A detailed outline of the various in vitro clotting tests discussed above are presented in Appendix C. The blood of various donors was used for the preliminary screening tests while, for the sake of consistency, this author's blood was used for the tests and results discussed later.

D. Membrane Surface Microphotographs

Microphotographs of membrane surfaces were prepared to study the surface irregularities and their influence on surface heparinization and compatibility. To accentuate these surface irregularities the samples were chromium shadowed. The chromium layer was deposited with a Kinney Shadow Evaporator at an angle of 27° with the plane of the sample. Typically a chromium source was evaporized in a 10^{-5} mm Hg vacuum producing gaseous chromium which deposited on the slide sample placed in the evaporator. The shadowed samples were observed through an optical microscope with direct lighting at resolutions of 450X and 100X.

E. CIH Membrane Stress-Strain Measurements

The stress-strain measurements of this study were made with a Table Model Instron testing machine manufactured by Instron Engineering Corporation of Canton, Massachusetts. This
machine consists essentially of a highly sensitive weight measuring system to detect and record the tensile or compression load placed on a test sample. A sample is placed between two jaws, one of which is stationary and attached to a load cell while the other or lower pulling jaw is attached to a moving crosshead. The crosshead is operated by two vertical screws driven by a synchronous motor through an elaborate gear system, designed to easily change crosshead speeds through a large range. The tensile force exerted on the load cell through the sample as a result of the crosshead movement is recorded by a single channel recorder, thus a plot of force versus strain is prepared automatically. With the wide range of crosshead and recorder speeds a large choice of magnification factors exist.

In particular reference to the stress-strain measurements of this study, the membranes were stretched while in a 37°C distilled water bath. All membrane samples were prepared with a standard template, 1/2" x 4". The initial distance between the jaws was a constant two inches and the crosshead speed remained constant throughout the measurements at .1 inches/min. No membrane fractures at the jaws were observed.

F. CIH - Membrane Permeability Measurements

Although an effective artificial kidney membrane requires many characteristics, one of primary importance is its ability
to pass urea efficiently. The exact cause for the uremic syndrome has yet to be understood, but the fact remains that a healthy adult produces 26 gms of urea a day and it must be removed from the blood stream by either natural or artificial kidneys. The ability of a membrane to pass urea can be ascertained by timing the rate of passage of this permeant from one well stirred chamber to another connected only by the test membrane. Such a cell has been called a membrane diffusion cell. A drawing of the diffusion cell used in this study can be seen in Figure 3-7 and a photograph of the cell can be seen in Figure 3-8. The cell is constructed of ordinary plexiglas cylinder and flat stock. It can be disassembled into two halves for mounting of a membrane between the two chambers. The membrane mounting requires a 1 inch diameter membrane, 1/2 inch diameter of which is exposed to the solutions. This cell quick mounts into a sturdy frame which positions the cell for the pulley driven agitators.

Single blade agitators with 3/4 inch by 1/4 inch impellers are driven with 1/10 hp motors. The agitator speed can be varied between 0 and 2500 rpm with Powerstats.

A .040" ID polyethylene tube is inserted into the bottom of each chamber for filling and emptying of the cell. B & D adaptors and valves are also attached to the polyethylene tubing to provide four-syringe connectors and thus facilitate
fluid handling.

Thermistor probes are placed into each cell to monitor the fluid temperature. Swagelock 1/8" tubing to 1/8" pipe fittings act as guides for the temperature probes and facilitate insertion and removal of the temperature probes.

The cell and frame were mounted in a light tight box for possible future adaption to a continuous scintillation counting system using a radioactive permeant. In such a system the right cell plate will be of solid scintillator light sealed directly to a PM tube. Thus a continuous count rate, which is directly related to the radioactivity or permeant mass in the well stirred cell, could be recorded. This system is not practical without an auxiliary amplifier between the preamp and the rate meter. This additional amplifier would allow the system to operate with lower high voltage, thus lower background, and with increased counting efficiency. Since the amplifier was not available, intermittent sampling and counting were performed by drawing a sample from the cell through a small hole in a well reflectorized solid scintillator. This scintillator was light sealed with mineral oil to the PM tube and appears to the right of light tight box in Figure 3-8.

Constant cell temperature was maintained by circulating cool air through the light tight box. The ambient air outside the cell box but inside the refrigerator was controlled by a
FIGURE 3-7 DIFFUSION CELL
FIGURE A
Membrane Diffusion Cell

FIGURE B
Assembled Diffusion Cell and Detection System

FIGURE 3-8 MEMBRANE PERMEABILITY MEASURING SYSTEM
mercury temperature sensing probe connected to a Sargent relay and in turn to the refrigerator.

The membrane diffusion coefficient can be obtained from measurements of the solution activity on one side of the membrane as a function of time. Assuming equal volumes in each compartment of the cell, the working equation then becomes:

\[
\ln \left( \frac{C_T - C_0}{2} \right) = -\frac{D_m A}{L} \frac{120}{V} T
\]  

(8)

where

- \( C_T \) = the activity in the compartment with outward diffusion of the solute, cpm
- \( C_0 \) = initial activity in same compartment, cpm
- \( D_m \) = average membrane diffusion coefficient for solute, cm\(^2\)/sec
- \( A \) = membrane area available for transport across, cm\(^2\)
- \( L \) = membrane thickness parallel to diffusion, cm
- \( V \) = volume of compartment, cm\(^3\)
- \( T \) = time since initial loading of compartment, min

The average membrane diffusion coefficient can then be obtained from the slope of a \( \left( \ln \left( \frac{C_T - C_0}{2} \right) \right. \) vs. \( T \) plot since the other constants are readily measurable.

G. In Vivo Studies with Heparinized Surfaces

Intravascular plastic prostheses were prepared from cel-
lulose acetate and placed in canine inferior vena cava to
test the effects of surface heparinization. Although the bulk
of the previous heparinization in vitro blood coagulation re-
sults were with a cellulose membrane substrate, the mechanics
of the in vivo compatibility testing required a more ridge and
less permeable material than the cellulose membrane. So cel-
lulose acetate was chosen as the canine vein replacements.
Prostheses 3 cm long, 1.0 cm OD and .8 cm ID were machined
from cellulose acetate test tubes injection molded with East-
man cellulose acetate powder No. 042A3700 M.H. The hepariniza-
tion procedure for cellulose membranes was extended to include
cellulose esters, i.e. cellulose acetate, cellulose acetate
butyrate, cellulose propionate etc., by first reducing or hy-
drolyzing the surface of the cellulose ester to cellulose.
The cellulose surface is then aminated and heparinized as with
the cellulose membrane. Numerous reducing or hydrolyzing
agents are available but preliminary studies with a few indi-
cated an alcoholic solution of an alkyl oxide gave the quickest
surface attack with minimum penetration into the substrate.
The reducing solution used in these studies consisted of a
1.0WT% sodium methylate in methanol solution. Its interaction
with cellulose acetate with subsequent washing in distilled
water appears as follows:
The surface deacetylated cellulose acetate prostheses were then reacted with ethylenimine in the same manner as the dialysis membranes. The prostheses were washed at least 175 hours following the amination to remove traces of polyethylenimine by-products and heparinized on the day of their use with a highly concentrated sodium heparin solution, 200mg/ml distilled water. Stress cracking and particularly surface bubbling were frequently observed with these prostheses, which created a severe test for the compatibility of the surface. Trauma caused by rough surface and flow characteristics usually results in an increased tendency for clotting. A photograph of a typical prosthesis surface is shown in Figure 3-9.

1. **Description of Testing Procedure**

   Numerous variables influence the thrombus formation and buildup on foreign surfaces when placed inside the alive animal. One obvious, but sometimes overlooked, influence is
the availability of blood to the foreign surface. While the tissue acceptance of the foreign surface is a challenging and difficult problem in its own right, one should be cautious about inferring thrombus resistivity from encouraging tissue acceptance results. Along these same lines, extrapolation and comparison of thrombus formation times in prostheses placed in various blood vessels throughout circulatory system should be made with great reservations until much more is known concerning the variables and their influence.

At the present time it is only possible to postulate that in vivo thrombus formation on foreign surfaces is a function of both surface compatibility and blood contact time per pass. Expressed analytically it might be:

\[
TFR = f \left( \frac{S.C.}{C.T.} \right)
\]

where:

- \(TFR\) = thrombus formation rate
- \(S.C.\) = surface compatibility
- \(C.T.\) = blood contact time/pass.

Judging from the above postulation, a prosthesis size and location should be selected such that it provides meaningful data concerning relative surface compatibilities. The contact times should be sufficiently long to result in consistent relatively rapid clotting of the controls (114,115,116,117).
Although Whiffen and Gott have mentioned contradictory control clotting times for prostheses placed in the canine chest inferior vena cava, preliminary studies with cellulose acetate prostheses, of a longer length than those of Whiffen and Gott, indicate no consistent control clotting time could be obtained. The times ranged from 10 up to and greater than 48 hours. In the artery side even longer patency times were observed.

When 3 cm prostheses were placed in the canine abdominal thoracic inferior vena cava below the renals, these prostheses of medical grade silicone rubber, cellulose acetate or regenerated cellulose stretched over teflon, were found to occlude completely in one hour to one hour and thirty minutes. A schematic of the canine circulatory system presented in Figure 3-10 will be of some assistance picturing the exact prosthesis location. The position below the renals represents one of low flow and thus of extreme severity, since only the blood returning from the right femoral region passes through the prostheses.

To insert a prosthesis in this position below the renals a portion of the vein is removed from an anesthetized mongrel dog and replaced by the rigid prosthesis with portions of the severed vein overlapping the prosthesis as shown in Figure 3-10. Prior to positioning of the prosthesis the right femoral vein was identified, isolated and catheterized for record-
ing of the venous pressure. The catheter was connected to a transducer via a small tube containing a heparin-saline solution for transmitting the pressure with minimal line coagulation problems. Output from the transducer was recorded continuously on a Sanborn 350 Recorder. The venous pressure was followed after the insertion of the prosthesis and was found to rise sharply as the cross sectional area of the prostheses occluded completely. If the prosthesis occluded completely but was cleared by the venous flow, this too could be observed with venous pressure measurements for the pressure would rise as the prostheses occluded and dropped suddenly as the emboli was thrown from the prosthesis.

The dogs were normally sacrificed when a venous pressure rise was observed or after a predetermined test duration, if no venous pressure rise was witnessed. In all cases the heart and lungs of the sacrificed animals were inspected for thrown emboli as well as the patency of the prosthesis.

H. Urea Diffusion Through Human Blood

Approximately 60-70 percent of the mass transfer resistance of the current clinical artificial kidneys lies on the blood side. This resistance, although formidable in current kidneys, will sharply limit the performance of new artificial kidneys of the same design, but with more permeable membranes. Therefore, emphasis should be placed not only on the membranes
FIGURE 3-9A

Material:
Cellulose Acetate 042A
50' Deacetylation +
1 1/2 h EtI, 73°C

Magnification:
40 X

WBC Time:
13 minutes

FIGURE 3-9B

Material:
Cellulose Acetate 042A,
50' Deacetylation +
36h EtO + 1 1/2h EtI
73°C

Magnification:
40 X

WBC Time:
70 minutes or greater

FIGURE 3-9 MICROPHOTOGRAPHS OF CAIH SURFACE, I.
FIGURE 3-10 SCHEMATIC OF CANINE INFERIOR VENA CAVA
but on the blood side configuration as well in the design of future artificial kidneys. A first step towards this improved design is the careful characterization of the blood side mass transfer coefficient for small solutes such as urea. This would allow accurate extrapolation to different blood channel designs and thus their evaluation.

Any characterization of this blood side resistance must include the diffusion coefficient of the solute through blood, i.e. $D_{\text{urea}}$ through blood. No experimental values of blood diffusivities were found in the literature. Theoretical approximations such as those by Prager (85, 86) and Wang (109) have been used in the past, but there is no a priori method of estimating their accuracy. In fact, one would be surprised if Prager's (86) treatment would give accurate values, since his assumption of zero diffusivity through the suspended solids probably does not apply to the red cells.

The purpose of this diffusivity section was to 1) develop a technique to measure the self diffusion constants in human blood solutions and 2) to provide an experimentally sound estimate of the urea diffusion constant in human blood to be used in the future correlations of blood side coefficient parameters.

Two methods are generally used for the measurement of
solute diffusivities, the diaphragm method or the capillary method. The diaphragm method (66) has the disadvantage of demanding a large quantity of material and thus being expensive for radioactive isotope studies with human blood. Isotopes offer decided advantages when analyzing for the solute with multicomponent blood. Additional disadvantages stem from the sensitivity of the blood to long term stirring and the pore size of the diaphragm required to accommodate the red cell. Therefore, the capillary method was used for this study.

A capillary tube with one end open was filled with blood solution containing a radioactively tagged solute, in this study urea, and the filled capillary was placed into contact with a reservoir of the blood solution but with no labeled solute present. The isotope content of the capillary was measured continuously as a function of time and recorded. The value of the diffusion constant was determined from the total capillary activity versus time data using the series expansion solution describing the one dimensional, unsteady state diffusion from the capillary.

According to Fick's second law in every point of the capillary tube

\[
\frac{dc}{dT} = D_s \frac{d^2c}{dx^2}
\]  

(10)
where \( C \) refers to solute concentration or radioactivity, \( T \) refers to time and \( X \) refers to distance along the capillary axis. The boundary conditions for the idea case are

\[
C(X,0) = \begin{cases} 
C_0 & \text{if } 0 \leq X \leq l \\
0 & \text{if } X > l
\end{cases}
\]

and \( C(l,T)=0 \) where \( l \) is the capillary length and \( T \) is any time. This latter boundary condition does not correspond exactly to the facts since during diffusion there is always a certain quantity of the isotope outside the capillary. This fact could be taken into account if the boundary condition \( C(l,T) = C(T) \) were known for the diffusion taking place outside the capillary and imposed on the ideal solution. The solution of Fick's equation with such a boundary condition is extremely difficult and should not be used to calculate \( D_s \) unless necessary.

Gergely, et al (31) modified the one dimensional model to include this effect by assuming a constant flux from the capillary and considering the diffusion from the capillary as a point source into a hemisphere. After extensive mathematics the authors derived an integral equation which was solved by numerical methods. A resubstitution of the capillary dimensions of this study produced an estimated error of no greater than 1.5 to 2.0%. The actual diffusion constant would be 1.5 to 2.0% larger than the measured constant using the ideal boundary
Fick's second law has been solved with the ideal boundary conditions and is given by:
\[
C(X,T) = 4 \frac{C_0}{\pi} \sum_{m=0}^{\infty} \frac{(-1)^m}{2m + 1} \exp \left( \frac{(2m+1)^2 \pi^2 D_s T}{4 \lambda^2} \right) \cos \left( \frac{(2m+1)\pi X}{2\lambda} \right) \tag{11}
\]

To obtain the ratio of the average concentration or radioactivity remaining in the capillary to the initial concentration or activity, \(C_0\), one integrates as follows:
\[
\frac{\bar{C}}{C_0} = \int C(X) dX = \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp \left[ -\frac{(2m+1)^2 \pi^2 D_s T}{4 \lambda^2} \right] \tag{12}
\]

From equation (12) it is possible to obtain the diffusion constant from average capillary activity versus time data. This can be done with the first term only if the series converges quickly. The importance of the terms beyond the first can be determined from Figure 3-11 for estimated diffusion constants and experiment duration. With a \(D(\text{urea})\) of as low as .25 \(\times 10^{-5}\) cm\(^2\)/sec compared to 1.2\(\times 10^{-5}\) cm\(^2\)/sec for urea in distilled water at 20\(^\circ\)C and an experiment duration of 18 hours or more, the use of the first term only gives a diffusion constant which is 6% low. With a more realistic \(D(\text{urea})\) of .5 \(\times 10^{-5}\) cm\(^2\)/sec and 18\(^+\) hours the diffusion constant is less than 3% low. Therefore the working equation becomes
\[
\frac{\bar{C}}{C_0} = \frac{8}{\pi^2} \exp \left( -\frac{15 \pi^2 \text{D} \text{urea} T_{\text{min}}}{\lambda^2} \right) \tag{13}
\]
\[ \ln \frac{\bar{c}}{c_0} = \ln \frac{8}{\pi^2} - \frac{15 \pi^2 D_{\text{urea}} T_{\text{min}}}{\lambda^2} \] (14)

A plot of \( \ln \frac{\bar{c}}{c_0} \) versus \( T_{\text{min}} \) gives a straight line after about 6 to 8 hours duration whose slope is \( \frac{-\pi^2 15}{\lambda^2} D_{\text{urea}} \).

Before discussing the actual experimental procedure one more restraint must be discussed. When measuring the diffusion constant through suspensions, the void fractions are limited to those values where sedimentation of the suspended particles is not excessive. For blood this means diffusivities must be measured at hematocrits of 60 or above. See Figure 3-12 for human erythrocyte sedimentation rates in whole blood. Although this procedure is limited to higher hematocrits and non suspensions, one can easily extrapolate from these higher hematocrits to the normal hematocrit of 40 to 45.

1. Diffusion Capillary and Instrumentation

The diffusion capillary for this study was center bored into a 1 3/4 inch diameter solid scintillator cylinder. The internal diameter of the capillary was .070 cm. and the length was 1.8 or 2.0 cm depending on the actual cell considered. The capillary cylinder mounted into a guide exposing the open end of the capillary to a 10 ml reservoir. See Figure 3-13A for a photograph of the separate components and Figure 3-13B for the completely assembled cell and reservoir.

The top, or face with open end of the capillary, of the
solid scintillator was reflectorized with reflective paint. The sides of the holder were also reflectorized and covered with electrical tape. Thus, all sides of the scintillator except the face light-sealed to the PM tube, reflect the light produced by the radioactivity in the capillary. Therefore, the light detected by the photomultiplier tube represents the average solute concentration in the capillary. The photomultiplier tube signal is amplified in the ratemeter and recorded as was previously discussed. The assembly of Figure 3-13B was placed inside the lead shield shown in Figure 3-4 and the entire assembly was kept at 20°C.

2. Procedure

The procedure for a typical diffusivity run was as follows:

1) Twenty-five ml. of fresh blood were drawn through a venous puncture and anticoagulated with trisodium citrate or heparin. This author's blood was used for all data reported here.

2) The blood was centrifuged and the hematocrit adjusted to the desired value.

3) A small aliquot (.2 ml) of the adjusted blood was mixed with 1 mg of radioactive urea purchased from New England Nuclear Corp. of Boston, Mass.

4) The capillary was filled with 10^\mu l of the radioactive
FIGURE 3-11  IMPORTANCE OF SECOND + TERMS IN THE CAPILLARY CELL SERIES EXPANSION
From:
"Syllabus of Laboratory Examinations in Clinical Diagnosis"
by Page & Culver

FIGURE 3-12 HUMAN ERYTHROCYTE SEDIMENTATION IN WHOLE BLOOD
FIGURE A
Separate Components (R to L)
PM Tube, Capillary Cell, Reservoir + Holder, Reservoir Top

FIGURE B
Assembled Reservoir - Cell - PM Tube

FIGURE 3-13 UREA DIFFUSIVITY MEASURING EQUIPMENT
blood with a #24 needle.

5) The capillary scintillator cylinder was then inserted into the holder and held in position with set screws.

6) The reservoir was then filled (10 ml) with the remaining non radioactive blood from the original 25 ml sample.

7) The assembled reservoir and capillary were light-sealed to the PM tube with mineral oil and placed inside the lead shield.

8) The high voltage to the preamp was slowly increased until the count rate was 250,000 cpm (1000-1100 volts). The output from the ratemeter was then automatically recorded for the duration of the run.

9) At the termination of the diffusion run the hemato-crit was rechecked and the reservoir and capillary rinsed with distilled water.

10) The background was then measured with no radioactivity in the capillary. Normal value was 500 cpm.
IV. RESULTS AND DISCUSSION

A. Tritiated Heparin

The primary purpose of this study was to develop a more compatible dialysis membrane than the regenerated cellulose films currently used in the artificial kidneys. Such a film would provide the necessary first step towards a compatible artificial kidney possessing obvious improvements over the existing ones. Whiffen and Gott, through their pioneering efforts with the GBH surface, have provided what seems to be an important clue to the possible requirements of a compatible surface. Their work seems to show that a surface layer of heparin imparts extended compatibility with human blood to a wide range of substrates. Whether or not this compatibility was a result of transient heparin could not be ascertained from their studies. Therefore, prior to the heparinization of regenerated cellulose films it seemed advisable to study the GBH surface under more clearly defined experimental conditions. Such studies would indicate whether an approach similar to the GBH method would be of value in the design of the nonmembrane parts for the artificial kidney. For such parts a permanent antithrombogenic effect would be more desirable than an anticoagulation effect due to transient surface heparin. To study this, the GBH surfaces were subjected to various wall shears as described in Section III A, to determine under what conditions, if any, the heparin migrates from the surface. The results of this study will be
presented later, but first, a few words concerning the radioactive heparin used during this study should be presented.

Radioactive heparin was selected for the surface heparin measurements. Radio-heparin can be synthesized through the ingestion of isotopes into animals, followed by tissue extraction, or by exposure to tritium gas according to the Wilsbach Technique (120). Experience with this technique has shown that exposure of organic compounds to tritium gas yields tritiated products of high activity, without extensive radiation damage. The tritiated products are a result of the exchange of hydrogen induced by tritium radiation when organic compounds are exposed to the tritium gas. This self-induced exchange reaction occurs at room temperature with sub-atmospheric pressures of tritium. For the purpose of this study, the conditions were chosen specifically to reduce degradation of the heparin. A low curie exposure with long exposure time was selected, i.e. an exposure of three curies for three weeks. This exposure produced sufficient activity to be readily detectable with the detection equipment discussed earlier, and was usually in the range of .2 millicurie per milligram of heparin. The exact exposure procedure can be found in Section III A.

To determine the activity of the tritiated heparin, a modified "protamine titration test" was used (128).
Samples of various protamine sulfate concentrations were mixed with a tritiated heparin solution of known concentration. An aliquot of the mixture was added to fresh plasma with subsequent thrombin time determinations. When the protamine sulfate exceeded the tritiated heparin present, the thrombin times were nearly normal. As the protamine sulfate present fell below the heparin level, the thrombin time lengthened, hence giving a distinct break in the curve and an accurate estimate of the original tritiated heparin activity. A typical curve for the Wilsbach type heparin used for this study can be seen in Figure 4-1. From this figure one can see that the anticoagulant activity of the tritiated heparin was at least in excess of 80% of the original and no doubt much closer to 100%, considering the break point or neutralization point.

One additional precaution which must be considered with tritium isotope studies is the ability of heparin's tritium to exchange with the hydrogens of water. The extent of this transfer can be witnessed in Figure 4-2. This figure shows the transfer of tritium from irreversibly adsorbed heparin to water molecules in the presence of agitation and during stagnation. The heparin was irreversibly bonded to cationic electrodialysis membranes supplied by the Ionics Corp. of Cambridge, Massachusetts. Figure 4-2 shows that with agitation about 85%
of heparin's original tritium is lost within 15 hours to water.

With no agitation, about 50% is lost in 34 hours. When exposed to air only, with 60% relative humidity, little or no tritium transfer from the heparin occurs in 30 days.

B. GBH Wall Shear Measurements

As mentioned earlier, the object of this wall shear study was to determine conditions under which heparin transferred from the GBH surface into solution. This would certainly shed light on the utility of GBH surfaces in extra-corporeal designs.

The heparin on the spindle before shear was determined with duplicate flat plates through the use of tritiated heparin. The experimental procedure was discussed in Section III-B-1. A typical surface adsorption curve with the tritiated heparin can be found in Figure 4-3. The surface adsorption is quite rapid and with a Lucite-GBH-surface a total heparin adsorption of $2 \mu g / cm^2$ is consistently observed.

The effect of wall shear with fresh platelet-poor-plasma and saline on the surface heparin of the GBH surface can be seen in Figure 4-4. In these studies the surface heparin was always fresh non-radioactive heparin. The wall shear of 9.4 dynes/cm$^2$ corresponds to the maximum wall shear, observed at the cannula, of the extracorporeal heart-lung machine circuit. A wall shear of 2.0 dynes/cm$^2$ corresponds to the normal wall
Wilsbach Procedure

500 mg exposed to 3 curies at 27 °C for 3 wks at 250 mm H₂

FIGURE 4-1 ANTICOAGULANT ACTIVITY OF TRITIATED HEPARIN
FIGURE 4-2 TRANSFER OF TRITIUM FROM HEPARIN TO WATER

Contact Time With Water, Hrs.

Tritium Exchange from Heparin to Water, % Original

- Agitated H₂O
- Stagnant H₂O

20°C
FIGURE 4-3  TYPICAL SURFACE ADSORPTION CURVE WITH H₃-HEPARIN
- 20°C -

○ - Fresh Plasma, Wall Shear 19 Dynes/cm²
△ - Fresh Plasma, Wall Shear 9.5 Dynes/cm²
□ - Fresh Plasma, Wall Shear 2.0 Dynes/cm²
■ - Fresh Plasma, Wall Shear 0.0 Dynes/cm²
□ - Saline, Wall Shear 9.5 Dynes/cm²

FIGURE 4-4  EFFECT OF WALL SHEAR ON GBH SURFACE HEPARIN

158
shear at the cannula of the artificial kidney circuit. The temperature for these wall shear studies was kept at 20°C rather than 37°C to reduce the possibility of plasma degradation.

The data of Figure 4-4 demonstrate a definite, interaction between the GBH surface and fresh plasma, but not between the GBH surface and saline. Considering the aforementioned heparin-protein interactions in solution and their postulated extension to heparin surfaces, the shear results with plasma were encouraging. However, as far as the use of GBH surfaces in extracorporeal circuits is concerned, the opposite is true. One can surmise from this data that the GBH surfaces might be expected to provide patency in regions of low shear by either a slow loss of heparin into the blood boundary layer or a build up of adsorbed blood components onto the GBH or graphite surface as the heparin is slowly leached from the surface. This leaching might provide the necessary anticoagulation during the initial adsorption period. If the blood flow through the prostheses is low enough, residual heparin might remain on the GBH surface, providing an antithrombogenic effect. In regions of high shear the GBH surface can be expected to quickly lose its initial heparin and hence show tendencies toward increased clotting over that of the low shear regions.

The weak link in the GBH surface appears to be the physical bond between the benzalkonium surfactant and the graphite.
Ionic's cationic membranes with similar quaternary ammonium groups were found to retain heparin in the presence of plasma at 37°C, and data to be discussed later indicates heparin is irreversibly bonded to the surface if the link between the quaternary ammonium and the substrate is chemical in nature.

If the physical link is indeed the weak one in the GBH surface, radioactive benzalkonium studies would be expected to indicate a loss of over 30% to 40% with implantation for greater than 24 hours.

C. Heparinization of Regenerated Cellulose Film

The previous results with the GBH surfaces suggest two possible mechanisms by which these surfaces might attain their alleged antithrombus properties: surface heparin release or long term surface heparin retention, although less than the initial surface load. If the latter plays a significant role in imparting the thrombus resistant properties to these surfaces in conditions of low wall shear, compatible surfaces are a real possibility. To ascertain the importance of an irreversibly adsorbed heparin surface, an entirely new research material other than the GBH surface was required. An obvious candidate for this surface heparinization study was regenerated cellulose film. Its abundance, semipermeable properties, and permeability has made it an undisputed choice for the dialysis membrane of the past as well as current artificial
kidneys. Its versatility and well researched chemical properties combined with its practical importance made it a natural for chemical modification and hence heparinization. Since the weakness of the GBH surface apparently lies in the physical bond between the graphite and the surfactant, the foremost requirement was that all bonds between the cellulose substrate and adsorbed heparin surface be chemical in nature. Numerous authors (36,92,98) have reported that heparin is readily adsorbed on various anion-exchange resin, notably Ecteola cellulose - an aminated cellulose with a distribution of amine groups from primary to quaternary, consequently the overall heparinization of the film was envisioned to encompass a surface amination of the cellulose film followed by a heparin adsorption to the basic amine sites on the film. Such a reaction scheme would guarantee that the heparin be chemically bonded to the surface, providing a system to study the interaction of an irreversibly heparinized surface with human blood.

Before discussing the various amination techniques for regenerated cellulose film, two other a priori requirements that must be imposed on these techniques must be noted. A successful amination technique must provide a uniform distribution of basic sites in sufficient number to provide at least one complete surface layer of heparin with practically no
cellulose substrate exposed, and must cause a minimum of sub-
strate degradation in order to preserve the original proper-
ties of the film.

A number of aminated cellulose derivatives have been syn-
thesized with two etherification processes. One of these in-
volves the action of chloroalkylamines upon alkali cellulose,
while the other is based on the reaction of the latter with
aminoalkyl hydrogen sulfates. The former reaction can be
represented by the equations

\[
\text{Cel-OH} + \text{NaOH} \rightarrow \text{Cel-OH-NaOH} \quad (15)
\]

\[
\text{Cel-OH} \cdot \text{NaOH} + \text{Cl} \cdot (\text{CH}_2)_2 \cdot \text{NRR'} \rightarrow \text{Cel-O-(CH}_2)_2 \cdot \text{NRR'} + \text{NaCl} \quad (16)
\]

where the alkali cellulose is represented by Cel-OH \cdot \text{NaOH} and
R's stand for alkyl groups (37).

The reaction of alkali cellulose with organic sulfates is
an alternative method for preparing cellulose aminoethers. Reac-
tions of this type can be represented by the equation

\[
\text{Cel-OH} \cdot \text{NaOH} + \text{HO} \cdot \text{SO}_2 \cdot (\text{CH})_2 \cdot \text{NRR'} \rightarrow \text{Cel-O(CH}_2)_2 \cdot \text{NRR'} + \text{NaHSO}_4 \quad (47).
\]

Although this latter reaction as well as the chloroalkylamine
method is complicated by the decomposition of the reagent by the
sodium hydroxide present, Jakubovic (50) was able to achieve a
nitrogen content of 1 to 2% when aminating wood cellulose.
This amounted to an exchange capacity of from .5 to 1.0 meg/gm
dry exchanger compared to 3 to 5 meg/gm for commercially avail-
able exchange resins. Jakubovic (50) also observed that chloroethyl-diethylamine and aminooethylhydrogen sulfate produced the highest degree of amination.

Jakubovic's conditions for the reaction of chloroethyl-diethylamine with alkali cellulose were followed for the amination of one mil thick regenerated cellulose film, and found to dissolve the film. Modification of his procedure finally did provide a technique whereby the film could be aminated and maintain its structural properties for the most part. The method consisted of soaking the film in 30-50% 2 chloroethyl(diethylamine - HCl for 30 to 60 minutes, followed by a brief drying period and immersion in 50% sodium hydroxide solution at 100°C for 1 minute. The membranes were then washed and refluxed with methylidode prior to heparinization, or simply heparinized. The high temperature sodium hydroxide treatment caused most of the severe damage to the film structure, but was necessary to obtain even the slightest adsorption of surface heparin. This sodium hydroxide treatment represented an optimum between surface amination and film structure because a decrease in temperature permitted longer contact times but gave practically no amination, while increased contact time at the higher temperature caused severe structural damage through the penetration of the sodium hydroxide into intracrystalline regions. This penetration resulted
in a gel-like structure with minimal strength.

Under the optimal conditions described above for the reaction of chloroethyl-diethylamine hydrochloride with alkali cellulose, a maximum adsorbed surface heparin layer of $0.33 \mu \text{gm/cm}^2$ was attained. Since this represents almost a ten-fold decrease in surface heparin from that of the GBH surface, no human blood coagulation tests were performed on the surface and alternate amination procedures were studied.

The amination technique utilizing the reaction of aminoethylhydrogen sulfate and alkali cellulose was found also to be heavily dependent on the preparation procedure for the alkali cellulose (91). Again high sodium hydroxide temperatures were required and optima reaction conditions gave a surface heparin coverage of $0.36 \mu \text{gm/cm}^2$ which again was considered inadequate.

A closer look at the interaction of the aminoethylhydrogen sulfate reactants gave an indication of a possible amination procedure which would not require the formation of alkali cellulose, and thus preserve the film structure to a much larger extent. In the presence of hot sodium hydroxide 2 aminoethyl hydrogen sulfate is converted to ethylenimine, bp $55^\circ$, which is a toxic but useful reagent since it is known to react like ethylene oxide with substances containing active hydrogens. The formation of ethylenimine via the above reac-
conceivably ethylenimine might be graft polymerized onto regenerated cellulose film to produce a surface aminated film with a high affinity for heparin. Such a reaction procedure has considerably more promise over the previous techniques because of the absence of sodium hydroxide. Before discussing the development of this amination procedure it might be helpful to digress briefly and consider the currently proposed mechanism of homogeneous ethylenimine polymerization. An understanding of this discussion will give insight into the heterogeneous graft polymerization between ethylenimine and the cellulose film.

Pure dry ethylenimine in the absence of a catalyst is very stable, even at 150°C. In the presence of acids,
alkylating agents, or water, ethylenimine has been observed to react at temperatures as low as 40° to 50°C. In fact the polymerization proceeds explosively if certain catalyst concentrations or certain temperatures are exceeded (53,59). Various bases which are known to catalyze the polymerization of ethylene oxide prove ineffective for the polymerization of ethylenimine.

Catalysts for the polymerization of ethylenimine, such as strong acids, boron trifluoride, 2 chloroethylamine, alkylating agents, benzyl chloride, dry carbon dioxide, silver and copper salts, weak acids and water, are capable of producing quaternary ethylenimmonium ions which in turn initiate the polymerization. The formation of ethylenimmonium ions is visualized as

\[
\text{RX} + \text{NH}_2 \rightarrow \text{RNH}^+ + \text{X}^-
\]

(20)

where R may be H.

Jones, et al, (54) suggested that the polymerization mechanism is an SN 2 displacement on the primary carbon of the ethylenimmonium or substituted ethylenimmonium ion. This mechanism can be represented by

\[
\begin{align*}
\text{H}_2\text{C} - \text{CH}_2 \text{NH}^+ \text{H}_2\text{C} - \text{CH}_2 + \text{H}_2\text{C} - \text{CH}_2 & \rightarrow \text{H}^+ \text{HN}^- \text{CH}_2\text{CH}_2\text{NHR} \\
\end{align*}
\]

(21)
The rate depends directly on the initial concentration of the catalyst and is initially of second order with respect to monomer concentration, but falls off markedly with increasing polymerization. This decrease in rate was observed not to be due to progressive termination by the anion, but is probably a result of the competition for protons between imine nitrogen and polymer amino groups. Therefore termination, i.e., deactivation of the reactive immonium ions, can be represented by

\[
\text{HN}^+ (\text{CH}_2\text{CH}_2\text{NH})_x \text{R} + B^- \rightarrow \text{N} \cdot (\text{CH}_2\text{CH}_2\text{NH})_x \text{R} + \text{HB}
\]

where B is any base, such as a monomer molecule, amino-nitrogen in a polymer, an anion, water, etc. Another possible termination mechanism might result from ring opening with an amino-nitrogen in the polymer molecule resulting in branched poly-ethylenimine. An example of this termination step could be the following
where the excess proton probably further reacts with the polymer to protonate one of the amino groups on the polymer. The possibility of the latter termination step immediately creates the further possibility of the additional reaction between two poly(ethylenimine) molecules. There is no reason why such a reaction should not occur, since it is no different from the polymerization of other N^− substituted ethylenimines. An example of this type addition might be

Thus equation (25) again leads to the distinct possibility of branched polyethylenimine, but because of the noted immobility of larger polymer molecules this latter branching and that resulting from the termination mechanism of equation (24) are probably prevalent only while dimers, trimers, or oligomers are present.

In summary, the homogeneous cationic polymerization of
ethyleneimine is exceedingly complicated, as can be seen from the innumerable reaction possibilities which arise from the above general type mechanisms. At best, the mechanism is poorly understood. The question of branching has thus far not been resolved, but consensus of opinion favors a relatively linear polymer with the possibility of oligomeric side chain branches. Regardless of the above uncertainties concerning the polymer structure, polyethyleneimine has one important well-defined characteristic: namely, the regularly repeating amino group which impart unusual and highly flexible ion exchange properties to the polymer. This regular spatial sequence of basic groups favors a firm overall adhesion of polyanions, since the individual bonds might be weak but their composite, due to the large number of individual bonds, is probably quite strong.

Having briefly discussed the homogeneous polymerization of ethylenimine, let us now turn our attention to heterogeneous graft polymerization of ethylenimine onto cellulose and regenerated cellulose films which comprises the initial step in the heparinization process.
Early amination studies of cellulose (18,25) with ethylenimine indicated that aminoethylcellulose with a nitrogen content of .5 to 1.8% could be reacted in the air-dried state with ethylenimine vapors at 70°C to give an aminocellulose with a nitrogen content of 5%. This amounts to an average of .7 atoms of nitrogen per anhydroglucose unit. Soffer and Carpenter (105) prepared aminoethylcellulose with nitrogen contents of 19 to 20% by reacting air-dried cellulose with liquid ethylenimine under pressure at 170°C. This gives a degree of substitution of 3.2. Hartman and Fujiwara (42) discovered a process for the production of aminoethylcellulose of over 20% nitrogen, which comprises the reaction of cellulose with ethylenimine in a non polar solvent in the presence of acid catalyst or organic halide containing active halogen atoms. The desired reaction temperature range was from about 90 to 130°C. At temperatures above 140°C in the presence of the acid catalyst much homogeneous liquid phase polymerization was observed as well as partial decomposition of the cellulose. All reaction systems of this technique are substantially water free during the amination, in contradistinction to the methods mentioned previously. In the former methods, air dried cellulose, which usually contains 6 to 10% water, was used as the starting material. In recent studies by Segal and Eggerton (102) little or no reaction between cellulose and dry ethylenimine - non
polar solvent solutions was observed at 80°C or 110°C. In the
presence of glacial acetic acid, molar ratio of 10:1 imine to
acid dissolved in refluxing benzene, ethylenimine was observed
to react readily with cellulose. With lower imine to acid
ratios, much homopolymerization appeared. This characteristic
necessitates a lower reaction temperature which in turn re-
results in a considerably reduced degree of cellulose amination.
One further pertinent observation of this study resulted from
X-ray diffractograms of imine-wet cellulose which showed that
ethylenimine does not penetrate the crystalline lattice of
cellulose.

From the above graft polymerization studies a few thoughts
concerning the mechanism can be derived. Graft polymerization
of ethylenimine onto cellulose appears to represent homopoly-
merization of ethylenimine in many aspects, which apparently
competes strongly for the monomer during graft polymerization.
The first step in the graft polymerization appears again to be
the formation of the immonium ion according to equation (20).
However, in the case of graft polymerization, the cellulose
hydroxyl groups furnish reaction sites for the immonium ion as
well as other ethylenimine monomers, amines, etc. Typical ex-
amples of the immonium-cellulose, substituted immonium-cellu-
lose, and immonium-aminoethyl cellulose reactions are repre-
sented by Equations 26, 27, and 28
The protons, $H^+$, on the right side of the above equations will probably react with the grafted polymer to form a partially protonated structure, thus leading to a decreased reaction rate with time even in the presence of excess ethylenimine monomers.

The exact characterization of the grafted imine polymer is again clouded by the innumerable reaction possibilities and its dependence on the heterogeneous reaction conditions. A few important generalities can be made, however, concerning this water soluble grafted polymer. Increased amination beyond 25% nitrogen greatly increases the solubility of the cellulose-imine polymer material in water. The grafted polymer cannot be removed from the cellulose by acids or by boiling alkalis. (16) Such a reaction enhances the anion exchange capacity of cellulose and has a high potential for uniform surface coverage because of its branching and polymerization tendencies.
Considering now the actual heparinization of one mil (dry thickness) regenerated cellulose dialysis film of the type currently used in the artificial kidney, four a priori process requirements come to mind. The process must: 1) provide an irreversibly adsorbed surface heparin coat of at least one monolayer over the entire membrane surface, 2) provide a heparin layer similar to 1) in the membrane pore entrances and immediate subsurface amorphous regions, which have a high possibility of interacting with the plasma clotting factors of the intrinsic clotting system, 3) maintain as nearly as possible the permeability properties of the original regenerated cellulose film, RCF, and 4) maintain as nearly as possible the strength characteristics of the original RCF. With consideration of the current state of the art in ethylenimine chemistry four separate ethylenimine-RCF processes were studied in hopes of finding one which met the above requirements. In three of the processes ethylenimine vapors were used to aminate while the fourth used liquid ethylenimine in a non polar solvent, toluene. The reaction temperatures were maintained in the lower range of those reported in the literature to minimize degradation of the RCF, especially in the presence of acid catalyst. Water was used as the catalyst in two schemes, one with an ethylene oxide pretreatment and one without, while hydrochloric acid and ammonium chloride were used separately.
for the other two schemes. The water and hydrochloric acid were employed to swell the membrane, allowing pore entrances and admorphus regions near the surface to be aminated. These catalysts acted as the proton donor in the initial immonium ion formation reaction. The ammonium chloride-toluene-ethylenimine reaction process was based on the studies of Hartmann and Fujiwara (42) and comprised their optimum reaction conditions producing maximum surface graft polymerization with a minimum of homopolymerization. Since RCF does not imbibe toluene, this reaction process should produce maximum surface heparinization with minimum internal change. This, of course, will favor requirements 1), 3) and 4) and will demonstrate the importance of requirement 2). The logic behind the ethylene oxide treatment is a result of studies by Satkowski and Hsu (97) and Hartman and Fujiwara (42) who demonstrated that ethylene oxide would react with alcohols and cellulose. Polyoxyethylation resembles amination with ethylenimine in that both are a result of ring opening and produce graft polymerization. The ethylene oxide reaction with cellulose is base catalyzed rather than acid catalyzed and is represented as

\[
\text{Cel} - \text{OH} + \text{MOH} \rightarrow \text{Cel-OH-MOH} \\
\text{Cel} - \text{OH} \cdot \text{MOH} \rightarrow \text{Cel-O}^-\text{M}^+ + \text{H}_2\text{O}
\]  

(29)
where \( M \) usually represents Na or K. In the absence of a catalyst the reaction with cellulose is seen to proceed at a much reduced rate. With long reaction times the ethylene oxide is thought to react with secondary cellulose hydroxyls to produce primary hydroxyls, thus increasing the probability for a uniform surface amination with ethylenimine. Because of the deleterious effects of a base on RCF and the long reaction times required, water was chosen as the catalyst.

The quantity of surface heparin adsorbed with the above reaction schemes can be seen in Figure 4-5. The effect of moisture content and reaction temperature is quite evident. The reaction rate of ethylenimine with water swollen cellulose is more than an order of magnitude faster at \( 85^\circ C \) than at \( 70^\circ C \). The leveling off of the \( 85^\circ C \) surface heparin curve reflects the use of only one initial water loading while the \( 70^\circ C \) reac-
tion had two resoakings in water. As the imbibed water evaporates from the membrane, it probably interacts with the ethylenimine vapors to form ethanol amine according to the following equation,

\[ \text{H}_2\text{O} + \text{CH}_2\text{CH}_2\text{→HO} - \text{CH}_2\text{CH}_2\text{NH}_2 \]  

(31)

This leads to a drying of the membrane and thus a stoppage of the graft polymerization reaction, as can be seen from the lower 70°C curve. A reintroduction of water into the membrane results in renewed graft polymerization.

The ethylene oxide pretreated membrane appears to surface aminate to a larger extent than the untreated membrane in the same reaction conditions. This could very well result from the elimination or reduction of the steric exclusion between the 2 and 3 hydroxyls after amination and partial polymerization at one of these hydroxyls. With the ethylene oxide treatment at 25°C very little ethylene oxide graft polymerization will result, but because of the poly hydroxyl effect of the cellulose and long reaction times, a noticeable amount of surface etherification must have taken place. This etherification will definitely separate the 2 and 3 hydroxyls and therefore should result in less steric hindrance between these groups during graft polymerization with ethylenimine.

The liquid phase amination produces a surface with about
the same exchange capacity as the others, but seems to be a little slower in the initial phases. The acid catalyzed reactions, however, produced some unexpected results. The surface heparin was observed to level much more quickly with reaction time than with the other reaction schemes and at a value considerably below those of the other schemes. This could not be a result of evaporation of the imbibed water, for it does not correspond closely enough with the \( \text{H}_2\text{O} (\text{Initial}) - 85^\circ\text{C} \) curve. A depletion of ethylenimine must be ruled out, for it was added continuously during the reaction procedure.

The same leveling of surface heparin with reaction time was observed with different hydrochloric acid concentrations as can be seen in Figure 4-6. The leveling value is an inverse function of catalyst loading, which leads one to conclude that the acid must somehow hinder the graft polymerization after the rapid initial amination. A plausible explanation might be that the graft polymerization is stopped by the protonation of the already grafted amine groups by the acid present. Since the immonium ion would show very little tendency to react with a protonated amine, it would no doubt continue to react with the cellulose film only so long as free hydroxyls exist. With this early termination and forced cellulose-imine reaction, a more uniform surface amination would be expected subsequently producing a more uniform sur-
face heparinization.

A comparison of the total adsorbed heparin on the cellulose-imine-heparin (CIH) membrane with that of the polymethylmethacrylate - GBH surface or the cellulose nitrate - GBH surface shows that about 5 to 6 times as much heparin is adsorbed by the CIH membrane than by the GBH membranes. This comparison can be seen in a more explicit fashion in Figure 4-7 which shows the surface heparin as a function of ethylenimine reaction time for the CIH surface and as a function of the benzalkonium soaking time in case of the GBH surface.

Having such data the immediate temptation is to calculate the theoretical surface heparin required for a monolayer and compare. Such calculations can be meaningful only if the system unknowns are considered carefully and the inferences paced accordingly. In the case of the GBH surface three uncertainties come in mind; namely those related to heparin's molecular configuration, its surface orientation on the GBH surface, and the graphite surface conformation itself.

A discussion of heparin's configuration at present must be, for the most part, conjectural. However, the presence of $\alpha(1 \rightarrow 4)$ linkages (23,24,107,108) in heparin has prompted suggestions that it could possess a helical structure similar to that of amylose. The helical structure theory would be strengthened should heparin be shown to contain only $\alpha(1 \rightarrow 4)$
linkages.

A certain amount of rigidity might be conferred onto the helical structure by a small number of intramolecular sulfate bridges of the type \( \text{CH - NH - SO}_2 - \text{O CH} \) (23). The presence of these bridges would probably indicate a collapsed helical structure for heparin. Besides this helical structure two extremes were considered in the monolayer calculations. The heparin was considered to be either a random coil or an extended linear molecule.

Concerning the size of these molecular configurations, heparin's molecular weight was taken as 15000, which corresponds to the median in the reported range of 12000 to 18000 molecular weight.

The orientation of the heparin molecule at the surface is a complete mystery, but for the sake of the calculations two conditions were assumed. It was hypothesized that the extremes are represented by an on-end attachment of heparin molecule which would resemble a bristle-like structure or by an axis attachment giving a corduroy type surface. The actual GBH surface probably lies between these extremes.

The final consideration must be of the surface itself. The imbedded graphite particles can produce a surface with from two to four times the area of a planar surface because of the spherical shape of the graphite particles and variations
in their penetration depth. If the average portion of the exposed graphite particle resembles a hemisphere, then the total heparin adsorption area will be twice the measured planar area. If the particles do not penetrate deeply at all, the total graphite surface exposed could range up to four times the planar area.

Although the potential for errors in the calculated surface heparin values listed in Table 4-1 is quite high, it is refreshing to observe that those for the collapsed helical coil and experimental value are of the same order of magnitude. To draw other conclusions from the calculation is difficult except for the calculated random coil values, which show the most deviation. This possibly indicates a poor heparin description.

The five fold or greater increase in surface heparin for the CIH membranes over the GBH surface could possibly be due to one or a combination of two possible causes: 1) graft polymerization to such an extent that an average of five or more layers of heparin are adsorbed to the membrane surface, or 2) amination of the pore entrances and subsurface amorphous regions followed by penetration and adsorption of the heparin molecule into these regions. If a bristle structure for the polyethylenimine-heparin surface complex is assumed, the grafted polymer would need to have an average size of 70 to
0- 108 h EtO + EtI Vapor 85°C

Δ - H₂O + EtI Vapor 85°C (initial)

Δ - H₂O + EtI Vapor 70°C (initial)

■ - H₂O + EtI Vapor 70°C (initial, 36h, 92h)

□ - NH₄Cl + Tol. + EtI, Liquid 80°C

○ - 2NHCl + EtI Vapor 85°C (initial)

EtI-Ethylenimine
EtO-Ethylene Oxide
RCF-Regen.Cellulose Film

FIGURE 4-5 EFFECT OF VARIOUS ETHYLENIMINE AND RCF REACTION SCHEMES ON THE QUANTITY OF ADSORBED HEPARIN

181
FIGURE 4-6  EFFECT OF CATALYST LOADING AND ETHYLENIMINE REACTION TIME ON THE QUANTITY OF ADSORBED HEPARIN
FIGURE 4-7 COMPARISON OF SURFACE HEPARIN - GBH VS. CIH PROCEDURES

- O - .2NHCl + EtI Vapor 85°C
- • - GBH - Cellulose Nitrate Soaking Time in Zep. Chlor. (.2%)
- □ - GBH - Polymethylmethacrylate Soaking Time in Zep. Chlor. (.2%)
<table>
<thead>
<tr>
<th>Heparin Configuration</th>
<th>Surface Heparin Orientation</th>
<th>Graphite Surface Conformation</th>
<th>Theoretically Adsorbed Heparin (Monolayer) ($\mu qm/cm^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extended Linear Molecular Configuration</td>
<td>On End</td>
<td>Planar</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemisphere Exposed</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sphere Exposed</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>Planar</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemisphere Exposed</td>
<td>.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sphere Exposed</td>
<td>.2</td>
</tr>
<tr>
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**TABLE 4-1**  CALCULATED SURFACE HEPARIN (MONOLAYER) - GBH SURFACE
80 mers corresponding to a molecular weight of 3000 to 3500 to produce a five layer heparin adsorption.

Although regenerated cellulose dialysis tubing normally acts as a barrier to heparin, a minor penetration of up to 4 to 5 microns into the membrane by the tritiated heparin could be detected because of the tritium range. Both causes probably contribute to the higher surface heparin contents of the CIH membranes but their relative contributions depend on the reaction conditions. With water or ethylene oxide catalyzed vapor systems or the non polar solvent amination system, the conditions are in favor of increased surface polymerization and cause 1). On the other hand, with the acid catalyzed system, swelling of the membrane was observed as well as a leveling of the adsorbed surface heparin which indicates an earlier termination of graft polymerization. These observations tend to bias in favor of cause 2) which corresponds to one of the a priori traits desired for the process. Anticipating results to come, the acid catalyzed reaction scheme might plausibly produce a more compatible surface, if indeed a heparinized surface is compatible.

More evidence in support of an early termination of the graft polymerization by protonation of the grafted amine with the acid catalyst is presented in Figure 4-8. The total nitrogen content, as obtained by micro-Kjeldahl, can be seen as a
function of reaction time for the various catalysts. Converse of that for the surface heparin, the nitrogen content is seen to increase with the acid catalyst concentration, indicating a more uniform surface amination and possibly a deeper penetration into the membrane. The acid catalyst produces significantly more amination than water and the liquid system during the observed reaction times. The lowest acid concentration produced about the same degree of amination as the ethylene oxide pretreatment.

An idea of the nitrogen distribution throughout the membrane can be derived from Figure 4-9. The surface heparin is plotted versus the nitrogen content. Again the non polar solvent and the water catalyzed amination processes react preferentially with the surface initially, but as the reaction continues the ethylenimine diffuses into the membrane and reaction proceeds both at the surface and in the interior. The toluene-ethylenimine system shows the strongest dependency between surface heparin and nitrogen content denoting a predominate surface reaction. The opposite appears to be the case for the acid catalyzed schemes. The surface heparin remains constant during further amination after an initial reaction period. This again lends support to the surface termination theory that the surface amines are believed to become protonated and act to repel the immonium ion rather than re-
FIGURE 4-8 AMINATION OF RCF USING ETHYLENIMINE WITH VARIOUS CATALYSTS
FIGURE 4-9 DISTRIBUTION OF AMINO NITROGEN WITH VARIOUS REACTION SCHEMES

Reaction Schemes 85°C

- NH₄Cl + Tol. + EtI(Liq) (80°C)
- H₂O + EtI Vapor
- 0.04NH₄Cl + EtI Vapor
- 0.2N HCl + EtI Vapor
- 0.4NH₄Cl + EtI Vapor
- Adsorbed PE I

--- EtO Pretreatment

Nitrogen Content, Wt. %

Surface Heparin, \( \mu \) gm/cm²
act with it, thus reducing the prospect of further extension of the grafted polymer.

The amination of the ethylene oxide pretreated membranes appears to be primarily a surface reaction, as can be seen from Figure 4-10. This is not surprising, considering that the ethylene oxide pretreatment was primarily a surface reaction because of its low moisture content and the very low diffusion rate of ethylene oxide in air dry cellulose. An additional factor which contributes to the situation is the low moisture content during the ethylenimine vapor phase reaction. The intent of this scheme was to confine the imine reaction principally to the surface to minimize substrate alteration. This can only be considered moderately successful, for nitrogen contents of up to 10% were attained, probably indicating more than just surface reaction.

If a minimum of polymerization is assumed, a 10% nitrogen content represents a degree of substitution of 1.75. With the acid catalyst concentrations of .04N, .2N and .4N the surface heparin was found to level at about 8.7%, 13% and 16.5% nitrogen, respectively. These values correspond to a possible maximum substitution of 1.4, 2.5 and 3.8 moles of ethylenimine per unit of anhydroglucose. (The calculated relationship between the degree of substitution, moles of ethylenimine to unit of anhydroglucose, and the nitrogen content is displayed in Fig-
ure 4-11.)

Since these substitution values represent a maximum only, to determine the extent of the internal amination is impossible until the extent of the graft polymerization is more fully understood. Internal amination, of itself, can not be generally labeled as deleterious at this time. A study of certain membrane characteristics before and after heparinization will elucidate the effect of amination. To arrive at a compatible functioning membrane, a balance between compatibility and membrane properties must be reached.

To summarize, the amination reaction results indicate that at least 5 times as much adsorbed heparin is obtained with the CIH surfaces as with the GBH. In contradiction to the GBH surface this heparin appears to be irreversibly bonded, since little or no heparin could ever be removed by vigorous wiping.

The amount adsorbed is a function of reaction schemes and conditions. With the acid catalyzed reactions less surface amination but more interior amination was observed than with the water catalyzed schemes, ethylene oxide pretreatment, and the non polar solvent system. The latter are listed in increasing order of surface amination. The acid induced graft polymerization termination theory seems to explain the amination data but no conclusion can be drawn definitely until
FIGURE 4-10  DISTRIBUTION OF AMINO NITROGEN WITH ETHYLENE OXIDE PRETREATMENT
FIGURE 4-11 THEORETICAL SUBSTITUTION OF CELLULOSE AS A FUNCTION OF NITROGEN CONTENT
more is known about the extent of the graft polymerization. 

A priori the acid catalyzed reactions would tend to give better surface coverage and deeper penetration since the free hydroxyls on and in the cellulose film constitute the active sites if the grafted polymer becomes protonated. In water catalyzed systems the grafted polymer and cellulose hydroxyls compete for the immonium ions. Since the exact requirements for a heparinized surface are not known, the only recourse at this time is to study the effect of these surfaces, with varying degrees of graft polymerization, on whole human blood. The compatibility will have to be used as an indicator of surface coverage and further measurements on membrane strength and permeability will indicate the effect of amination on membrane structure.

D. In Vitro Compatibility Studies with Human Blood

The object of the in vitro compatibility studies with human blood was to ascertain the effects of irreversible surface heparinization on the foreign surface-human blood interaction. Prior to the actual performance of the blood coagulation tests, it was necessary to review potential artifacts and evaluate their importance. The primary consideration is the surface release of heparin, as in the GBH system. A small amount of heparin, $1\mu$gm/ml, is sufficient to alter the whole blood coagulation time by several minutes. One way to test the permanency
of the surface-heparin bond is to incubate citrated platelet-
poor or platelet-rich plasma with the surface for the expected
duration of the coagulation experiment and test the plasma for
the presence of heparin with the thrombin time.\textsuperscript{1} A negative
test for heparin with this procedure, as was the case with
the CIH surfaces, is sufficient to preclude the interference
of surface heparin by release from the surface, at least dur-
ing coagulation tests. Care must be taken in extrapolating
this zero wall shear data to predict CIH's performance in
artificial organs, where wall shears as high as 9.4 dynes/cm\textsuperscript{2}
can be experienced in the heart-lung machine. The shear dura-
tion is considerably longer than the previous incubation. One
way of obtaining a more meaningful evaluation of the irreversi-
bility of CIH's adsorbed heparin is to subject its surface to
the maximum heart-lung shear for a duration in excess of the
current clinical operation. The Couette shear cell of Section
III A was again used and the shear results with platelet-poor
plasma and saline are shown in Figure 4-12.

The shear results show no heparin loss from the CIH sur-
face for either fresh plasma or saline shearing solutions with

\textsuperscript{1} The thrombin time along with other routine coagulation
tests have been discussed previously in Section III-C of this
report. The reader is referred to this section if a more
thorough understanding of the coagulation tests is desired.
a wall shear of 9.4 dynes/cm² for a period of 15 hours. The thrombin time was normal for all sample aliquots from the shear cell, indicating the virtual absence of heparin in solution. These results had been expected since the H³-heparin of the CIH membranes could never be wiped or rubbed from the surface with a wet or damp cloth applied vigorously. In light of the tightness with which heparin is bonded to the surface at 20°C and its continued firm adhesion at 37°C incubation, the conclusion may be safely drawn that the heparin of the CIH surface is indeed irreversibly bonded for all practical biological purposes.

Again considering the GBH and CIH surface shear results, the failure in the GBH laminate seemingly must be the physical graphite-surfactant bond since the heparin-surfactant and heparin-protonated amine bonds are similar in nature. A question might be raised concerning the number of the latter bonds per heparin molecule versus the former and the effect of the difference, if it is significant. At this time neither surface structure is beyond the conjectural stage, but if the heparin release was a result of heparin-amine or surfactant severance, at least a minimum heparin release from the CIH surface would be expected. This is so because of the random adsorption of the heparin onto the CIH surface, which dictates a distribution with a finite number of heparin molecules having
a similar overall heparin-to-nitrogen bond strength as those of GBH surface.

The second and final group of potential artifacts to be studied were related to the amination reactants and their reaction by-products. The reactants of particular interest are ethylenimine and polyethylenimine. To ascertain their effect on human plasma, aliquots of varying concentrations of the commercial materials were added to plasma and tested for coagulation. Three different coagulation tests were employed to provide information on the nature of the interaction as well as the specific position of interference in the coagulation mechanism. The results of the coagulation tests as a function of impurity concentration in parts per million can be seen in Figures 4-13, 4-14, and 4-15.

The ethylenimine used for these impurity studies was the same as that used for the cellulose modification, while the polyethylenimine, PEI, was supplied by the Dow Chemical Company of Midland, Michigan, and the L79 organo-silicone cationic surfactant was supplied by Union Carbide Corporation of Tonawanda, N.Y.

Figure 4-13 shows that methanol has very little effect on human blood coagulation even in concentrations as high as 1000 PPM, while ethylenimine can be seen to prolong the intrinsic or cephalin clotting time starting at 20 to 50 PPM. Ethyl-
enimine also prolongs the extrinsic or prothrombin clotting
time as well as the thrombin time at concentrations near 200
PPM.

A more potent anticoagulant activity can be seen in Fig-
ure 4-14 for polyethylenimine of 1400 molecular weight and a
similar sized organo-silicone cationic surfactant, L79.
Figure 4-15 indicates that polyethylenimine of 6000 average
molecular weight has nearly the same anticoagulant activity
per unit weight as the 1400 average molecular weight PEI of
Figure 4-14. The 100,000 molecular weight PEI appears to
have less anticoagulant activity per unit weight than the
lower molecular weight PEI. Currently very little is known
concerning the size of the grafted PEI polymer, but this
author does not expect its molecular weight to exceed 6000
because of the amount of adsorbed heparin measured to date.
The practical importance of the data presented in the pre-
ceeding three Figures is immediately obvious. If the amin-
ated surfaces are not washed sufficiently prior to heparini-
zation and testing, artifacts in the form of PEI anticoagu-
lants will over-shadow the effect of heparinization. Because
of the high solubility of PEI in aqueous solutions, the wash-
ing solution was usually distilled water. Results to be dis-
cussed show that a washing time in excess of 120 hours at room
temperature is sufficient to remove all but minute traces of
PEI from CIH dialysis membranes.

Two methods are available to detect the presence of excess PEI impurity: an extended prothrombin time or an excessive erythrocyte sedimentation rate. The PEI impurity interferes with portions of coagulation system in differing degrees of severity. Assuming the cascade coagulation theory (9) is a valid representation of the coagulation mechanism, the anticoagulant activity of PEI appears to be inversely related to the clotting factor level, i.e. PEI affects the cephalin time at far lower concentrations than those showing an effect on the thrombin time. The PEI influence on the prothrombin time lies intermediate between that of the cephalin and thrombin time. However, since the cephalin time is more sensitive to smaller changes in the level of other clotting factors than is the prothrombin time, the latter was selected as an indicator of PEI presence.

Another less quantitative measure of PEI presence can be obtained by observing the erythrocyte sedimentation rate. This rate increases many fold in the presence of PEI or other synthetic high molecular weight polymers, but is insensitive to PEI concentrations below 200 PPM. Therefore, the best analytical tool for the measuring of PEI in plasma seems to be the prothrombin time.

With the heparin and PEI artifacts understood, attention
Shear Temperature 20°C

Total Heparin on Surface-
500 - 700 μgms

CIH Reaction Procedures
1) H₂O + 48h EtI Vapor 85°C
2) .2N HCl + 6h EtI Vapor 85°C

Fresh Plasma 9.4 Dynes/cm²
Saline 9.4 Dynes/cm²

FIGURE 4-12 EFFECT OF WALL SHEAR ON SURFACE HEPARIN OF CIH MEMBRANES
FIGURE 4-13 EFFECT OF ETHYLENIMINE AND METHANOL ON HUMAN PLASMA

- Ethylenimine
- Methanol
a - Cephalin Time
b - Prothrombin Time
c - Thrombin Time

Normal Cephalin Time
Normal Prothrombin Time
Normal Thrombin Time

Third Point off scale
FIGURE 4-14  EFFECT OF POLYETHYLENIMINE AND CATIONIC SURFACTANTS ON HUMAN PLASMA
FIGURE 4-15 EFFECT OF POLYETHYLENIMINE ON HUMAN PLASMA
must be turned to the actual compatibility of the heparinized surface. CIH dialysis tubes were prepared by the four reaction schemes mentioned earlier and washed for at least 160 hours in agitated fresh distilled water prior to heparinization. This wash duration is sufficient to produce the required membrane cleanliness, i.e. freedom from diffusible PEI for all practical purposes, and is necessary to achieve normal prothrombin times with plasma incubated in the CIH tubes. The extensive washing time is no doubt required because of the slow diffusion of the PEI from the interior of the membrane and its tendency to adhere to the membrane surface. During the washing, care should be taken to avoid undue contact with the atmosphere in order to prevent the formation of cellulose-amine-carbonates and to avoid contamination with complexing metal ions such as copper which forms a strong bluish complex with the amine groups on the cellulose.

After the washing period the membranes were heparinized with a 100 to 200 Mg/ml aqueous heparin solution for two to five minutes followed by forty to fifty saline washes to remove any loose heparin. These washes have proven to be sufficient, for at no time has plasma incubated in the CIH tubes had extended thrombin times. Thus one can state conclusively that the heparin is irreversibly adsorbed by the converted cellulose.
The compatibility of the heparinized dialysis tubing was
determined by observing whole blood clotting times in the
tubes incubated at 35°C in a dry bath. The results of these
clotting tests are presented in Figure 4-16, plotted against
surface heparin. The normal whole blood clotting times for
glass, dialysis tubing with plasticizer removed, and iminized
cellulose dialysis tubing, can be seen on the ordinate to be
approximately 6, 20 and 30 minutes, respectively. The WBC
times for tubes prepared with only water as the catalyst or
with the toluene - NH₄Cl system are represented by the lower
curve which is seen to level off at a WBC time of around 60
minutes. The CIH surface resulting from these processes is
envisioned to have large PEI units attached to the cellulose
surface.

A minimum of internal amination should exist: thus
leading to a situation where either certain unreacted areas
are exposed on swelling in aqueous blood, possibly pore entran-
ces, or the long chain PEI is wrapping around or bending over
to cover most of the active sites on the heparin molecules.
If the latter be the case, it should be relieved somewhat by
increasing the soaking heparin concentration. Such an increase
in heparin would increase the probability of more heparin mole-
cules being adsorbed per grafted polymer by decreasing the pos-
sibility of multiple imine-heparin links per heparin molecule.
This should result in more heparin active sights being exposed to the blood. The data to the far right of the lower curve represent the increased heparin concentration. An improvement in WBC times results from the increased heparin concentration but still represents only marginal improvement over existing materials. One interesting point, however, is that the WBC time seems to be independent of the surface heparin at this stage, which no doubt indicates a significant portion of the surface is not heparinized. The surface heparin values for the asymptotic portion of the curve were acquired by $H^3$-heparin delution and measurement. Because of the inherent errors in such a technique, it is necessary to be skeptical of the exact surface heparin values, although the above thoughts concerning the unreacted-site-theory and multiple-link-adsorption still seem valid.

The middle curve and that nearest the ordinate of Figure 4-16 represent the ethylene oxide pretreatment process and the .04N hydrochloric acid catalyzed process, respectively. The WBC times for the ethylene oxide pretreatment process seem again to show a leveling tendency with increasing surface heparin, but at a much higher compatibility level than that of the water catalyzed process. The significance of this leveling will be discussed later. A similar leveling was not observed for the WBC times with the .04N HCl process, at least within the
range of observed WBC times of this study. The most compatible membrane produced with the .04N HCl process had a WBC time of 90 minutes.

When the concentration of the acid catalyst was increased to .2N and .4N HCl the results were encouraging. WBC times in these instances were consistently above 110 minutes with surface heparin approximately 10 to 12 \( \mu \) gm/cm\(^2\). The data of Figure 4-16 indicate amination of RCF with ethylenimine in the presence of .2N or greater hydrochloric acid with subsequent heparinization produces a surface with a powerful antithrombogenic effect. As was shown earlier this effect is not a result of loss of surface heparin. Similar surfaces produced with alternate catalysts show an antithrombogenic tendency but not as pronounced as that with higher concentrations of acid catalyst. The leveling tendency of WBC times with surface heparin, observed with water as a catalyst, might be a result of coagulation initiated by unreacted cellulose surfaces regions.

E. CIH Membrane Surface Microphotographs

To elucidate more fully the role of the acid catalyst and to substantiate the unreacted-surface-theory mentioned above, microphotographs were prepared of the membrane surfaces. The samples were shadowed with chromium prior to photographing to bring out in more detail the surface irregularities. Microphotographs of particular interest to the compatibility studies
are presented in Figures 4-17 to 4-22. Before discussing the significance of these photographs, a brief introduction to some recent cellulose crystallization studies might prove of value.

Considering the small size of the crystalline regions (crystallites or micelles) as measured from the width of the X-ray reflections, the fringe micelle theory was proposed as a morphological model for partially crystalline cellulose. This model implies that long cellulose chains are running through several crystalline micelles which are surrounded by disordered (amorphous) regions. This model appears to apply to commercial types of regenerated cellulose, i.e. cellophane and dialysis tubing. In fact, the fringe micelle concept has been gradually applied to crystalline synthetic polymers of linear chain structure, i.e. polyamids, polyesters, and polyethylene.

In recent years many types of linear polymers have been successfully obtained as lamellar single crystals of microscopic size (10 μ to 100 μ) when they are crystallized slowly, usually from dilute solutions (30,83,89). The same has been demonstrated for regenerated cellulose by Raney and Noe(90) in 1961 for cellulose from aqueous solutions of soluble cellulose acetate at 90°C and pH 8.0. They observed in the optical microscope that the cellulose particles had lamellar struc-
tures. In the early stages of the crystallization, the particles were thin and showed no birefringence. The particles grew gradually larger and thicker until they finally developed into slightly birefringent, somewhat irregular, diamond-shaped crystals of 20-30μ width. The gradual thickening of the crystals and the growth lines observed indicate these crystals are also lamellar and formed principally in the same manner as the single crystals of synthetic polymers.

These past researches aid greatly in clarifying the microphotographs of this study. Figure 4-17 A and B show that crystalline regions of about 40μ in length are present on the surface of the regenerated cellulose dialysis tubing in large numbers. Earlier mention was made that because of the unavailability of the cellulose hydroxyls in the crystalline region, these regions have been observed to be much less reactive than the amorphous regions. The earlier discussion pertained to the micelles of the fringe micelle theory, but should be equally true for the single crystals observed on the dialysis film. This thought adds another dimension to the surface heparinization difficulties which could completely overshadow the quantity of surface heparin. In keeping with the unreacted-surface-theory of an earlier section, these crystals if present in sufficient number in the final CIH surface could very well be the unheparinized surface which initiates coagulation. The remaining microphotographs do indeed show
Normal Prothrombin & Thrombin Times were observed with plasma incubated in duplicate tubes.

**Figure 4-16** COMPATIBILITY OF HEPARINIZED CELLULOSE DIALYSIS TUBING (CIH) WITH HUMAN BLOOD
FIGURE 17-A

Material:
#20 Dialysis Tubing,
Union Carbide Corp.

Magnification:
450 X

WBC Time
20 minutes

FIGURE 17-B

Material:
Same as Fig. 17-A

Magnification:
100 X

WBC Time
20 minutes

FIGURE 4-17 MICROPHOTOGRAPHS OF REGENERATED CELLULOSE MEMBRANE SURFACE
FIGURE 18-A

Material:
RCF + H₂O + EtI Vapor
85°C, 13h

Magnification:
450 X

WBC Time:
30 minutes

FIGURE 18-B

Material:
RCF + H₂O + EtI Vapor
85°C, 48h

Magnification:
150 X

WBC Time:
50 minutes

FIGURE 4-18 MICROPHOTOGRAPHS OF CIH MEMBRANE SURFACE, I.
FIGURE 4-19A

Material:
RCF + H₂O + EtI Vapor,
85°C, 108h

Magnification:
450 X

WBC Times:
60 minutes

FIGURE 4-19B

Material:
RCF-EtO (108h), EtI Vapor 85°C, 8h.

Magnification:
150 X

WBC Times:
60 minutes

FIGURE 4-19 MICROPHOTOGRAPHS OF CIH MEMBRANE SURFACE, II.
FIGURE 4-20A

Material:
RCF- .04N HCl + EtI vapor, 85°C, 12h

Magnification:
100 X

WBC Time:
60 minutes

FIGURE 4-20B

Material:
Same as Fig. 4-20A

Magnification:
450 X

WBC Time:
60 minutes

FIGURE 4-20 MICROPHOTOGRAPHS OF CIH MEMBRANE SURFACE, III.
FIGURE 4-21A

Material:
RCF + .2N HCl + EtI
Vapor 85°C, 12h

Magnification:
100 X

WBC Time:
> 90 minutes

FIGURE 4-21B

Material:
Same as Figure 4-21A
but different sample

Magnification:
100 X

WBC Time:
> 90 minutes
FIGURE 4-22A

**Material:**
RCF - Same as Figure 4-21A

**Magnification:**
450 X

**WBC Time:**
> 90 minutes

FIGURE 4-22B

**Material:**
RCF - #8 Dialysis Tubing + .2N HCl + EtI Vapor
85°C, 12h

**Magnification:**
450 X

**WBC Time:**
Not Performed

FIGURE 4-22 MICROPHOTOGRAPHS OF CIH MEMBRANE SURFACE, V
the presence of the crystals in CIH surfaces which were partially compatible, and the complete absence in CIH surfaces which have been found compatible for 110 minutes or longer.

Figures 4-18A, 4-18B, and 4-19A show a decided increase in the density of surface crystals with increasing amination duration. This crystallization might well be expected since the membrane is being dried and annealed at 85°C for a period of 108h in the presence of EtI. Such an increase in surface crystallization along with a slow amination reaction would produce more unreacted surface area, although the surface heparin level might rise. To predict a priori the effect of this situation on the overall compatibility picture is difficult but in this study little if any improvement was noticed with further amination beyond 36-48 hours with water as the catalyst.

With the ethylene oxide pretreatment and the .04N HCl catalyst the amination times were of short enough duration to prevent additional crystallization, but the processes were not of the nature to remove the original crystals. It is felt that again these crystals, see Figures 4-19B, 4-20A and 4-20B, were instrumental in producing the coagulation observed with these CIH surfaces. The halo effect seen in Figure 4-20A and 4-20B is probably a result of the chromium shadowing of a moat-like structure around the crystal. This moat, if not a shadowing artifact, might be related to the acid penetration into the
crystalline regions.

Additional and more conclusive proof concerning the effect of the surface crystals can be gathered from Figures 4-21A and B and 4-22A and B. The photographs are of three different CIH surfaces whose WBC times were all in excess of 110 minutes. The fourth surface, Figure 4-22B, was prepared with the same procedure as the above three but with a thicker substrate, #8 dialysis tubing, which has been used as an in vivo prosthesis material. The complete dearth of crystals on the first three CIH surfaces which were found to be compatible with human blood certainly lends credibility to the above unreacted-surface-theory and discussion. Figure 4-22B was included to caution against the wide-spread use of the acid catalyzed process for all types of cellulose substrates without regard to initial or final surface properties.

The dissolution of the crystalline regions by the hydrochloric acid appears to be an important co-reaction along with the immonium ion production. The dissolution no doubt is a result of acid penetration into the large crystals through structural flaws which are more prevalent in these larger crystals. Most strong acids and bases produce both intracrystalline or intercrystalline swelling of cellulose, although the former requires stronger conditions than the latter (83). The swelling is usually typed as limited or unlimited. The latter
results in solution of the cellulose while the former produces
a definite swollen compound. Usually strong acids such as
72% sulfuric acid and 83% phosphoric acid are required to
produce unlimited swelling of the intracrystalline regions. However, weak concentrations of strong acids, 0.2N HCl, in
the presence of oxygen and at high temperatures, 80-900C,
will produce severe degradation of the cellulose structure.
In the absence of the oxygen the degradation is not as severe,
but still pronounced. With the oxygen present the cellulose
is subject to general oxidation, while with .2N HCl at high
temperatures the acid-sensitive 1→4, β-glucosidic linkages
are readily attacked and hydrolyzed, thus the average molecu-
lar weight of the cellulose and RCF strength are reduced(76).

In this acid catalyzed amination the reduction of mem-
brane strength because of acid presence at high temperature
can be reasoned to be less severe than the above because of
the competition for the proton between the imine, grafted
amine and glucosidic linkage. Since the acid catalyst is a
necessity with the currently available dialysis tubes or mem-
branes, it was necessary to determine experimentally the ef-
fect of acid catalyst on the CIH membrane structure. The
membrane stress-strain characteristics were measured and will
be discussed later. Clearly then, a balance between membrane
strength and compatibility is a prerequisite to a successful
application of the membrane.

Before continuing with the discussion of membrane structure let us turn our attention back to the surface heparin-blood interaction and consider a possible mechanism by which heparin could render surfaces more compatible with blood.

F. Proposed Interaction Between Surface Heparin and Human Blood

For many years prior to Lister's classical paper in 1863 and for the past one hundred years since its publication, many very able men have contributed their energies to understanding blood coagulation. The fact that normal blood remains fluid while contained in the vascular tree but clots quickly upon contact with foreign tissues or surfaces, has intrigued researchers for over a century and still continues to do so. To explain this phenomenon two general schools of thought have arisen, 1) the fluidity of the recirculating blood is maintained by a circulating anticoagulant which is overpowered by a stimulus produced by or derived from the foreign surface and 2) the intima of the vascular tree is of such high compatibility with blood that the delicate coagulation mechanism is not triggered by continued blood-intima contact. It would be a presumptuous overextension of the results of this study to claim a strong case for either of the above theories. However, a few interesting facts have arisen from the CIH in vitro com-
compatibility studies.

Additional *in vitro* data, Figure 4-23, shows the effect of the CIH tubes on fresh whole human blood. Fresh human blood was incubated in CIH tubes for varying durations and then transferred and incubated in glass test tubes. The WBC times in the glass tubes were observed and are displayed in Figure 4-23. Blood incubated in CIH tubes for 30 minutes was observed to clot fully in glass in about 15 minutes, however that incubated for 60 minutes showed only the formation of a partial clot when placed in glass. Whole blood incubated in the CIH tubes for 90 minutes was found to be permanently anticoagulated, which is suspected to be a result of the adsorption of plasma clotting constituents onto the heparinized surface. If Factor IX were involved in the adsorption, its level would need to be reduced to below 5 per cent (94) before the decreased activity would extend the WBC clotting time. Transfer of the clotting factors to the CIH surface was probably enhanced by the intermitant tilting of the tube during incubation. Concomittant with clotting factor adsorption might be the additional anticoagulant effect produced by the deactivation of Factor VIII, a labile precursor of the intrinsic system (94).
To test for the absence of clotting factors the CIH tubes must be as free as possible of diffusible by-products which will interfere with the sensitive cephalin or prothrombin clotting test but not the WBC test. Thus thorough washing of CIH tubes is required prior to contact with fresh citrated plasma, used to determine the adsorption deficiency. If the plasma deficiency following contact with the CIH tubes is correctable with normal plasma one can assume a minimal amount of anticoagulant is present. Figure 4-24 shows the effect of the CIH tubes on fresh citrated plasma with only 20 hours of washing. The PEI impurity can be observed to extend the clotting time of the intrinsic and extrinsic clotting systems. Since the clotting times are not correctable with normal plasma an anticoagulant effect is believed present.

The effect of an additional 100 hours of washing, following the amination, but prior to heparinization, can be observed in Figure 4-25. A small amount of impurity could still be extracted from the membrane on the first two plasma incubations but was on the order of 10 PPM or less, since the prothrombin time was not extended with either incubation. The third incubation seemed only to produce a protein deficiency in the plasma, which was correctable with normal plasma for both the duplicate tubes. Fresh whole blood was then placed in the CIH tubes, following a saline rinse, and incubated for
90 minutes. The blood was then transferred to a glass test tube and incubated. No clot was formed in 35 minutes, but a partial clot did appear in less than three hours in contradiction to the permanent anticoagulation observed with the earlier 90 minute incubation. Judging from the diverse WBC times for tubes giving the same PEI impurity effect and the lack of a PEI impurity effect with the above tubes having a WBC time in excess of 90 minutes, the presence of minimal amounts of PEI, >120 hour washing, can be assumed to have a negligible effect on the WBC tests and the interpretation of the overall compatibility data. However, the impurity can be bothersome when trying to relate loss in intrinsic system activity to CIH surface contact.

The partial anticoagulation observed after the above 90 minute incubation of fresh whole blood in the thrice plasma contacted CIH tube could be a result of 1) additional adsorption of clotting factors onto the heparin surface, 2) degradation of labile clotting factors required for normal coagulation or 3) metabolic degradation producing an environment alien to coagulation. To pinpoint the cause requires extensive in vitro testing with, hopefully, a system more amendable to these studies than the CIH dialysis tube.

However with 360 hours of washing prior to heparinization interaction between fresh citrated plasma and the CIH surface
For CIH Tubes:

0.2N HCl + EtI 2-12h
85°C, 160 h wash

FIGURE 4-23  EFFECT OF CIH TUBES ON FRESH WHOLE HUMAN BLOOD

WBC for Fresh Human Blood Drawn into Glass

Δ - Partial Clot
○ - Total Clot
Clotting times on plasma incubated in CIH tubes: 110 - Clotting times on 1 part incub. plasma to 1 part normal plasma

a - Kaolin-Cephalin times on incubated plasma
b - Prothrombin times on incubated plasma
c - Thrombin times on incubated plasma

Normal Kaolin-Cephalin Time

Normal Prothrombin Time

Normal Thrombin Time

FIGURE 4-24 INTERACTION OF CIH TUBES WITH FRESH HUMAN PLASMA - 20h WASHING PRIOR TO HEPARINIZATION
0 - .2N HCl, 2h EtI 85°C, 120h Wash
N - Not Correctable with Normal Plasma
1:1 - 1 part incubated Plasma to 1 part normal plasma

a - Kaolin-Cephalin time on incubated plasma
b - Prothrombin time on incubated plasma
c - Thrombin times on incubated plasma

Whole Blood Clotting time on tubes after 3rd incubation

WBC > 90' - into glass at 90', > 35' no clot, but did clot however < 3 hrs

FIGURE 4-25 INTERACTION OF CIH TUBES WITH FRESH HUMAN PLASMA - 120 h WASHING PRIOR TO HEPARINIZATION
FIGURE 4-26 INTERACTION OF CIH TUBES WITH FRESH HUMAN PLASMA
360h WASHING PRIOR TO HEPARINIZATION
was demonstrated to produce, see Figure 4-26, an extension of the intrinsic system clotting time which was correctable with normal plasma. This gives great credibility to the surface heparin-protein complex theory discussed above and indicates that the protein or proteins most likely to complex with the surface heparin are in the intrinsic system. Since the extrinsic system or prothrombin time was normal, the possible complexing candidates are limited to Factors VIII, IX, XI, and XII. Considering O'Brien's researches, Factor IX would be favored; however, this remains to be proven.

Another observation which tends to support the above theory concerns the neutralization of the GBH surface. In contrast to the in vivo results reported by Whiffen, et al. (118), a protamine sulfate rinse, 10 mg/ml, prior to contacting the GBH surface with fresh blood reduces the WBC time from greater than 10 hours to 36-40 minutes for the GBH surface versus 14 minutes for a graphite surface. The surface heparin is again shown to be active and can be neutralized with protamine. A possible explanation for the apparent contradiction between the in vitro results of this study and those of Whiffen et al. can be found in the test procedures. Whiffen studied the effect of an anti-heparin environment in vivo for a duration of two hours with a maximum protamine sulfate dose of 3 Mg/kg above the exogenous circulating heparin dose. Such in vivo
experiments are inconclusive since there is no way to estimate
the time required to diffuse the protamine to the GBH surface
and observations longer than two hours are required to detect
neutralization of the GBH surface. Studies with cellulose
acetate, CA, prostheses, to be reported later in this report,
indicate CA prostheses when placed in the thoracic inferior
vena cava require much longer than two hours to occlude. With
a WBC time of 36-40 minutes for the protamine-GBH surface com-
pared to 14-18 minutes for the CA surface, one would expect a
neutralized GBH surface to show less thrombus formation than
the CA prosthesis. Hence very little, if any, thrombus build-
up would be expected in two hours even if the GBH surface were
completely neutralized by protamine sulfate.

Additional evidence of surface heparin activity results
from the observation of lengthened thrombin clotting times in
irreversibly heparinized and well rinsed tubes. The extended
thrombin times appear to be a consequence of surface heparin-
thrombin complexing which reduces the potency of the thrombin
in solution. A similar type complexing has been noted for
heparin and thrombin in solution (9, p.103).

An altogether different approach to the surface heparin
interaction phenomenon involving adsorption studies with radio-
active plasma can offer valuable backup information concerning
the previous theory. Tritiated plasma was prepared by subject-
ing lyophilized human plasma to the Wilsbach treatment for three weeks at 3 curies. The tritium labeling is general and hence by itself it can only demonstrate the activity or passivity of the heparin surface. This serves as a critical test of the adsorption theory and should do much to substantiate or dispel the current theory that views heparin, by itself, as a completely passive surface.

The results of this study presented in Figure 4-27 certainly enhance the previous surface heparin-complex theory, for the heparinized surface is seen to combine quite strongly with a plasma component or components. The adsorption onto the heparin surface levels off after 30 minutes of vigorous agitation indicating a saturation of the surface with plasma components. The total level of adsorbed plasma components, believed to be proteins and probably clotting factors because of experience with heparin isolation techniques (98,99) and previous in vitro data, is very nearly equal to the weight of heparin adsorbed per cm$^2$. Cellulose, silicone rubber, and cellulose-imine surfaces adsorb plasma components to a varying degree but not in the same quantity as the heparinized surface. CIH surfaces which are contacted with protamine sulfate prior to tritiated plasma contact are seen to adsorb about half the quantity of plasma components as the untreated CIH membrane. This might be a result of partial removal of the
protamine from the heparin surface or a result of adsorption onto the protamine surface. Regardless, these results are again consistent with the in vitro clotting data in that protamine does indeed have an effect on the heparinized surface. The same cannot be said for a CIH surface soaked in CaCl₂ prior to the H₃⁻-plasma contact. The plasma constituents are seen to replace the calcium counter ion from the heparin surface, thus this further indicates that the anticoagulant and compatible properties of the CIH membrane are probably not due to calcium chelation. The cellulose-imine surface is also seen to have about a third as much adsorbing capacity as the heparin surface. Both the silicone rubber and cellulose surface are seen to strongly adsorb plasma components, but to a much lesser degree than the heparinized surface.

In summary, this study indicates through two separate approaches that a heparinized surface is indeed active, at least initially. In addition, the CIH surface was found to extend the intrinsic system clotting time of fresh citrated plasma when incubated with the surface. Since the kaolin-cephalin clotting tests were used to test the intrinsic system, the extended clotting time indicates a deficiency in one or more coagulation factors of this clotting system. These clotting factors as well as the other coagulation factors are
FIGURE 4-27 ADSORPTION OF PLASMA COMPONENTS ONTO HEPARINIZED SURFACES

- ○.4N HCl EtI Vapor 12h 85°C, 3 wk H₂O wash, Hep 250 mg/ml
- □ Same as ○ except 30' soak 1.0N CaCl₂ & rinse
- ● Same as ○ except 30' soak protamine (10mg/ml) rinse
- ■ Same as ○ except no Heparinization
- ○ Silicone Rubber - Dow's medical grade silastic
- □ #8 Dialysis Tubing, Union Carbide Corp.
believed to be B-globulin proteins. Since the prothrombin
and thrombin times are normal for the incubated plasma ali-
quot, apparently the major coagulation deficiency must be in
the intrinsic system, although a certain amount of factor ad-
sorption from the rest of the coagulation mechanism cannot be
definitely ruled out. Normally intrinsic clotting factor ac-
tivities of less than 40 to 50% cause an increase in the
kaolin-cephalin time while an activity of less than 5% for
the same components is required to produce a long WBC time.
Possibly with saturation the surface complex might rearrange
with the formation of a stabler heparin-protein complex, pos-
sibly that between heparin and factor IX, considering the work
of O'Brien.

In the discussion thus far the heparin complexing com-
ponent extracted from fresh plasma has been alluded to be pro-
tein. This assumption is based mainly on the results of pre-
vious studies indicating that heparin preferentially complexes
with proteins and is readily found in nature in just such a
state (13,14,98). This complex is believed to be the result
of ionic bonding between the anionic heparin and the proteins.

Another indirect indication resulting from this study is
that certain coagulation factors, thought to be B-globulin
proteins as mentioned above, have reduced activities after con-
tact with heparinized surface, causing a deficiency in the clot-
ting mechanism. This deficiency can be readily corrected by the addition of normal plasma to the deficient plasma aliquot. It therefore seems reasonable to assume that the CIH surface heparin has the ability to complex with at least one of the coagulation factors and quite possibly with plasma components not now well understood.

With the initial anticoagulation by the heparinized surface having now been explored and hopefully understood, to evaluate the potential of these surfaces for long term antithrombogenic properties becomes necessary. Considering the protein adsorption theory discussed above, it seems unlikely that in vitro clotting tests could be used to elucidate these long term properties. This hypothesis arises from an extension of the adsorption theory to a completely saturated surface where the "protein" is postulated to be irreversibly adsorbed under normal plasma conditions in a non denatured manner. This heparin-viable "protein" complex produces an outer surface of great similarity to the actual blood itself, which, because of this property, would be thought to have a greatly reduced tendency to initiate clotting through the intrinsic system. The distinguishing feature of this surface is postulated to be its ability to adsorb "proteins" with little or no denaturing effect. This probably is a result of heparin's particular site configuration and type of active site. Although the
non-denaturing property of the CIH surface was not demonstrated specifically in this study, this property is demonstrated daily by the heparin molecule in solution. The neutralization of heparin with protamine sulfate is the case in mind, where the addition of protamine sulfate produces a return to normal hemostasis by allegedly replacing all or part of the "protein" in the heparin-"protein" complex. This release must produce a "protein" of sufficient activity to reinstate the clotting mechanism, thus leading to the belief that the "protein" must have been complexed with heparin in a non-denaturing manner. Due to the demonstrated similarity between CIH heparin and that in solution, the same non-denaturing properties would be expected to apply to the CIH surface.

Returning now to the thought of in vitro testing of these surfaces -- since it is known that a gaseous interface causes protein denaturing of blood, in vitro testing would present the difficulty of saturating the heparin surface and of conducting the clotting tests without excessive gaseous contact. Hence the gaseous denaturing effect would be present at all times to contaminate the results. In conclusion then, in vitro clotting tests apparently have great utility when studying the specific heparin surface-blood interaction, but should bow to a more natural system, such as in vivo testing, to ascertain the extent of the heparin surface's
long term compatibility.

Prior to discussing the preliminary in vivo studies with the heparinized surfaces mention should be made that although this study dealt exclusively with heparin, the possibility of producing the same effect with other synthetic or natural chemicals cannot be ruled out, i.e. heparinoids of the sulfonated dextran type.

G. Preliminary In Vivo Compatibility Studies in Canine Veins

Since the usefulness of the in vitro testing to determine long term compatibility is limited, as discussed in the previous section, the use of in vivo testing techniques must be substituted. To the chemical engineer this is analogous to the familiar pilot plant stage of chemical process development. Normally, in vivo compatibility testing consists of blood vessel replacement with a prosthetic conduit. The extent to which the prostheses stay patent for long periods of time is dependent on their design, surface compatibility, and location in the circulatory system.

Considering design, an ideal prosthesis should be fabricated so as to have a minimum of stagnation points and blunt obstructions. The entrance regions should be bevelled and all surfaces in contact with blood should be as highly polished as possible to provide smooth undisturbed flow patterns. Although many other specifications must be considered in the development
of long term synthetic vascular replacements, such as carcino-
genicity, and antigenicity, they need not be considered here
because the main purpose of these in vivo studies was to de-
termine the relative surface compatibility of heparinized test
sections (113). However, the importance of the prosthetic de-
sign and location in the circulatory system on the in vivo
clotting time should not be underestimated. Prosthetic sur-
face roughness no doubt has the tendency to decrease the in
vivo clotting time because of the possible damage to plate-
lets, thus aggravating the situation. In addition, stagnant
regions on the downward side of the raised roughness favors
thrombus. In these stagnant regions coagulation is probably
more easily initiated because of the longer contact time be-
tween the blood and the surface.

As mentioned earlier, the prosthesis location is of con-
siderable importance when evaluating the relative surface com-
patibilities among prostheses. Generally in experimental de-
sign it is desirable to have the control clot consistently in
a short period of time. Since a finite time is required for
the diffusion of contact factors to the foreign surface and
their activation by the surface, in vivo clotting times will
generally decrease with increasing contact time per pass. The
contact time per pass can be varied by prosthesis design or
vessel location. Judging from this, one would expect longer
in vivo clotting times, with similar prosthesis size and design, when the prosthesis is placed near the heart or in other regions of high blood flow.

For the sake of continuity it would be desirable to test CIH membranes in vivo. However, because of their permeability and thickness, it is difficult to use such an unsupported membrane for blood vessel replacements. An alternative prosthesis was developed consisting of a CIH dialysis tube lining the inside of a rigid teflon support tube and overlapping the ends of the teflon. Such a prosthesis allowed the blood to contact only the CIH membrane, but fluid pockets between the membrane and teflon wall were produced during the implantation, as a result of water diffusion through the membrane. These pockets produced flow obstructions, but blood flow through the prosthesis was possible in the absence of occlusion, if the amination scheme used only water as the catalyst. But, as could be seen from the in vitro data, this reaction scheme was undesirable for commercial regenerated cellulose dialysis tubing, probably because of surface crystalline irregularities. With the more desirable acid catalyzed amination, the membranes were found to swell to about twice their original thickness and to such an extent that the entire lumen of the prosthesis was obstructed by membrane. Other smaller sized dialysis tubing was not commercially available and required a two to three
month set up time to produce. This, of course, was contingent on a guaranteed large demand. At this time two alternatives were available: 1) select an adhesive which would permanently bond the CIH membrane to a suitable substrate, thus eliminating the swelling problems with the acid catalyzed CIH membranes or 2) choose an alternate material to regenerated cellulose film which can be surface heparinized by a process similar to that for the RCF and which has the necessary water impermeability and strength required of the prosthesis. Cellulose esters are likely candidates for the second alternative, although any number of water resistant adhesives might be suitable for the first alternative. The screening program for the selection of the appropriate adhesive and its progress thus far will be discussed at the end of this section.

Cellulose acetate was chosen as the prosthesis material because of its low permeability and high strength characteristics. Also, future extensions of the heparinization procedure to deacetylated cellulose acetate thin films may be desirable. Preliminary work at this laboratory indicates the procedure is amendable to the acetate films. The deacetylated cellulose acetate films might be of future value as artificial kidney membranes because of their allegedly higher permeability to urea than RCF. For the prosthesis, however, it is desirable only to deacetylate the surface to retain as much of the sub-
strate properties as possible. The surface deacetylation provides the cellulosic type surface required for reaction between the substrate and the ethylenimine. Many reducing agents are available to perform the deacetylation—lithium aluminum hydride, excess of acid or base, acid hydrolysis with alkyl phosphoric acid esters, and alcoholic solutions of a sodium alkoxide, to name only a few (10, 44, 29).

A saturated solution of sodium methoxide was used for the surface reductions of this study and its reaction with the surface can be represented as

\[
\text{NaMeOH + O-CCH}_3 \rightarrow \text{ONa + CH}_3\text{OCCH}_3
\]

This reaction system was selected because of the rapid surface attack and limited penetration into the substrate. It is still not the ideal surface reducing agent because of the penetration of the methanol into the substrate during the time required for deacetylation. This penetration causes surface precipitation when the methanol is extracted during the water washing step.
This surface precipitation makes the cellulose acetate translucent rather than transparent as was the original.

Following the deacetylation and rinse, the prostheses are reacted with ethylenimine or pretreated with ethylene oxide and then reacted with ethylenimine. The ethylenimine reaction temperature must be kept in the range of 70 to 80°C to reduce the occurrence of stress cracking. The prostheses' walls are normally under considerable stress as a result of their fabrication from injection molded test tubes. Water and hydrochloric acid were again used as the ethylenimine catalyst. With the ethylene oxide pretreatment there was possibly a shallower penetration of the ethylenimine during the reaction, which produced a smoother surface following the final washing. Those surfaces with no EtO pretreatment were found to bubble up and split open during the final wash.

Figure 4-28 shows the effect of surface deacetylation and ethylene oxide pretreatment on the surface heparin adsorbed after 1.5 hours of ethylenimine reaction time at 73°C. Water was the catalyst for the upper curve while the lower curve was a result of pretreatment with ethylene oxide, followed by reaction of the air dried pretreated prosthesis with ethylenimine vapor. Figure 4-28 shows that in the presence of traces of water, ethylene oxide will catalyze the amination reaction, but not to the extent that water will. Figure 4-29 also shows
Material:
Tenn. Eastman - 042A

Surface Deacetylation Time, Minutes

- Sat. NaOCH₃, H₂O wash 20h, EtOH 24h
  EtI vapor 73°C 1.5h, vac. dry 60°C.

- Sat. NaOCH₃, H₂O wash 30h, EtI vapor
  73°C wet 1 1/2 h, vac. dry 60°C.

FIGURE 4-28 SURFACE HEPARINIZATION OF CELLULOSE ACETATE
Figure 4-29 EFFECT OF REACTION IMPURITIES ON PROTHROMBIN TIME OF PLASMA INCUBATED IN CAIH TUBES
that the penetration of the ethylenimine into the ester substrate is a function of EtI reaction time and EtO pretreatment. The extended prothrombin times on plasma incubated in the cellulose acetate-imine-heparin (CAIH) tubes is thought to be a result of leaching of reaction by-products or reactants from the substrate. The impurity is probably again polyethylenimine (PEI). If PEI is the impurity, one would expect its buildup in the substrate with ethylenimine reaction; such was the case. With the EtO pretreatment the substrate is air dried prior to reaction with EtI. This procedure appears to reduce the EtI penetration or possibly the in situ polymerization, to give a quicker impurity clean up time for the CAIH tube.

The problem of penetration can be seen even more graphically in Figures 3-9 and 4-32. With just ten minutes of deacetylation with the sodium methoxide-methanol solution, large blisters appeared on the CAIH surface following amination and washing. With 50 minutes of deacetylation the blisters were less pronounced but of the same order of magnitude. The thin films over the blisters have been observed to flake off, thus exposing the substrate. Surface microphotographs reveal the blister size in Figure 4-32. The out of focus portion of each photograph is a result of photographing half a test tube. These blisters and flaking are severe with the injection molded CA tubes but non existant with cast films.
The blister size can be reduced by more than an order of magnitude if the test tube surface is pretreated with ethylene oxide at room temperature for 36 or more hours. The improved surface can be seen in Figure 3-9 in comparison with the non EtO treated surface. EtO is thought to diffuse into the substrate and possibly react, but more importantly it seems to act as a plasticizer preventing surface rupture and blister formation. Although the EtO treatment is a vast improvement over the regular surface, additional improvements are necessary. A few words concerning these improvements will follow the in vivo results.

Prior to the testing of CAIH surfaces in vivo, whole blood clotting tests were performed on them as a rough screening check. The results of these in vitro tests are presented in Figure 4-30. A definite improvement in the WBC time seems apparent with additional deacetylation, provided the prosthesis receives an EtO pretreatment. With no EtO pretreatment additional surface deacetylation produces a decreased WBC time. This is probably a result of surface blistering and flaking which exposes the cellulose acetate substrate. The data also indicate that deacetylation longer than 50 minutes is required to produce surfaces comparable to the CIH surface of the pre-
ceding section. Since the question of impurity artifacts is always present when conducting this type of study, it is encouraging to notice the inverse relationship between impurities and WBC times in Figure 4-31 and that the thrombin times were always normal indicating no loss of heparin from the surface. If the presence of PEI impurities, as evidenced by the extended prothrombin time, was a dominant factor on the WBC times the WBC time could be expected to become longer as the prothrombin time, on incubated aliquots of plasma, became extended. Just the inverse of this can be seen in Figure 4-31, again indicating that some effect, other than impurities, notably surface compatibility, must have the dominant influence on the WBC test.

Considering now the actual prosthesis placement and testing, Whiffen and Gott (114,115,116,117) indicated that their 1 cm long controls would be totally occluded, or nearly so, within two hours when placed within the canine thoracic inferior vena cava near the heart. The prostheses were positioned in the inferior vena cava (IVC) through a right atriotomy. A schematic of the canine inferior vena cava (IVC) can be seen in Figure 3-10. Section A of this figure shows in more detail the positioning of the prostheses in the thoracic IVC.

In this study cellulose acetate prostheses (Eastman 042A)
three cm long were placed in the thoracic IVC by the same procedure used by Whiffen and Gott. The clotting results of this study were somewhat at variance with those of Whiffen and Gott who reported that controls of polycarbonate, graphite and other plastics occlude fully in two hours. The cellulose acetate controls of this study were found to be only partially occluded after implantation times of from 24 to 72 hours. Because of the wide range of control clotting times for this IVC position, a section of IVC with lower blood flow was selected for the prosthesis implantations. With lower blood flood through about the same size prosthesis, a longer contact time per pass should be observed and concomitant with this would be a higher tendency toward clotting.

The 3 cm lengths had to be placed below the renal veins before a consistent in vivo clotting time was observed in the controls. The walls of the prostheses were machined down to an OD of 1 cm with an ID of .8 cm in order that the implants could be easily placed into the IVC as shown in Section B of Figure 3-10. When placed below the renal veins prostheses of regenerated cellulose dialysis tubing supported by teflon, cellulose acetate and medical grade silicone rubber were all found to occlude fully in from 1 to 1.5 hours. Clots were found extending from both ends of the prosthesis. To prevent an unnoticed dislodging of an embolus from the prosthesis,
Material:
Eastman Cellulose Acetate
042A MH

Δ- EtO Pretreatment 24h, room temp. + EtI 73°C vapor + heparin
○ EtI 73°C vapor + heparin

Cellulose acetate + Imine only

FIGURE 4-30  EFFECT OF SURFACE DEACETYLATION ON WHOLE BLOOD CLOTTING TIME
FIGURE 4-31 EFFECT OF EXTENDED PROTHROMBIN TIME ON WHOLE BLOOD CLOTTING TIME

248
FIGURE 4-32A

Material:
Cellulose Acetate 042A,
+ 10' Deacetylation +
1 1/2h, EtI, 73°C

Magnification:
40 X

FIGURE 4-32B

Material:
Cellulose Acetate 042A,
+ 50' Deacetylation +
1 1/2 h, EtI, 73°C

Magnification:
40 X

WBC Time:
13 minutes

FIGURE 4-32 MICROPHOTOGRAPHS OF CAIH SURFACE, II.
the venous pressure upstream of the implant was continuously monitored. Venous pressure build up followed by a sudden decline was associated with the release of an embolus from the prosthesis to the lung.

The in vivo clotting results for the CIH membranes supported by teflon are presented in Figure 4-33. The reaction conditions for these CIH membranes are not optimal and represent early in vivo attempts. Because of the impressive amount of adsorbed heparin measured with these reaction conditions, EtI temp. 73°C and water as the catalyst, a quick decision was made to study the membrane compatibility in vivo to circumvent the tedious in vitro testing. The in vivo clotting times were found to go through a maximum and fall off with increasing surface heparin. This discouraging performance was later found, through in vitro testing and surface microphotographs, to be a result of surface crystallization and, no doubt, heparin inactivation by long chain PEI polymers which could easily flex about and tie up many active sites on the heparin molecule. In vitro WBC times with amination at 85° and H₂O as a catalyst never exceeded 60 minutes and microphotographs of the 128h EtI, 73°C reacted membranes of this study showed much surface crystallization. Figure 4-19A adequately represents this surface also. Besides the surface characteristics of these CIH membranes, these prostheses had the added
disadvantage of building up fluid between the teflon and the membrane causing a partial closing of the lumen. This effect can do nothing to enhance the life of the prosthesis. With acid catalyzed aminations, which were the most promising in vitro, this buildup of fluid was severe enough to totally occlude the lumen in one hour. A method of adhering the acid catalyzed CIH membrane to a suitable substrate for moderate periods of time in an aqueous environment was considered to be the paramount obstacle to further in vivo testing of the CIH membranes.

Scouting studies for a suitable adhesive indicate that an epoxy type resin, Allico All-Bond, is the most likely candidate thus far. Recent experiments with CIH laminate prostheses, produced by Harvard Instruments, Inc. of Needham, Massachusetts, indicate that the membranes remain bonded to thin wall stainless steel tubes for periods in excess of three days when subjected to in vivo type conditions. Additional emphasis on fabrication techniques will certainly result in operative CIH laminate conduits of a flexible and rigid nature.

For the purposes of this study an improvement in compatibility over that witnessed in Figure 4-33 was desirable. For this reason cellulose acetate prostheses were prepared by the methods discussed earlier. The in vivo clotting results with the CAIH materials can be seen in Figure 4-34. Because of the
in vitro results reported earlier, only the EtO pretreatment process was used to heparinize the prostheses. The purpose of this preliminary study was to verify the in vitro results rather than to optimize the process variables. Therefore the EtI reaction time was kept at 73°C for all prostheses aminations. All prostheses shown in Figure 4-34 were washed for 175 to 200 hours following the amination in order to remove any reaction impurities and were heparinized prior to use with a sodium heparinate solution of 200 Mg of heparin mer ml of distilled water for 15 to 30 minutes.

With only 60 minutes of deacetylation, 36h of EtO treatment, and no acid present during the amination, the in vivo whole blood clotting time was found to be around 4 hours. With 90 minutes of deacetylation and the same conditions as above, except with 2.5 hour of EtI reaction rather than 1.5 hour, the clotting time was around 5 hours.

While the results of this prosthesis implantation indicated a slight improvement with additional deacetylation, a substantial improvement in prosthesis compatibility did not result until an acid catalyst was incorporated into the EtI treatment as in the CIH treatment. The other sections of the process were identical to those above except that the pre-treated prostheses were soaked in hydrochloric acid prior to the EtI treatment. At the end of six hours one of the pros-
**Reaction Scheme**

RCF + H₂O + EtI 73°C Vapor for hours designated on curve

**FIGURE 4-33** IN VIVO CLOTTING TIMES OF CIH PROSTHESES PLACED IN THE CANINE ABDOMINAL IVC BELOW THE RENALS
All Heparinized
-Et I 73°C-

○1 - C.A. Control 3 cm
(full clot)

○2 - C.A. - EtO 36h, EtI 1 1/2h,
3 cm. (full clot)

○3 - C.A. - EtO 36h, EtI 2 1/2h,
3 cm. (full clot)

○4 - C.A. - EtO 36h, EtI 1 1/2
(.2HCl), 3 cm (no clot)

○5 - C.A. - EtO 36h, .2NHCl EtI
1 1/2 h, 3 cm (10% clot
one end)

○6 - C.A. Controls - 1.2 cm
(partial clot)

○7 - Medical Grade Silicone
Rubber "Silastic" 3 cm
(full clot)

FIGURE 4-34 IN VIVO CLOTTING TIMES OF CAIH PROSTHESES PLACED IN
THE ABDOMINAL IVC BELOW RENALS OF DOG

254
theses was removed and found to be completely patent with no emboli present in the lungs. The other was removed after 8 1/2 hours and found to contain a small clot in one end of the prosthesis. The venous pressure was normal during these latter tests for the entire time.

Although these patency times are, in themselves, not particularly impressive, they become so when compared to the silicone rubber and acetate controls and when considered in the context of the entire study. Again there seems to be a certain consistency between the in vivo and in vitro results if the former conditions are severe enough to differentiate between the surfaces tested.

In summary, this study again indicates that an irreversibly adsorbed heparin layer gives extended compatibility with human blood. Judging from the in vitro and adsorption data, this compatibility is most likely a result of adsorption onto the heparin surface. At this time insufficient data exist to identify the exact nature of the heparin adsorbate, but evidence points towards one or more clotting factors of the intrinsic clotting system.

In order to ascertain the long term compatibility of irreversibly adsorbed heparin, much additional in vivo testing is required. Because of the rough surface of the CAIH prosthesis and the inability to adhere the CIH membrane to a rigid
substrate, a certain amount of developmental research is required prior to the actual implantation of prostheses with potentials of greater than 10 hours.

H. CIH Membrane Stress-Strain Measurements

The most widely used mechanical test is the stress-strain test, although it is one of the least understood from a theoretical point of view(79). When applying the results of this test, the testing conditions and thermal history of the specimen should be kept in mind, especially if the material tested is a plastic. If the stress-strain tests are conducted at uniform conditions, they will reflect the effect of past history on the test sample's strength. Therefore such measurements offer a way of quantitatively comparing the strength of normal regenerated cellulose films with the CIH membranes produced by the various processes.

Normally the stress-strain measurements are made in tension by stretching the specimen at a uniform rate and simultaneously measuring the force on the specimen. The test is continued until it breaks. The strain, increase in length divided by the original length, is normally determined from the change in length as measured from the change in width between the jaws holding the specimen. Serious errors can result if the specimen is allowed to slip in the jaws during the measurements. The stress, force per unit area on the specimen,
is usually calculated from the total load on the sample as measured by strain gauges connected to the stationary jaw. The cross-sectional area is normally taken as the original cross-sectional area of the specimen.

The stress-strain curve of a polymeric material depends on the rate at which the sample is elongated. Common rates of elongation are generally in the range of .01 to 20 inches per minute. With high rates of elongation the material usually breaks at or before the yield point -- that point where the stress goes through a maximum. Brittle materials generally behave in a similar manner. With a low rate of strain, non brittle materials usually show a yield point if near the glass transition point. Measurements at temperatures well below the glass transition temperature rarely show a yield stress.

The testing of this experiment was performed on membranes at 37°C and in distilled water. Thus the regenerated cellulose membranes were swollen with water at a temperature well below the glass-transition temperature. Off hand then a yield stress could not be expected which was seen to be the case with rates of elongation ranging from .02 inches/minute to .2 inches/minute. The stress-strain curves for these two rates of strain, as well as values between them, were identical, within the limits of experimental error.
Therefore a rate of elongation of .1 inch/minute was used for the measurements.

The force-strain curves for CIH membranes prepared at various reaction conditions are compared to the force-strain curve of regenerated cellulose membrane in Figures 4-35, 4-36, 4-37 and 4-38. Figure 4-35 shows the effect of EtI reaction time on the force-strain curves with water as the catalyst. Figures 4-36, 4-37 and 4-38 show the same effect but with acid catalysts of .04N HCl, .2N HCl and .4N HCl, respectively.

With water as a catalyst, the breaking force was seen to decrease by about 15% with EtI reaction times up to 25 hours, while the breaking strain remained around .30. With a 48 hour reaction time the breaking force dropped by 35%. The force-strain curves for .04N HCl resembled the water catalyzed curves but started to show slightly higher break strains near a 24h EtI reaction time.

With .2N HCl as the catalyst, acid degradation of the membrane became evident by 24 hours of EtI reaction at 85°C. At 12 hours of reaction with EtI, the breaking force dropped only by 15-20%. With this same acid loading in air at 85°C, the membrane was degraded completely in 1-2 hours. It is essential then to completely exhaust all of the air from the reactor kettle prior to the addition of heat and EtI.

Of equal importance, no atmospheric leaks should be al-
FIGURE 4-35 FORCE-STRAIN CURVES FOR CIH MEMBRANES-H₂O CATALYZED
FIGURE 4-36  FORCE-STRAIN CURVES FOR CIH MEMBRANES - .04N HCl CATALYZED
FIGURE 4-37 FORCE-STRAIN CURVES FOR CIH MEMBRANES- .2N HCl CATALYZED

- Union Carbide #20 Dialysis Tubing
- #20 D.T., .2N HCl, 2h EtI Vapor 85°C
- #20 D.T., .2N HCl, 6h EtI Vapor 85°C
- #20 D.T., .2N HCl, 12h EtI Vapor 85°C
- #20 D.T., .2N HCl, 24h EtI Vapor 85°C

△ - Break Force & Break Strain
FIGURE 4-38  FORCE-STRAIN CURVES FOR CIH MEMBRANES-.4N HCl CATALYZED
Figure 4-38 shows that with .4N HCl as a catalyst, excessive degradation started at about 12 hours of EtI reaction. This indicates that an excess of acid is present beyond that which is necessary to protonate the amines and reacts with the glucosidic groups of the cellulose membrane.

The force-strain data demonstrates that with catalyst loadings of .04, .2 and .4N HCl, the maximum EtI reaction time at 85°C should be 24, 12, and 6 hours, respectively. Since catalyst loadings of .2N HCl and greater with EtI reaction times of 2 hours or more gave satisfactory WBC times, apparently a range of acceptable reaction conditions are available. Therefore the choice of the optimal reactions conditions must consider membrane swelling and permeability as well.

Although the breaking force-breaking strain measurements give a good indication of the EtI process degradation of the overall membrane strength, they tell little about those characteristics based on membrane thickness.

Figure 4-40 shows that the amination process produces

2. These reaction times are based on a maximum decrease in breaking strength of 15 to 20%. See Figure 4-39 for a graphical representation.
significant swelling when catalyst loadings of 0.2N HCl or more are used. With lesser acid concentrations the swelling is not so pronounced and possibly with fewer surface irregularities, a lower acid concentration could be used, resulting in less swelling. Using commercial grade dialysis tubing, however, will no doubt require the higher acid concentrations. Therefore if a reduction in swelling became necessary because of the increased membrane resistance due to the added thickness, crosslinking the membrane prior to amination might be a solution. Of course, one cannot predict a priori if this tightening up of the membrane would improve the overall permeability or not, but it certainly would improve the mechanical data based on cross sectional area, such as stress. The importance of the stress data is questionable if the overall permeability of the CIH membrane is comparable to the original RCF. If, however, the CIH permeability is lower because of the thicker membrane, the use of the stress-strain data to calculate what the breaking force of thinner, more permeable CIH membranes is necessary.

The stress-strain curves for various reaction conditions can be seen in Figure 4-41, and the breaking stress-reaction time relationships can be seen in Figure 4-42. The dichotomy between the RCF and CIH membrane properties shown in these figures is a result of swelling. Apparently this swelling is
Note: #20 DT, .2 & .4N HCl at 85°C disintegrates in 2 hrs.
Note: #20 DT, .04N HCl at 85°C disintegrates in 6 hrs.
Note: #20 DT, 85°C H₂O 100 hr

FIGURE 4-39 EFFECT OF REACTION CONDITIONS ON MEMBRANE BREAKING FORCE
FIGURE 4-40  EFFECT OF REACTION CONDITIONS ON MEMBRANE THICKNESS
Young's Modulus

- 2200 PSI
- 2400 PSI
- 3200 PSI
- 3600 PSI
- 8800 PSI

- #20 D.T., .4HCl, 2h EtI Vapor 85°C
- #20 D.T., .04NHCl, 24h EtI Vapor
- #20 D.T., 2N HCl, 6h EtI Vapor 85°C
- #20 D.T., 2N HCl, 2h EtI Vapor
- #20 D.T., Union Carbide

FIGURE 4-41 STRESS-STRAIN CURVES FOR VARIOUS REACTION CONDITIONS
FIGURE 4-42  EFFECT OF REACTION CONDITIONS ON MEMBRANE BREAKING STRESS

- Union Carbide #20 Dialysis Tubing
- #20DT, H₂O, EtI Vapor 85°C
- #20DT, .04N HCl, EtI Vapor 85°C
- #20DT, .2N HCl, EtI Vapor 85°C
- #20DT; .4N HCl EtI Vapor 85°C
the result of large crystalline dissolution and amination, but probably not micelle penetration since the CIH membranes seem to retain their overall strength. Acid hydrolysis of the glucosidic linkage probably accounts for much of the later degradation. Figure 4-42 indicates that this degradation is linear with reaction time after an initially rapid swelling period.

I. CIH Membrane Permeability Measurements

Since the primary function of the hemodialyzer is to remove waste metabolites from the uremic's blood, any membrane considered as a candidate for this operation must at least have a high permeability to urea, the principle waste metabolite excreted by the kidney. The most desirable membrane for this application would pass urea at an infinite rate; at this time, however, such a membrane is not available. Since regenerated cellulose dialysis films have received wide-spread acceptance as the artificial kidney membrane, the permeability of the newly developed CIH membranes will be compared to that of the cellulose membranes. The comparison is also consistent with the original objects of modifying the compatibility of the cellulose films but not their important structural properties.

The CIH membranes having a minimum reaction time and acid concentration, but still showing compatibility, were used for
the comparison. Since adsorption studies with tagged plasma indicated an adsorption onto the heparinized surface, all membranes were soaked in fresh citrated plasma prior to measuring their urea permeability. The permeability was measured at two different overall urea concentrations, 6 Mg% and 400 Mg% which totally encompass the range of interest in the artificial kidney. The use of radioactive urea allowed this flexibility, for a driving force of only 6 Mg% was required in the cell.

Since these measurements were to serve only as an initial screening of the new membranes to find the effects of the reaction conditions on the original membranes, all urea diffusion measurements were performed at 20°C with saline as the solution. After additional optimization of the reaction conditions and considerably more in vivo testing, a more thorough investigation of the CIH membrane's transport properties would be fruitful, especially for later design. But at this time a complete study of these transport properties would be premature and possibly of little value if alternate reaction conditions were selected.

One prime consideration in any membrane permeability measurements is the importance of the liquid side resistances on both sides of the membrane. Two methods of accounting for this resistance are available: reduction of the resistance
on both sides with sufficient agitation, or measurement of the membrane permeability at different agitation rates and extrapolation to infinite agitation through the use of a modified Wilson Type Plot, shown in Figure 2-4. The former was chosen for this study because of the insensitivity of the saline solution to agitation in the range of interest. Marangozis and Johnson (72) have developed correlations for mass transfer data in liquid-solid systems in agitated vessels which show the effect of agitation on the fluid side coefficient at the solid or membrane interface. The most appropriate correlation is displayed in Figure 4-43. With the agitation speed of 1200 RPM the total liquid side resistant should be around 5%, which is quite acceptable for these membrane studies.

With membrane diffusion studies using radioactive tracers, to convert the activity in counts per minutes to weight concentration is not necessary and the actual membrane diffusion coefficient can be calculated directly from the activity measurement versus time. The working equation is displayed in Figure 4-44, accompanied by a semilog plot of the actual data. The derivation of the working equation can be found in Appendix H. The membrane mass transfer coefficients are directly proportional to the slope on the semi-log plot and the membrane diffusion coefficient results from division of
the transfer coefficients by the swollen membrane thickness. Figure 4-44 shows that the transfer coefficients, for $K_m$, for both the CIH and RCF membranes are about the same, within the experimental error. Thus, the urea clearance of an artificial kidney with a CIH membrane should approach that of current hemodialyzers. As far as actual membrane resistance per unit thickness is concerned, the swollen CIH membrane seems to offer less resistance than the regular cellulose membrane. The membrane diffusivity values listed in Table 4-1 show this clearly.

In anticipation of some urea diffusion results to follow, it is interesting to compare the membrane diffusion coefficient, $D_m$, with the diffusion coefficients of urea through blood and saline. $D_m$ for the CIH membrane is about 40% of the diffusion coefficient in saline and about 60-70% of that in blood. These results are at 20°C and probably would not hold precisely at the normal hemodialysis temperature. The results of this section indicate that the CIH membrane has passed another necessary condition for its eventual incorporation into the artificial kidney.
<table>
<thead>
<tr>
<th>Km cm/sec</th>
<th>Dm cm²/sec</th>
<th>k fraction H₂O</th>
<th>Dm cm²/sec</th>
<th>Membrane</th>
<th>Thickness Mils</th>
<th>Solvent-Saline</th>
<th>Solute-Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.9x10⁻⁴</td>
<td>.20x10⁻⁵</td>
<td>.50</td>
<td>.4x10⁻⁵</td>
<td>Regen. Cellulose</td>
<td>2.0-2.1</td>
<td>0 Mg%</td>
<td>6 Mg%</td>
</tr>
<tr>
<td>5.7x10⁻⁴</td>
<td>.28x10⁻⁵</td>
<td>.50</td>
<td>.5x10⁻⁵</td>
<td>&quot;</td>
<td>2.0-2.1</td>
<td>400 Mg%</td>
<td>&quot;</td>
</tr>
<tr>
<td>3.9x10⁻⁴</td>
<td>.45x10⁻⁵</td>
<td>.67</td>
<td>.7x10⁻⁵</td>
<td>CIH + Plasma</td>
<td>4.5-4.7</td>
<td>400 Mg%</td>
<td>6 Mg%</td>
</tr>
<tr>
<td>4.8x10⁻⁴</td>
<td>.54x10⁻⁵</td>
<td>.67</td>
<td>.8x10⁻⁵</td>
<td>CIH + Plasma</td>
<td>4.2-4.3</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Saline</td>
<td>--</td>
<td>400 Mg%</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
<td>--</td>
<td>500 Mg%</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blood (H=45)</td>
<td>--</td>
<td>500 Mg%</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

TABLE 4-2 UREA DIFFUSION THRU MEMBRANES AND SOLUTIONS - 20°C
**Temp = 20°C**
**Solution = Saline**

- CIH + Plasma (Urea 6mg%) (4.2 miles)
- CIH + Plasma (Urea 400mg%) (4.6 miles)
- DuPont PD215 (Urea 6mg%) (2.1 miles)
- Union Carbide #20 DT (Urea 400mg%) (2.0 miles)

**Working Equation:**
\[
\ln \left( \frac{CPM}{CPM_{\text{initial}}} \right) = -K_m \cdot T_{\text{min}}
\]

**FIGURE 4-44 UREA MEMBRANE DIFFUSION RESULTS**
As far as the sufficient conditions go, the ability of this membrane to reject proteins of the albumin size, pass electrolytes rapidly, and lend itself to a thickness reduction will ultimately determine its suitability for hemodialysis. These factors must be balanced against the advantages accrued because of CIH membrane compatibility.

J. Diffusion of Urea Thru Human Blood

With the ever escalating demand for human blood processing equipment of the artificial kidney and heart-lung machine type the engineering profession, especially chemical engineers, are being called upon to contribute their energies to the area of equipment design. The chemical engineer is particularly suited to this task because of his knowledge of the dialysis transfer process and his familiarity with polymeric materials. The bulk of this report thus far has dealt with the development of improved materials for eventual incorporation into artificial kidney designs. Very little in this study has been mentioned concerning the actual design of such units but a very real need for design improvements does exist, as is evidenced by the low overall efficiencies mentioned in Section II.

To adequately sustain the lives of the 5000 uremic patients who die each year in this country alone, an artificial kidney must be developed which will efficiently and inexpensively substitute for their normal renal function. To keep the oper-
ating cost to a minimum the future artificial kidney should be designed for home lay operation with completely fail safe features. The development of suitable materials which are more friendly towards human blood would constitute a major step toward the realization of such a unit. A coupling of these materials with an effective design would no doubt produce the desired result.

Assuming passive hemodialysis continues its dominant role in uremic treatment, the design of an effective dialyzer must provide for a considerably lower blood side mass transfer resistance. To characterize this resistance in well defined blood channels and extrapolate to proposed blood channel configurations for evaluation, the actual diffusion coefficient of the solutes in question thru blood must be known to a reasonable degree of certainty. With a known solute diffusivity the buildup of concentration gradients can be calculated. For those designs advocating intermittent mixing of the blood within the channel by membrane agitation, the calculations for concentration gradient buildup will contribute much to the optimization of such a design. The most promising approaches to the theoretical description of solute diffusion coefficient in suspensions similar to blood and protein solutions have been conducted by Prager(85,86) and Wang(109). Prager, in his statistical treatment of diffusion through an in-
homogeneous medium, has derived an equation for determining
diffusion coefficients through two materials of different permeability, isotropically dispersed in each other. Wang, on the
other hand has derived a model for predicting solute diffusivities in protein solutions taking into account the obstruction effect and the hydration effect of the proteins. Assuming Wang's model will predict diffusivities in plasma there is considerable doubt as to the applicability of the Prager treatment. In those cases where a numerical value can be calculated by the Prager equation, one is forced to assume complete impermeability for one of the two phases. This probably does not describe the red cell adequately since the red cell is known to be permeable to most waste metabolites(119).

The purpose of this portion of the study was to develop a technique to experimentally measure the diffusivity of small molecules through human blood. The actual measurements were performed with urea as the solute for different hematocrits and anticoagulants. This data should be of value in future artificial kidney designs and can be used as an evaluation of the applicability of current solute transport models to blood.

The equipment and procedure used throughout this study were discussed in Section III-H. The final working equation which describes the one dimensional diffusion of the capillary cell can be represented as

\[ \text{Equation} \]
\[
\ln \frac{c}{c_0} = \frac{-15 \pi^2}{\lambda^2} D \text{ (urea)} T \text{ min}
\]
if all terms in the series expansion but the first one are dropped. As seen from previous discussion all terms beyond the first cease to be of importance with a diffusivity run of greater than 6 to 8 hours. Therefore, the self diffusion coefficient can be determined from the slope of equation (32), since the length of the capillary can be readily measured. Because of the problem with human erythrocyte settling at hematocrits below 60, the diffusivities were determined for higher hematocrits and extrapolated to the normal hematocrits of 44-45.

The data from this study is plotted according to equation (32) in Figures 4-45 to 4-51. Figure 4-45 shows that the capillary cell calibrates to within ± 5% of the literature diffusivity for urea thru distilled water, which is 1.20 x 10^{-5} \text{ cm}^2/\text{sec at 20°C}, for both distilled water and saline. The data in Figure 4-46 shows that with the same anticoagulant to blood dilution the nature of anticoagulant, i.e., heparin or trisodium citrate, has little effect on the diffusivity of urea through fresh plasma. The plasma and blood for this study were never more than seven hours old at the beginning of the diffusion run and all samples were donated by this author for the sake of consistency. The citrate solution used was always 3.8% trisodium citrate and the heparin solution had a concentration of 2 Mg/ml. Both
FIGURE 4-45 DIFFUSIVITY OF UREA THROUGH SALINE AND DISTILLED WATER
FIGURE 4-46 DIFFUSIVITY OF UREA THROUGH HUMAN PLASMA

Temperature = 20°C
Urea = 500 mg %
Cell #2 = $f = 1.8$ cm

$\ln \frac{c}{c_0}$ vs $T$ min

FIGURE 4-47 DIFFUSIVITY OF UREA THROUGH CITRATED HUMAN BLOOD - HEMATOCRIT OF 50

Temperature = 20°C
Urea = 500 mg %
Cell #3 = $f = 2.0$ cm

$\ln \frac{c}{c_0}$ vs $T$ min
FIGURE 4-48 DIFFUSIVITY OF UREA THROUGH CITRATED HUMAN BLOOD - HEMATOCRIT OF 65

Temperature - 20°C
Urea - 500 mg %
Cell #2 - $l = 1.8$ cm

$\ln \frac{c}{c_0}$ Vs T min

Time, Hours

FIGURE 4-49 DIFFUSIVITY OF UREA THROUGH HEPARINIZED HUMAN BLOOD - HEMATOCRIT OF 70

Temperature - 20°C
Urea - 500 mg %
Cell #3 - $l = 2.0$ cm

$\ln \frac{c}{c_0}$ Vs T min

Time, Hours
**FIGURE 4-50** DIFFUSIVITY OF UREA THROUGH HUMAN RED CELL - SALINE SUSPENSION - HEMATOCRIT OF 70.

ln \( \frac{c}{C_0} \) vs T min

Temperature = 20°C
Urea = 500 Mg %
Cell #1 = \( \delta = 1.8 \) cm

**FIGURE 4-51** DIFFUSIVITY OF UREA THROUGH CITRATED HUMAN BLOOD - HEMATOCRIT OF 78

ln \( \frac{c}{C_0} \) vs T min

Temperature = 20°C
Urea = 500 Mg %
Cell #3 = \( \delta = 2.0 \) cm
Normal Adult Hematocrit

Temperature 20°C

FIGURE 4-52 UREA DIFFUSION COEFFICIENTS FOR HUMAN BLOOD
FIGURE 4-53 COMPARISON OF THEORETICALLY PREDICTED AND EXPERIMENTALLY MEASURED UREA DIFFUSION COEFFICIENTS FOR HUMAN BLOOD

\[ D(\text{urea}) \propto \frac{1}{H} \]

- Experimentally measured
- Prager's Equation

\[ D(\text{plasma}) = 0.9 \times 10^{-5} \text{cm}^2/\text{sec} \]

Temperature 20°C
were used in a 1 to 10 dilution with the freshly drawn blood for the purposes of anticoagulation. Figures 4-47 through 4-51 show the results with human blood. The effect of red cell sedimentation can be observed in Figure 4-47 with a hematocrit of 50. A comparison of Figures 4-49 and 4-50 shows that the substitution of saline for the suspending plasma produces an increased diffusivity, as would be expected considering the higher diffusivity of urea through saline compared to that of plasma.

A plot of the urea diffusivity as a function of the hematocrit can be seen in Figure 4-52. An extrapolation of the diffusivities from the higher hematocrits gives a urea diffusion coefficient of $0.75 \times 10^{-5}$ cm$^2$/sec through normal whole human blood. As in the case of plasma the anticoagulant appears to have little or no effect on the urea diffusivity. The diffusion coefficient through heparinized blood with an hematocrit of 70 was seen to fall on the diffusivity curve for the citrated blood.

A comparison of the experimentally measured data with the predicted values from the Prager model can be seen in Figure 4-53. As expected the Prager approach, assuming impermeability of the red cell, yields lower values than the experimental data. The shape of the curve calculated with the Prager model is in further contradiction with respect to its shape.
and slope. Due to the low particle-particle interactions with void fractions greater than 60 or hematocrits less than 40 one would expect the particles or red cells to have a lesser influence as seen in the experimental curve. The rapid drop in dif-
fusivity with low hematocrit predicted by the Prager equation is questionable. Another phenomenon peculiar to the red cell, that of membrane flickering, might contribute to the discrep-
ancy also.

An alternate approach to the detailed physical model might be one based on the predominant blood component concen-
trations. Since the permeable red cell membrane is known to comprise about 2 to 5% of the red cell dry weight with haemo-
globin comprising another 90%, a more appropriate diffusion model might consider the blood as a protein solution of haemo-
globin and the other plasma proteins.

An approach similar to Wang's based on the protein con-
centration might then provide a more accurate model for diffu-
sion in blood. Since Wang's derivation is based on dilute protein solutions and predicts a linear relationship between protein concentration and diffusivity, which obviously does not hold for blood at high hematocrits, a simple substitution into Wang's equations is not acceptable. The basic derivation must be studied and extended to concentrated protein solutions.
A model where the diffusivity is assumed inversely proportional to the blood viscosity appears to result in urea diffusivities similar to those of the Prager model, but of consistently lower values. Diffusivities calculated from typical hematocrit-viscosity data for blood (38, p. 345) can be seen in Figure 4-53.

Although no simple model has been found which adequately describes solute diffusivity through human blood, this study indicates that the urea self diffusion coefficient in normal blood is higher than that predicted by existing models and can be taken as about $0.75 \times 10^{-5}$ cm$^2$/sec at $20^\circ$C for most design purposes.

K. Design Considerations for a Compatible Artificial Kidney

The overall results of this study indicate that the possibility of producing a highly reliable compatible artificial kidney, and for that matter heart-lung machine, is coming within focus. Irreversibly adsorbed heparin has been shown to enhance the compatibility of a number of substrates by actively interacting with blood to form a protein-like layer over the heparin. The degree of passivity of this "intact" protein-like layer to human blood has not yet been completely established. Much additional in vivo testing is required to establish the limits of compatibility, if indeed such limits do exist.
Assuming for the moment that the limit of compatibility can be established, one would expect that a successful design should have: 1) no unheparinized surfaces in contact with blood, including lead tubing and catheters, 2) no areas conducive to stagnation such as poorly designed header entrances or junctions, 3) a minimum of surface contact area, especially that not directly involved in dialysis, 4) fail safe features, and 5) of course, maximum removal efficiency. These requirements, including the fabrication considerations, indicate the first compatible artificial organ prototypes might be a single channel coil type with intermittent or continuous external vibration or agitation of the coil to disrupt the concentration gradients in the channel, which result from non obstructed forced flow. The patient's blood would enter the coil by means of a catheter and short lead tubing which gradually flared to the channel dimensions. A possibility of producing the lead tubing and membrane from the same CIH membrane now exists, since the basis of a technique to bond CIH films to rigid and flexible substrates has been developed.

One final note concerning the depletion of plasma factors from the patient during hemodialysis through their adsorption onto the heparinized surface. If only Factor IX is selectively adsorbed by the heparinized surface, one can expect that a 10,000-15,000 cm$^2$ heparinized surface would adsorb 80 to 120
Mg of Factor IX, sufficient to totally deplete the patient's Factor IX activity, if no regeneration occurs. If other factors adsorb besides Factor IX or the regeneration rate of the depleted factors is substantial, reduced coagulation activity should be much less severe and, in fact, this depletion phenomenon might be used to good advantage. If, however, this phenomenon must be avoided, the heparinized surface could be treated with a plasma factor rich solution or bank blood prior to hemodialysis.
Conclusions

A) Artificial Kidney Performance

1. The current artificial kidneys appear to be operating at 20-30% of their membrane urea removal capacity.

2. These efficiencies apply equally well to extracorporeal hemodialysis units dialyzing actual patient blood or saline as a blood simulant. Thus an excessive increase in membrane resistant due to the adsorption of blood components onto the membrane must be ruled out.

3. The major resistance to urea transport appears to exist in the blood.

B) Graphite-Benzalkonium-Heparin Surface

1. About 2 µgm/cm² of adsorbed heparin is present on the Lucite-GBH surface. This value is of the same order of magnitude as that calculated for a monolayer of heparin assuming a collapsed helical coil configuration for heparin.

2. Surface heparin can be desorbed from the GBH surface by fresh plasma with little or no fluid shear at the surface, but not with saline even at wall shears of 9.5 dyne/cm², suggesting strong complexing power of the plasma proteins.

3. The heparin removal rate from the GBH surface is a function of fluid shear at the surface when the shearing solution is plasma.

4. The weak bond in the GBH surface is probably the
physical bond between the graphite particle and the benzal-konium organic tail.

C) Heparinization of Regenerated Cellulose Films

1. Heparin can be irreversibly bonded to regenerated cellulose dialysis tubing following amination with ethylenimine.

2. An acid catalyst must be present during the amination to give effective surface amination if surface crystalline regions are present on the substrate. The total adsorbed surface heparin ranges from $9 \times 10^6 \text{ gm/cm}^2$ to $12 \times 10^6 \text{ gm/cm}^2$ depending on the acid concentration of the solution initially used to swell the cellulose.

3. Such heparinized surfaces give WBC times in excess of 100 minutes whereas the controls are normally 20 minutes.

4. The surface heparin, when adsorbed in the preferred manner, interacts with plasma and whole blood. This interaction appears to be a result of complexing between the surface heparin and one or more intrinsic clotting factors.

5. The adsorption of clotting factors onto the heparinized surface is, in many cases, sufficient to anticoagulant the contacting aliquot of blood. Of course, the degree of saturation of the heparin surface dictates the surface adsorption power.
6. Tritiated plasma adsorption studies with heparinized surfaces indicate that the heparin surface can be saturated with plasma components.

7. The heparin surface appears to adsorb about its own weight in plasma components whereas medical grade silicone rubber and RCF adsorb far less, but a definite amount of plasma components.

8. Treatment of a heparinized surface with a protamine sulfate solution renders the antithrombogenic nature of the surface much less effective.

9. In vivo experiments with heparinized cellulose acetate prostheses placed in veins with low blood flow in dogs indicate that an irreversibly adsorbed heparin surface, when saturated with plasma components, possesses superior antithrombogenic properties.

10. The structural properties of the CIH membranes are comparable to those of regenerated cellulose if the acid catalyst concentration or amination times is not excessive.

11. The breaking force and breaking strains can be maintained with 10 to 15% of those for regenerated cellulose while the compatibility of the CIH surface is far superior to that for RCF.

12. Because of the membrane swelling during amination the breaking stress and Young's modulus are about 50% lower.
than those for regenerated cellulose.

13. The urea membrane transfer coefficients for CIH membranes saturated with fresh plasma are comparable to those of regenerated cellulose membranes.

14. As a result of amination and swelling of the CIH membranes, the CIH membrane diffusivity for urea is about twice that for regenerated cellulose or $0.5 \times 10^{-5}$ cm$^2$/sec.

15. For the purposes of mass transfer correlations or engineering designs the diffusivity of urea through human blood was experimentally determined to be $0.75 \times 10^{-5}$ cm$^2$/sec at $20^\circ$C.
VI. RECOMMENDATIONS

1. Since a time consuming rinse is now required to clean up the CIH membranes of diffusible impurities, an ultrafiltration washing procedure which would probably wash out the impurities quicker, appears advantageous and should be developed.

2. The need to establish the limit of compatibility of the CIH surface needs little enunciation, but the fabrication of suitable prostheses with the CIH membrane is not that obvious. A thin walled laminate of CIH membrane adhered to a rigid thin wall support structure is envisioned.

3. Since the CIH prosthesis may eventually have potential applications in long term intravascular implantations, the toxicity of the material should be studied to possibly uncover yet unknown rejection situations.

4. The reaction conditions for the amination of cellulose acetate and regenerated cellulose film should be optimized to give improved compatible materials. Higher amination temperatures and shorter reaction times are desirable. Other reducing agents, such as lithium aluminum hydride, should be studied for their possible use as deacetylation agents.

5. Serious thought should be given to the idea of extending this same type reaction scheme to other potentially useful artificial organ materials.
APPENDIX A

Derivation of Relationship Between Overall Membrane Mass Transfer Coefficient and Dialysance

Assumptions:
1) Membrane acts as sieve
2) Concentration in dialysate \( \approx 0 \)
3) Neglect radial gradients of concentration in blood

Mass Balance on Differential Element

\[ Q_B \; dC = - dA \; K_m \; C \]

\[ \frac{dC}{C} = - K_m \frac{dA}{Q_B}, \text{ integrating} \]

\[ \ln \left( \frac{C_0}{C_1} \right) = -A \frac{K_m}{Q_B} \]

Now "Dialysance" is defined as,

\[ D_y = Q_B \left( \frac{C_1 - C_o}{C_1} \right) \]

Therefore

\[ D_y = Q_B \left( 1 - e^{-\frac{AK_m}{Q_B}} \right) \]

or

\[ K_m = - \frac{Q_B}{A} \left[ \ln \left( 1 - \frac{D_y}{Q_B} \right) \right] \]
Concentration, (milliequivalents/liter) (38, p. 92)

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**TABLE A-1** TYPICAL SOLUTE CONCENTRATIONS OF PLASMA, URINE, AND ARTIFICIAL KIDNEY DIALYSATE
APPENDIX B

Conversion of Urea to Ionic Products

The removal of urea from human blood or other solutions by means of ion exchange resins or electrodialysis is almost totally ineffective because of the neutral nature of the urea molecule. If this waste metabolite could be effectively converted to an ionic form, the converted urea could be efficiently removed from solvents by the above methods, be the solvents blood or dialysate. A continuous removal of urea from the dialysate side of the artificial kidney would greatly reduce the dialysate handling problem and increase urea dialysis efficiency by maintaining a maximum urea driving force across the membrane.

One possible technique for producing ionic products from urea might utilize the well known enzymatic urease-urea reaction. High molecular weight urease might be separated by a membrane from the blood but allowed to react with urea which diffuses or is forced through the membrane. Urea in the presence of active urease is rapidly converted to ionic products, namely $\text{NH}_4^+$, $\text{CO}_3^-$ or $\text{CO}_2$ depending on the exact $\text{p}_\text{H}$ conditions. These products could be efficiently adsorbed by a suitable ion exchange resin to render the urease containing solution virtually free of urea. This technique appears to have many immediately obvious potentials in the area of uremia therapy and should be investigated to its fullest.
APPENDIX C

In Vitro Blood Coagulation Test Procedures

1. Whole Blood Clotting Test

This test should be carried out in a clean saline rinsed test tube. Venous blood is conveyed directly from the vein to the test tube through a siliconized #18-19 gage needle and a small length of plastic tubing. Care should be taken to see that the needle is very sharp and enters the vein directly. Contamination with tissue juices will invalidate the test by setting off the extrinsic clotting system rather than the intrinsic clotting system.

The whole blood clotting time refers to the interval between the initial test tube loading (about 1 ml) and the appearance of a clot with intermittent tilting every 30 seconds. The WBC test should be performed at 37°C for maximum reproducibility since this is the optimum coagulation temperature.

If the proper precautions are followed the WBC time can be reproducible to within 5 to 10% for a given surface material with normal blood.

2. Thrombin Test

The thrombin test and those to follow are performed on citrated plasma rather than whole blood. Sodium citrate is added to chelate the calcium ion, thus anticoagulating the plasma or blood. Citrated plasma is prepared by collecting
whole blood into a dry plastic syringe into which was placed 3.8% trisodium citrate solution (Na$_3$C$_6$H$_5$O$_7$ - 2H$_2$O) in the proportion of nine parts of blood to one part of citrate solution. The blood is drawn through a clean venous puncture and mixed with the anticoagulant as soon as possible. To obtain platelet-rich plasma (PRP) the blood is centrifuged for 5 minutes at 1500 rpm while to obtain platelet-poor plasma (PPP) the blood is centrifuged at 3000 rpm for 30 minutes or at 15000 rpm for 5 minutes. For the thrombin test .1 ml of thrombin solution (10 thrombin units/ml) is added to .2 ml of PPP and incubated at 37°C tilting every two seconds. The normal thrombin time for plasma is 13-14 seconds with a 10 unit/ml thrombin concentration. This test is only sensitive to fibrinogen and can be performed successfully on old plasma.

3. **Prothrombin Test**

The prothrombin test, as mentioned earlier, is a measure of the extrinsic system functioning. Tissue extract and M/40 calcium chloride are added to fresh citrated PPP with subsequent incubation and tilting at 37°C. Fresh plasma must be used, since factor V is labile and loses much of its activity in a matter of three to four hours at room temperature and even more rapidly at 37°C.

Commercial prothrombin reagents are available which contain not only the tissue extract but the calcium chloride as well. The recommended prothrombin test procedure with these
reagents is to add .2ml of the commercial reagent to .1 ml of PPP. The normal clotting time is 13 to 14 seconds.

4. **Kaolin-Cephalin Test**

The kaolin-cephalin clotting test gives an indication as to the functioning of the intrinsic clotting system. Kaolin provides the necessary surface for activation of the Hageman factor complex while cephalin supplies the phospholipids normally released from the platelets. The kaolin-cephalin reagent is usually prepared by the individual research laboratories and the general procedure for the test is as follows:

1. .1 ml of kaolin-cephalin solution and .1 ml of saline are added to .1 ml of PPP and .1 ml of pH 7.4 buffer.

2. the total mixture is incubated 10 minutes at 37°C with occasional tilting.

3. At ten minutes .1 ml of M/40 calcium chloride is added. The kaolin-cephalin time is measured from the addition of calcium chloride. Normal kaolin-cephalin times with fresh plasma are about 45 to 50 seconds depending on the exact nature of the original kaolin-cephalin solution.
APPENDIX D

Couette Wall Shear Cell

Derivation of Design Equation

θ  Momentum Balance

\[ \frac{d}{dr} \left( \frac{1}{r} \frac{d(rV_\theta)}{dr} \right) \]

integration gives

\[ C_2 \frac{r^2}{2} = r V_\theta + C_3 \]

where \( C_2 \) and \( C_3 \) are integration constants

Substitution of the boundary conditions gives

\[ C_3 = \frac{R^2}{2} C_2 \quad \text{and} \quad C_2 = \frac{2k^2 \mu}{k^2 - 1} \]

now

\[ rV_\theta = \frac{\mu}{1 - \frac{1}{k^2}} \left( r^2 - R^2 \right) \]

Since

\[ \tau_{re} = -\mu \left[ r \frac{d (V_\theta)}{dr} \right] \]

it easily follows

\[ \tau_{re} = -2 \frac{\mu \mu'}{1 - \frac{1}{k^2}} \cdot \frac{1}{r^2} \]

Now the wall shear at the spindle is related to the couette design and spindle speed by

\[ \tau_{\text{spindle wall}} = \frac{2 \mu \mu'}{1 - k^2} \]

Calculation of Cannula Wall Shear Heart-Lung Machine

\[ \tau_{\text{wall}} = 4 \frac{\mu Q_B}{\pi R^3} \]

\[ Q_B = \frac{5500}{60} = 92 \, \text{cm}^3/\text{sec} \]

\[ R = .64 \, \text{cm} \]
therefore
\[ \tau_{\text{wall}} \text{ shear} = (4)(3)(.695)(92)(10^{-2}) = 9.5 \text{ dynes/cm}^2 \]

Artificial Kidney

\[ Q_B = \frac{150}{60} = 2.5 \text{ cm}^3/\text{min} \]

\[ R = .32 \text{ cm} \]

\[ \tau_{\text{wall}} = (4)(3)(.695)(2.5)(10^{-2}) = 2.0 \text{ dynes/cm}^2 \]

Calculation of Annulus Outside Dimension

Spindle Speed - 600 rpm
Solution - Saline
Temperature - 20°C
\[ \tau_{\text{wall}} = 9.5 \text{ dynes/cm}^2 \]

\[ \mu = .01 \text{ poise} \]

\[ \omega = 62.8 \text{ radians/sec} \]

\[ \tau_{\text{wall}} = \frac{2\mu\omega}{1-k^2} \]

9.5 - 9.5 \( k^2 \) = 2(0.01)(62.80)

\[ k^2 = .87 \]

\[ k = .93 \]

I.D. = k O.D. Now I.D. = .830" for dialysis tubing

therefore O.D. = .900"

Calculation of Spindle Speed

Plasma - Heart-Lung Shear -20°C-

\[ \mu_{\text{plasma}} = 2.0 \text{ H}_2\text{O} \]
therefore \( \omega = 300 \text{ RPM} \)

Plasma - Artificial Kidney  
\(-20^\circ\text{C}\)  
\[ \tau_{\text{wall}} = 2.0 \text{ dynes/cm}^2 \]

therefore \( \omega = 60 \text{ RPM} \)

Water - Artificial Kidney  
\[ \tau_{\text{wall}} = 2.0 \text{ dynes/cm}^2 \]

therefore \( \omega = 120 \text{ RPM} \)

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303
APPENDIX E

Calculations Pertaining to $^3\text{H}$ - Heparin Detection

Calculation of Tritium Efficiency For Solid Scintillator Detector

- High voltage setting - 1200 V
- Sample distance from crystal - 10 mils

Sample calculation:

\[
\text{cpm} = \frac{7.2 \mu \text{c/planchet}}{\text{(ave of three planchets)}} = 78000 \text{ CPM}
\]

Therefore, Efficiency = \[
\frac{78000}{7.2 \times 2.2 \times 10^6} = 0.0049
\]

Average detection efficiency with 9 separate measurements = 0.0052 or 0.5%

Calculation of Maximum Tritium Range in Regenerated Cellulose

- Tritium - 0.018 MEV (Maximum Beta)
- Range (§7, p.18) of such betas in aluminum equals 0.6 Mg/cm$^2$

Now \[
R(g/cm^2)_{\text{zi}} = R(g/cm^2)_{\text{z2}}
\]

Therefore, 0.6 Mg/cm$^2$ = $\gamma X$

$\gamma$ (Regenerated Cellulose) = 1100 Mg/cm$^2$

Therefore, \[
X = \frac{0.6}{1100} = 5.5 \times 10^{-4} \text{ cm}
\]

or Maximum tritium beta range in cellulose is 0.0055 cm or 5.5 microns. The normal air dried regenerated cellulose membrane has a thickness of 25 to 30 $\mu$.
APPENDIX F

Ethylendimine Health Hazards

Ethylendimine is a highly toxic material requiring special handling precautions. No one should attempt to handle or use it without fully acquainting himself with its dangers, special precautionary measures, and appropriate first aid treatment.

Skin and Eye Contact

Ethylendimine is corrosive to the skin and is readily absorbed in toxic amounts. Contacts with high concentrations of ethylendimine vapor (100 PPM) may cause severe eye and skin irritation. Liquid ethylendimine contacts are capable of causing severe burns and may result in sufficient absorption into the body to cause serious illness. Immediate and sustained flushing of the wound (at least 30 minutes) with water will help to reduce these effects.

Swallowing and Vapor Inhalation

Ethylendimine has a high acute oral toxicity. Serious illness may result from ingestion of relatively small amounts. After accidental ingestion the patient should drink large amounts of milk or water immediately. A physician should be called immediately.

Flammability and Stability

Ethylendimine in either liquid or vapor form is extremely flammable. Its vapors may explode when exposed to electric...
sparks, static electricity, excess heat, or an open flame. Explosion hazards may be reduced or prevented by dilution with an inert gas or proper electrical grounding.

Undiluted ethylenimine can polymerize violently in the presence of acids or acid-forming materials. Therefore extreme care must be taken to avoid contact with these materials.
APPENDIX G

Heparin Monolayer Calculations

1. Molecular Configurational Dimensions

   \[ \bar{M}_w = 15000 \]
   \[ \bar{M}_w = 267 \]
   No. of Mers/Molecule = 56

   a) Extended Linear Configuration

      Estimated Width = 10 Å
      Estimated Length/Mer = 10 Å
      Estimated Overall Length = 560 Å

   b)Collapsed Helical Coil Configuration

      Estimated Width = 10 - 15 Å
      Estimated Length/Mer = 2.5-4.0 Å
      Estimated Overall Length = 70 -110 Å

   c) Random Coil Configuration

      Anhydroglucose Unit Length - 5 Å

      \[ r^2 = c^2 n \quad (17, \text{p. } 413) \quad n = 56 \]
      \[ c = 3l = 15 \]
      \[ r^2 = 12600 \quad \text{or} \quad r = 112 Å \]
      \[ D_{\text{effective}} = (.8)(2)(112) = 195 Å \]

2. Typical Monolayer Calculation

   a) Collapsed Helical Coil - axially on surface

      Area/molecule = 1200 Å²

      Surface Heparin = \[ \frac{10^{16}}{1200} \times \frac{15000}{6.02 \times 10^{23}} = .2 \mu \text{gm/cm}^2 \]
APPENDIX H

Membrane Diffusion Calculations

1) Liquid Side Resistance

The influence of the liquid side resistance for the membrane diffusion cell was estimated with the correlation suggested by Marangozis and Johnson (72) for mass transfer at flat surfaces. The results of these calculations are presented in Figure 4-43 and sample calculations have been presented by Jouris (52).

2) Working Equation

The working equation for the membrane diffusion studies, 
\[
\ln \left( \frac{\text{CPM} - \text{CPM(Initial)}}{2} \right) = -10K_m \cdot T,
\]

has been derived by Jouris (52) and the reader is referred to his work for the derivation.
APPENDIX I

Urea Diffusion in Human Blood

a) Calculation of Reservoir End Effects

From (31):

\[
\varepsilon(R, D(\text{urea}) T) = R \left[ 0.806 - 0.604(D(\text{urea}) T) + 0.1658(D(\text{urea}) T)^2 
- 0.0172(D(\text{urea}) T)^3 - 0.000436(D(\text{urea}) T)^4 \right]
\]

For 20 to 24 hour run with:

\[ T \approx 10^5 \text{ sec} \]

\[ D(\text{urea}) = 10^{-5} \text{ cm}^2/\text{sec} \]

\[ R = 0.070 \text{ cm} \quad \text{therefore} \]

\[ \varepsilon(R, D(\text{urea}) T) = 0.017 \]

Now:

\[ D(\text{urea}) \text{ Meas.} \left[ 1 - \varepsilon(R, D(\text{urea}) T) \right] D(\text{urea}) \text{ Actual} \]

\[ D(\text{urea}) \text{ Meas.} = 0.98 D(\text{urea}) \text{ actual} \]

b) Calculation of \( D(\text{urea}) \) from \( \ln \frac{C}{C_0} \) vs. \( T \text{ min} \).

Typical calculation \( D(\text{urea}) \) in saline 20°C.

slope = \( \ln \frac{0.71}{0.40} = 0.570 \times 10^{-3} \)

\[ (21.75-5.00) \times 60 \]

\[ D(\text{urea}) = \frac{0.570 \times 10^{-3}}{45.8} = 1.23 \times 10^{-5} \text{ cm}^2/\text{sec} \]
DATA

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c) Calculation of D(urea) from Prager's Model (85, 86)

\[
D(\text{urea}) = D(\text{plasma}) \sqrt{p} \left( \frac{\sigma_{rc} + \sigma_{p} + \ln \left( \frac{\sigma_{p}}{\sigma_{rc}} \right)}{2 \left( \sigma_{rc} + \sigma_{p} + \ln \left( \frac{\sigma_{p}}{\sigma_{rc}} \right) \right) - \frac{1}{2} \sigma_{p} \ln \left( \frac{\sigma_{p}}{\sigma_{rc}} \right)^{2}} \right)
\]

\[ H = \sigma_{rc} (-2\% at \ max) \]

\[ H = 40: \sigma_{rc} = 40, \ \sigma_{p} = 60 \]

\[ \sigma_{rc} = \text{red cells} \]

\[ D(\text{plasma}) = .9 \times 10^{-5} \]

\[ D(\text{urea}) = (.9 \times 10^{-5})(.6) \left( \frac{.4 + 6(-.52)}{2(.09) - .3(.25)} \right) = .46 \times 10^{-5} \text{cm}^2/\text{sec} \]
APPENDIX J

LITERATURE CITATIONS


94. Salzman, E.W. Massachusetts General Hospital, Boston, Massachusetts. Personal communication.


97. Satkowski, W.B. and Hsu, C.G. "Polyoxyethylation of Alcohol." Industrial and Engineering Chemistry. 49:1875,


NOMENCLATURE

A - area, cm$^2$
C - concentration, Wt/cm$^3$
Cb - concentration in dialysate bath
Cl - Inlet concentration
Co - Outlet concentration
Ct - Concentration as a function of time
D - Diffusion coefficient, cm$^2$/sec
Db - Diffusion coefficient thru blood, cm$^2$/sec
Dm - Diffusion coefficient thru a membrane, cm$^2$/sec
Dy - Dialysance, ml/min
h - Channel thickness, cm
K - Mass transfer coefficient, cm/sec
Kov - Overall mass transfer coefficient, cm/sec
Kbl - Blood-side mass transfer coefficient, cm/sec
Ko - Dialysate-side mass transfer coefficient, cm/sec
L - Membrane thickness, cm
MW - Molecular weight, (weight average)
Rbl - Blood-side mass transfer resistance, sec/cm
Ro - Dialysate-side mass transfer resistance, sec/cm
Rm - Membrane mass transfer resistance, sec/cm
r - radius, cm
Qb - Blood flow rate, ml/min.
T - time
V = Volume, cm³

Ve = Angular velocity, cm/sec

ρ = density, mg/cm³

σ = Volume fraction

μ = Viscosity, poise

ω = Angular velocity, radians/sec

Γ = Shear stress, dynes/cm²

Γwall = Shear stress at the wall, dynes/cm²

μc = Microcuries

**ABBREVIATIONS**

CAIH = Heparinized Cellulose Acetate

CIH = Heparinized Regenerated Cellulose

CPM = counts per minutes

EtI = Ethylenimine

EtO = Ethylene Oxide

GBH = Graphite-Benzalkonium-Heparin

H = Hematocrit

IVC = Inferior Vena Cava

PEI = Polyethylenimine

RCF = Regenerated Cellulose Film

WBC = Whole Blood Clotting(test)
BIOGRAPHICAL NOTE

The author was born on September 4, 1940 in Connersville, Indiana. Here, he received his early schooling. The author entered Purdue University in 1958 and graduated in June, 1962 with a Bachelor of Science in Chemical Engineering. While at Purdue he was elected to Tau Beta Pi, Omega Chi Epsilon, Phi Eta Sigma, and Omicron Delta Kappa.

The author entered the graduate school at M.I.T. in the fall of 1962. During his graduate studies he has held a National Science Foundation Fellowship, except for a period of summer employment in 1963 with Esso Production Research Co. of Houston, Texas. While at M.I.T. he received a Masters of Science in Chemical Engineering in June 1963 and was elected to the society of Sigma Xi in 1965. Recently, February 5, 1966, the author married the former Ruth Caldwell. Plans beyond an extensive honeymoon have not yet become definite.