TRANSPORT OF FLUID AND SOLUTES IN THE BODY

A Compartmental Model Approach

By

Cristina C. Gyenge

B. A. Sc. (Chemical Engineering) University 'Babes-Bolyai', Cluj, Romania, 1990

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE STUDIES
CHEMICAL and BIOLOGICAL ENGINEERING

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
September, 2000
© Cristina C. Gyenge, 2000
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemical and Biological Engineering

The University of British Columbia
Vancouver, Canada

Date 6/ October 2000

DE-6 (2/88)
Abstract

A mathematical model is formulated to study the transport and redistribution of fluid, proteins and small ions between the circulating blood, interstitium and cells. To achieve this task, the human/animal body was schematically divided into two distinct compartments, namely the plasma and interstitium. Two additional cellular compartments representing the red blood cells and generalized tissue cells were introduced as sub-compartments embedded in the two extracellular compartments.

Two major sites of exchange are accounted for to characterize the movement of materials between these four fluid compartments. The microvascular exchange system (MVES) involves the movements of fluid, proteins and small ions from plasma into the interstitium across the capillary membrane as well as the return of these materials from the interstitial space back into plasma via the lymphatic system. Across the cellular membrane separating the intra- and extracellular compartments, there are dynamic exchanges of fluid and the three important ions, Na\(^+\), K\(^+\) and Cl\(^-\). These exchanges are assumed to occur by both passive and active mechanisms.

The general model consists of a large set of time-dependent differential-algebraic equations that must be solved simultaneously to predict both clinically measurable data (e.g., plasma and blood volumes, plasma solute concentrations, and osmotic pressures) and experimentally difficult or impossible to measure variables (e.g., intracellular volumes and small ion concentrations, cellular transmembrane potentials, and transmembrane fluid shifts). The solution of these equations is carried out by the use of numerical methods.

To describe mass exchange within the MVES and across the cell membranes, the transport characteristics of the principle resistances encountered by the exchanging materials must be known. The set of transport parameters needed to describe fluid and protein exchanges across the capillary membrane and within the lymphatic system were estimated previously from human data by other researchers in our group. As part of the present work, the transport parameters related to the movement of small ions across the capillary membrane (i.e., the reflection coefficient, \(\sigma_{\text{ION}}\), and the permeability-surface area product, \(\text{PS}_{\text{ION}}\)) were estimated using data from studies in which animals were successively infused with iso-osmolar saline (NS)
and hyperosmolar saline (HS) solutions. Also, the transport parameters associated with cellular exchange (i.e., the cell membrane permeabilities for sodium, potassium and chloride, $p_{Na}$, $p_{K}$ and $p_{Cl}$, as well as the rate of the sodium-potassium pump, $RP$, were determined from the steady-state equations that describe cell volume regulation, together with the known normal distribution of ions between the intra- and extracellular fluids. Additional transport parameters required to accommodate external infusions of macromolecular species such as dextran were obtained from the literature.

The validation of the model with these newly introduced parameters was carried out by comparing model-predicted results with experimental data from animals and humans that had undergone different resuscitation protocols (i.e., different rates and volumes of fluid administration using different types of infusates (NS, Ringer's solution (RS), HS or hyperosmotic saline/dextran solution (HSD)). Considering the physiological complexity of the body, the model-predicted results compared very well with the experimental data in the majority of cases simulated.

As a subset of this study, mathematical expressions are developed to describe the excretion of fluid and small ions by the kidney. The formulation of this renal model is based on the physiological role of the kidney in maintaining the plasma volume and plasma sodium concentration at their normal values. Thus, it is assumed that the kidney responds via a negative feedback to any changes in these two values from their normal set-points. The generalized four-compartment model that includes the 'kidney module' was tested using experimental animal and human data involving infusions of NS or HS solutions, or non-treated hemorrhages. The model predictions were generally in very good agreement with the measured results for all the cases simulated.

Finally, the applicability of the model to the study of hemorrhagic shock was exemplified through a series of simulations that describe the distinct stages in the progression of shock. Empirical equations were proposed to characterize the release of glucose and other solutes that occur during the compensatory (hemodilution) phase of hemorrhage, as well as the disturbed cellular transport that takes place during the decompensatory (hemoconcentration) stage of shock. The weaknesses and strengths of the model to clarify certain mechanisms related to hemorrhagic shock were underlined.
Table of Contents

Abstract ii
List of Figures ix
List of Tables xv
Acknowledgments xviii

Chapter 1: INTRODUCTION 1

Chapter 2: PHYSIOLOGICAL OVERVIEW OF THE COMPARTMENTS OF STUDY 7

2.1 Introduction ......................................................... 7
2.2 Water Distribution in Fluid Compartments ................................ 7
2.3 Distribution of Solutes between Different Fluid Compartments .......... 9
2.4 Characterization of the Vascular Compartment .......................... 11
  2.4.1 Cardiovascular system and vascular segments ....................... 11
  2.4.2 Composition of blood ........................................... 15
    2.4.2.1 Cellular fractions of blood .................................. 16
    2.4.2.2 Plasma ..................................................... 16
2.5 Characterization of the Interstitial Compartment ......................... 17
  2.5.1 Structure of the interstitium .................................... 17
    2.5.1.1 Interstitial fluid .......................................... 18
    2.5.1.2 Structural molecules present in interstitium ............... 18
  2.5.2 Physical characteristics of the interstitium ....................... 19
    2.5.2.1 Volume exclusion ............................................ 19
    2.5.2.2 Interstitial compliance .................................... 20
2.6 Characterization of the Lymphatic System ............................... 21

Chapter 3: TRANSPORT ACROSS THE CAPILLARY AND CELLULAR MEMBRANES 24

3.1 Introduction ................................................................ 24

PART I: TRANSCAPILLARY EXCHANGE 24

3.2 Structure and Function of the Microcirculation ......................... 24
3.3 The Capillary Wall and its Transport Pathways .......................... 25
3.4 Transcapillary Transport Equations .................................... 27
  3.4.1 Fluid transport across the capillary wall .......................... 27
  3.4.2 Protein transport across the capillary wall ....................... 31
  3.4.3 Transport of small ions across the capillary ...................... 32

PART II: CELLULAR EXCHANGE ...........................................
8.4.2.2 Renal response to hyperosmolar (HS) or hyperosmolar/ hyperoncotic (HSD) infusions ............................................. 166
8.4.2.3 The renal response to hypovolemia caused by external hemorrhage (HEM) .................................................. 167
8.5 Model description .......................................................... 169
  8.5.1 General assumptions ................................................. 169
  8.5.2 Model equations ..................................................... 171
  8.5.3 Initial conditions for renal excretion in humans ............... 177
  8.5.4 Parameter estimation .............................................. 178
    8.5.4.1 Estimation of \( k_E^u \) using data from Watenpaugh et al. 178
    8.5.4.2 Estimation of \( k_D^u \) using data from Lucas and Ledgerwood 179
    8.5.4.3 Estimation of \( k_{Na}^u \), \( k_K^u \) and \( k_{Cl} \) using data from Tølløfsrud et al. 182
    8.5.4.4 Estimation of \( k_c \) based on the data of Cannon et al. 184
  8.5.5 Model validation and discussion ................................ 191
    8.5.5.1 Validation based on NS or HSD infusions in normal swine 192
    8.5.5.2 Validation based on data from mild hemorrhage followed by HSD administration ............................................. 201
    8.5.5.3 Validation based on data from graded hemorrhages in pigs 206
8.6 Conclusions ..................................................................... 207

Chapter 9: APPLICATION OF THE MODEL TO THE STUDY OF HEMORRHAGE 212
  9.1 Introduction ................................................................ 212
  9.2 Hemorrhagic Shock ....................................................... 213
    9.2.1 Classification of hemorrhagic shock ............................ 213
    9.2.2 Progression of hemorrhagic shock ............................... 215
    9.2.3 Hemodilution phase .................................................. 217
    9.2.4 Hemoconcentration phase ......................................... 222
      9.2.4.1 Cellular damage during shock .............................. 223
  9.3 Application of the model to the study of hemorrhage .......... 228
    9.3.1 Proposed time-course of events for modeling hemorrhage 228
    9.3.2 Model description .................................................. 230
    9.3.3 Simulated hemorrhage scenarios ............................... 235
      9.3.3.1 Simulation of hemorrhage in the absence of glucose release 
          9.3.3.2 Simulations of glucose release and osmolarity increase after blood removal 246
      9.3.3.3 Cellular defect in hemorrhagic shock .................... 257
      9.3.3.4 Application of the model in identifying the hemodilution and hemoconcentration stages of hemorrhage 273

Chapter 10: CONCLUSIONS AND RECOMMENDATIONS 276
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Distribution of body fluids in compartments as a percentage of body weight for a 70 kg man.</td>
<td>8</td>
</tr>
<tr>
<td>2-2</td>
<td>Distribution of blood volume in different segments of the circulatory system.</td>
<td>13</td>
</tr>
<tr>
<td>2-3</td>
<td>Distribution of cardiac output to several organs in normal human at rest.</td>
<td>15</td>
</tr>
<tr>
<td>2-4</td>
<td>The change from normal of interstitial fluid volume $\Delta V_{IT}$ (ml/g dry weight) vs. the change from normal of interstitial pressure, $\Delta P_{IT}$ (mmHg), for experiments with dogs.</td>
<td>21</td>
</tr>
<tr>
<td>2-5</td>
<td>Schematic diagram of the initial lymphatic and collecting vessels.</td>
<td>23</td>
</tr>
<tr>
<td>3-1</td>
<td>Transport of different types of solutes (see below) through the multiple pathways identified for transcapillary exchange.</td>
<td>26</td>
</tr>
<tr>
<td>3-2</td>
<td>The Starling forces that contribute to fluid exchange across the capillary membrane.</td>
<td>29</td>
</tr>
<tr>
<td>3-3</td>
<td>Schematic representation of the Donnan partition of small ions across the capillary.</td>
<td>31</td>
</tr>
<tr>
<td>3-4</td>
<td>Distribution of ionic species across the cellular membrane as a consequence of the different types of transport mechanisms.</td>
<td>41</td>
</tr>
<tr>
<td>4-1</td>
<td>Schematic of a general model of fluid, protein, small ion and additional solute (introduced through infusion) exchange between plasma interstitium and cells.</td>
<td>56</td>
</tr>
<tr>
<td>4-2</td>
<td>The 'most-likely' human compliance curve with its three distinct segments for dehydration, moderate hydration and overhydration.</td>
<td>73</td>
</tr>
<tr>
<td>6-1</td>
<td>Combined sum-of-squares-of-differences between the model predictions and Wolf's [1982] plasma volume and plasma osmolarity measurements as a function of $PS_{ION}/PS$ and $\sigma_{ION}$.</td>
<td>107</td>
</tr>
<tr>
<td>6-2</td>
<td>Comparison of the model predictions for plasma volume changes vs. time with experimental data from Wolf [1982].</td>
<td>108</td>
</tr>
</tbody>
</table>
6-3 Comparison of the model predictions for changes in plasma osmolarity vs. time with experimental data from Wolf [1982].

6-4 Comparison of the model predictions for blood volume change vs. time with experimental data from Manning and Guyton [1980].

6-5 Comparison of the model predictions for plasma protein concentration change vs. time with experimental data from Manning and Guyton [1980].

6-6 Comparison of the model predictions for extracellular volume change vs. time with experimental data from Manning and Guyton [1980].

6-7 Starling forces: Model predictions performed according to the experimental protocol described by Wolf [1982] for the NS infusion.

6-8 Model predictions for the relative transcapillary flow (i.e., $J_{IT}/J_{IT,NL}$) vs. time, for RS infusions [Manning and Guyton, 1980].

6-9 Model predictions for changes in cell volume vs. time following HS infusion [Onarheim, 1995].

6-10 Model predictions for the relative transcapillary fluid flow vs. time after HS infusion based on the studies of Onarheim [1995] and Wolf [1982].

7-1 A schematic diagram depicting the compartments and flows which comprise the human model.

7-2 Model predictions and experimental data [Watenpaugh et al. 1992] for changes in plasma volume ($V_{PL}$), the colloid osmotic pressure of plasma ($\pi_{P,PL}$) interstitial fluid volume ($V_{IT}$), hematocrit (Hct) and the net transcapillary fluid flow, after NS infusion.

7-3 Model predictions and experimental data involving HSD infusion in normovolemic humans by Tøløfsrud et al. [1997].

7-4 Model predictions and experimental data involving HSD infusion in hypovolemic humans by Tøløfsrud et al. [1997].

7-5 Model predictions for the hypovolemic condition described in Tøløfsrud et al. [1997] for interstitial fluid volume ($V_{IT}$), red blood cell volume ($V_{RBC}$) and interstitial cell volume ($V_{TC}$).

7-6 Model predictions for the hypovolemic condition described in Tøløfsrud et al. [1997] for transcapillary fluid exchange ($J_{IT}$) and lymph flow ($J_{L}$).
7-7 Model predictions of blood volume dynamics for three HSD infusion times.

8-1 Differences between a cortical nephron and a juxtamedullary nephron.

8-2 Schematic of a functional nephron indicating the renal corpuscle composed of the glomerulus and Bowman's capsule and a series of tubules.

8-3 The three main mechanisms of urine formation.

8-4 Schematic representation of kidney part of the overall model of whole-body fluid and solute exchange.

8-5 Average experimental urinary output reported by Watenpaugh et al. [1992] vs. average model predicted urinary outputs obtained by using the estimated parameter $k_u^E$.

8-6 Calculated urinary output rate vs. change in plasma volume obtained by using the estimated $k_u^D$ and $k_u^E$ constants.

8-7 Comparison between the computed averages urinary rates and the experimentally measured values for the normovolemic HSD case investigated by Tøllofsrud et al. [1997].

8-8 Comparison between the computed averages for sodium excretion rates and the corresponding experimentally measured values for the normovolemic HSD case investigated by Tøllofsrud et al. [1997].

8-9 Comparison between the computed averages for sodium concentration in urine and the experimentally measured values for the normovolemic HSD case investigated by Tøllofsrud et al. [1997].

8-10 Comparison between the computed averages for potassium excretion rates and the corresponding experimentally measured values for the normovolemic HSD case investigated by Tøllofsrud et al. [1997].

8-11 Comparison between the computed averages for potassium concentration in urine and the experimentally measured values for the normovolemic HSD case investigated by Tøllofsrud et al. [1997].

8-12 Comparison between the computed averages for chloride excretion rates and the corresponding experimentally measured values for the normovolemic HSD case investigated by Tøllofsrud et al. [1997].
8-13 Comparison between the computed averages for chloride concentration in urine and the experimentally measured values for the normovolemic HSD case investigated by Tølløfsrud et al. [1997].

8-14 Least-squares fitting of the experimental data for the change in $\mathrm{C^{2+}}$ excretion rate vs. the change in sodium excretion rate at peak natriuresis after HS fluid infusions.

8-15 Comparison between model predictions and experimental data for Hct values from the study of Sondeen et al. involving NS infusion in swine.

8-16 Model-predicted for continuous changes in plasma volume and the comparison between model predictions and experimental data for the urinary output values from the study of Sondeen et al. [1990(a)] involving NS infusion in swine.

8-17 Experimental data from Sondeen et al. [1990(a)] and model-predicted changes in the concentration of sodium in plasma and the relative change in the sodium excretion rate after NS infusion in swine.

8-18 Comparison between model predictions and experimental data for Hct values from the study of Sondeen et al. [1990(a)] involving HSD infusion in swine.

8-19 Model-predicted for continuous changes in plasma volume and the comparison between model predictions and experimental data for the urinary output values from the study of Sondeen et al. [1990(a)] involving HSD infusion in swine.

8-20 Experimental data from Sondeen et al. and model-predicted changes in the concentration of sodium in plasma and the relative change in the sodium excretion rate after HSD infusion in swine.

8-21 Comparison between the computed average urinary rates and experimentally measured values for the hypovolemic HSD case investigated by Tølløfsrud et al.

8-22 Model-predicted sodium renal clearances of sodium following HSD infusions for normovolemic humans (solid line) and mildly hemorrhaged humans (dashed line) based on the hypovolemic HSD case investigated by Tølløfsrud et al. [1997].

8-23 Urinary output profile predicted by the model for increasing degrees of hemorrhage and the comparison between the computed average urinary rates with the experimentally measured values based on the work of Sondeen et al. [1990(b)].
Sodium excretion rate profile predicted by the model for increasing degrees of hemorrhage and the comparison between the computed average sodium excretion rates with the experimentally measured values reported by Sondeen et al. [1990(b)].

Comparison between the model-predicted renal clearance of sodium and the values calculated using measured data from Sondeen et al. [1990(b)].

The change in arterial pressure after different degrees of hemorrhage.

Changes in blood pressure, transcellular membrane potential and plasma and interstitial potassium concentrations as a function of time post-hemorrhage.

A schematic diagram depicting the compartments and flows, which comprise the hemorrhage model, is presented.

Predicted changes in the compartmental fluid volumes after a 10% and a 30% hemorrhage of long duration.

Model predictions for normalized transcapillary and lymphatic fluid flow rates after a 10% and a 30% hemorrhage of long duration.

Model predictions for the capillary and interstitial hydrostatic pressures after a 10% or a 30% hemorrhage of long duration.

Model predictions for the colloid osmotic pressures of plasma and interstitium after a 10% and a 30% hemorrhage of long duration.

Model predictions for Hct from studies involving 10% and 30% hemorrhages of short or long duration.

Model predictions for the compartmental fluid volumes from studies involving 10% and 30% hemorrhages of short or long duration.

Model predictions for fluid excretion rates after 10% and 30% hemorrhages of short or long duration.

Comparison between model-predicted and experimental results of Gann et al. for a 10% hemorrhage.

Comparison between model-predicted and experimental results of Gann et al. for a 20% hemorrhage.

Comparison between model-predicted and experimental results of Gann et al. for a 30% hemorrhage.
9-14 Model predictions of BVR in the presence and absence of solute release to plasma after 10, 20 and 30% hemorrhages.

9-15 Simulation protocol for a severe 34% hemorrhage based on the experiments of Illner and Shires [1980].

9-16 Changes in the membrane potential of interstitial cells after an increase in $p_{\text{Na}_{TC}}$ or a decrease in $\text{RP}_{TC}$.

9-17 Model-predicted changes in the compartmental fluid volumes after a shock-producing hemorrhage which corresponds to the experiment of Illner and Shires [1980].

9-18 The normalized transcapillary and lymph flows after a hemorrhage which corresponds to the experiment of Illner and Shires [1980].

9-19 Comparison of the model predictions with the experimental results of Illner and Shires [1980] for changes in tissue cell concentrations of sodium, potassium and chloride.

9-20 Comparison of the model predictions with the experimental results of Illner and Shires [1980] for changes in plasma concentrations of sodium, potassium and chloride.

9-21 Model-predicted changes of the cell membrane potential of RBCs after a severe hemorrhage followed by an increase in $p_{\text{Na}_{TC}}$ or a decrease in $\text{RP}_{TC}$.

9-22 Model predictions for the Donnan distribution across the capillary after a severe hemorrhage.

9-23 Model predictions for a hypothetical scenario where a severe external loss of blood is followed by an initial 2 h of compensation and a subsequent 2 h of continuous poisoning of the Na$^+$-K$^+$-pump.
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Normal physiological concentration ranges and average values for small ions and proteins showing their partition between the intra- and extracellular compartments.</td>
<td>10</td>
</tr>
<tr>
<td>2-2</td>
<td>Characteristics and function of different vessels of the circulation.</td>
<td>13</td>
</tr>
<tr>
<td>2-3</td>
<td>Average geometric properties of normal red blood cells in humans.</td>
<td>16</td>
</tr>
<tr>
<td>3-1</td>
<td>The permeabilities for red blood cells and skeletal muscle cells.</td>
<td>37</td>
</tr>
<tr>
<td>4-1</td>
<td>Initial steady-state compartmental values for the 70-kg &quot;Reference&quot; man.</td>
<td>81</td>
</tr>
<tr>
<td>4-2</td>
<td>Steady-state values for small ions and proteins showing their partition between intra- and extracellular compartments.</td>
<td>84</td>
</tr>
<tr>
<td>4-3</td>
<td>Calculated and literature values for steady-state cellular membrane parameters.</td>
<td>87</td>
</tr>
<tr>
<td>5-1</td>
<td>Cash-Karp coefficients for the Runge-Kutta-Fehlberg method.</td>
<td>95</td>
</tr>
<tr>
<td>6-1</td>
<td>Examples of compositions for isotonic and hypertonic fluids used most commonly for experimental infusion studies.</td>
<td>99</td>
</tr>
<tr>
<td>6-2</td>
<td>Resuscitation protocols used by Wolf [1982].</td>
<td>101</td>
</tr>
<tr>
<td>6-3</td>
<td>Initial steady-state compartmental values for Wolf's [1982] dogs.</td>
<td>103</td>
</tr>
<tr>
<td>6-4</td>
<td>Initial steady-state compartmental values common to humans and all experimental animals.</td>
<td>105</td>
</tr>
<tr>
<td>6-5</td>
<td>Initial steady-state compartmental values used in animal validation studies.</td>
<td>111</td>
</tr>
<tr>
<td>6-6</td>
<td>Interstitial compliance relationships for 'scaled rat' [Onarheim, 1995] and 'Scaled dog' [Guyton and Manning, 1980].</td>
<td>112</td>
</tr>
<tr>
<td>6-7</td>
<td>Resuscitation protocols for model validation based on dog studies by [Guyton and Manning, 1980] and rat studies by [Onarheim, 1995].</td>
<td>112</td>
</tr>
<tr>
<td>6-8</td>
<td>Comparison between model predictions and Onarheim's [1995] experimental data at 60 minutes after fluid infusion.</td>
<td>113</td>
</tr>
</tbody>
</table>
7-1 Inputs and outputs used for the model for the infusion of NS solution in human subjects [Watenpaugh et al., 1992].

7-2 Inputs and outputs used for the model for the infusion of HSD solution in human subjects. Data corresponds to the normovolemic case, NV, as reported in the original study of Tølløfsrud et al. [1997].

7-3 Inputs and outputs used for the model for the infusion of HSD solution in human subjects. Data corresponds to the hypovolemic case, HV, as reported in the original study of Tølløfsrud et al. [1997].

7-4 Comparison between the model predictions and experimental results for the changes in plasma osmolarity for the experiments of Tølløfsrud et al. [1997].

8-1 Average concentrations of sodium, potassium, chloride and glucose in the protein-free filtrate and in the urine.

8-2 Diagrams of renal and vascular responses to fluid infusion and hemorrhage.

8-3 Vascular and renal responses to different degrees of hemorrhage.

8-4 Initial values for renal excretion in a 70-kg normal human

8-5 Set of estimated parameters of the kidney model obtained by least-square fitting technique.

8-6 Renal excretion of fluid, sodium, potassium and chloride after a 10% hemorrhage followed by 4 ml/kg HSD infusion: comparison between model predictions and experimental data from the study of Tølløfsrud et al. [1997].

9-1 Summary of changes in plasma osmolarity, plasma glucose and other plasma solutes for a severe, non-resuscitated hemorrhage of 35% in cats [Jarhult, 1975].

9-2 The rate of refill of plasma volume at different times after a non-resuscitated 35% hemorrhage in cats Jarhult [1975] and humans (estimated based on Jarhult's data).

9-3 Experimental studies showing the direct correlation between the decrease in arterial pressure and cell membrane depolarization for different animal species.

9-4 Factors hypothesized to be responsible for: cellular swelling, cellular sodium and chloride content increase, and cellular potassium decrease during hemorrhagic shock.
9-5 Literature information regarding the percentage of blood loss where hypovolemic shock was first detected.

9-6 Initial compartmental values for glucose amounts and the transcapillary transport parameters.

9-7 Conditions used for simulating mild and severe hemorrhages.

9-8 Model predictions for small ion concentration in plasma, interstitial fluid and interstitial cells in control (t = 0 h) and during shock characterized by a 23% $V_m$ depolarization (t = 2.95 h).
Acknowledgements

It would be very difficult to adequately thank everyone who either has helped make this work possible or had been an important part of my life during my stay in this university. First of all, I would like to thank to my two supervisors, Professors J. L. Bert and B. D. Bowen, for giving me the opportunity to pursue this work and for their continuous guidance.

Dr. Bert was instrumental in making this thesis possible. He was responsible for keeping things on track and for being an important driving force in surpassing many obstacles encountered during this project. On both professional and personal levels, I have learned a lot from him, but like any other student most probably not enough. If I were to single out an important piece of advice I received from him it would be ... keep it simple. Keeping it simple was the most complex task I was called upon to master and, unfortunately, it is still a work in progress. I take this opportunity, therefore, to thank him for all the useful advice, time, understanding and continuous effort put into my development during these past few years.

Gertrude Stein once said, 'a difference, to be a difference, must make a difference'. Among the many people whom I had the fortune to meet during my work here, Dr. Bowen was the person who made that difference. I would like to extend my sincere thanks to him for his guidance, tireless patience and effort put into making this work better.

Several friends and colleagues brought a lot of enjoyment to our life. Although they are too many to be enumerated, I am very grateful for their presence. My kind thoughts are especially extended to a dear friend, Antonio. Our lively conversations, exchange of ideas and good times are dearly missed. Also, the staff in the Chemical and Biological Engineering Department was always gracious and prompt in helping me in times of need. I thank them for this and for creating a pleasant environment in our department.

I would also like to thank my parents for their support and love. It must be very difficult for a parent to let an offspring fly when the time comes. It must be also very difficult to visualize your child through occasional photographs and scribbled lines. I thank them for understanding that all this effort was necessary in order for me to become who I am.

Last but not least, I am greatly indebted to Elod for making everything possible. His kind presence, unconditional love, continuous support and encouragement gave me the unique opportunity to understand the unbelievable depth and force of still waters. Thank you Elod for being here.
Chapter 1: INTRODUCTION

To gain an improved understanding of how the body responds to different perturbations, scientists have attempted to simulate the behaviour of the real system through the use of models. In fact, experimental measurements are not ultimately directed toward simply acquiring an extensive collection of data; rather, their purpose is to achieve a 'model image' of how and why the body functions the way it does under normal conditions, or how it can be assisted in resuming its normal function under perturbed circumstances. A variety of models have been developed to serve this purpose. Such models can be qualitative (e.g., a conceptual description of the cellular membrane), experimental (e.g., use of animal models for studying hemorrhage) or mathematical (e.g., mass balances to investigate the redistribution of materials within the body). Each of these examples can, in turn, give rise to more and more complex representations of the system depending, ultimately, on the purpose of the model. All models are idealized speculative representations of reality and, hence, have to be constantly questioned and tested against the information obtained from the real system. As science progresses, and as the collection of experimental data provides more and more useful information, any model needs to be subjected to continuous changes and improvements or, if proven unsatisfactory, discarded in favor of more realistic descriptions.

In the present work, a mathematical model that aims to approximate and interpret the complex dynamics of material exchange within an animal or human body is presented. Specifically, this work focuses on the distribution and transport of fluid, proteins and small ions between the plasma, interstitium and cells, based on the two important sites of exchange within the body, namely the microvascular exchange system (MVES) and the transport across the cell membranes.

When the body is at steady state, there is an unchanging balance of fluids, proteins and small ions, characterized by no net movement of materials across the capillary membrane, that separates the vascular and interstitial compartments, as well as across the cellular membrane that separates the intra- and extracellular spaces. Over a reasonable (e.g., 24 h) averaging period, the external inputs to the body in the form of food, fluids, etc., exactly balance the outputs from the body in the form of renal excretions, sensible/insensible losses, etc. However, extrinsic disturbances to the system (such as hemorrhage or burns) or intrinsic ones (such as venous
constriction, venous dilation, hypoproteinemia, or cellular damage) compromise the normal physiological fluid distribution between the vascular, interstitial and cellular compartments, and furthermore, influence the renal elimination of fluid and solutes from the body. All of these disturbances have a direct effect on the health and well-being of the body.

In order to better understand the interplay of different mechanisms and forces acting at the capillary and cellular levels under both normal and perturbed conditions, mathematical models have been used as an important complement to experimental studies. These models, which are now commonly-used tools for describing physiological systems, can be categorized into two general classes: 1) compartmental models and 2) distributed models.

In the former case, the system is defined by a series of compartments and sub-compartments that are assumed to be homogenous and well-mixed. Thus, fluid and solutes entering the compartment are instantaneously dispersed throughout its entire volume. Fluid and solutes are exchanged between compartments at rates that are directly proportional to a driving force (mechanical, chemical or electrochemical) and inversely proportional to the resistances encountered in crossing a given boundary. Mass balance equations for fluid and each of the solutes (e.g., proteins, ions) involved, combined with auxiliary transport equations describing mass-exchange rates or the properties of the individual compartments (colloid osmotic pressure, pressure-volume relationship, etc.) are written to characterize the system. The mathematical formulation of a compartmental model can be described by a set of time-dependent ordinary differential equations. The degree of complexity of the model depends on the number of compartments considered and the number of transported species accounted for in these models. One major disadvantage of these types of models is their inability to provide any information about local gradients and mass transport behavior within an individual compartment.

Distributed models, on the other hand, try to account for spatial heterogeneity within a compartment. In these types of models, the compartment is usually a specific organ or even a small part of an organ. Volume averaging procedures are applied and a continuum representation of the system is obtained. Usually this leads to a set of non-linear, coupled, partial differential equations which necessitate a far more complex mathematical description than the compartmental models and a more demanding computational effort. For these reasons, distributed models are difficult to employ when dealing with a large number of transporting species, especially when the information available with respect to the properties of a given compartment is scarce.
Previous studies by our group have approached the representation of the MVES system through both compartmental [e.g., Bert et al., 1988] and distributed [e.g., Taylor, 1996] models. Because of our interest in developing a model that can be used to simulate the behaviour of the whole body in the presence of externally-applied perturbations, a compartmental approach was adopted here.

Much of the previous effort in developing compartmental models has been directed towards mathematically describing the transport properties of the capillary wall [Curry, 1986; Haraldson, 1986], simulating whole body fluid and plasma protein exchange under both normal and pathological states [Guyton et al., 1973; Wiederhielm, 1979; Arturson et al., 1984; Bert et al., 1988; Carlson et al., 1996] and predicting the effect of resuscitation with iso- and hyperosmolar solutions [Wolf, 1982; Mazzoni et al., 1988]. Other models, fewer in number, have also focussed on the topic of transport across the cellular membrane of an isolated cell [Jakobsson, 1980; Gordon and MacKnight, 1991].

Each of the models cited above provides insight into various aspects of either the MVES or transport between the intra- and extracellular media. However, in all the cases cited, the mathematical models are limited in scope. Common weaknesses of many of these models are incomplete and/or out-of-date descriptions of both the MVES and cellular transports. For example, although Wolf [1982] attempted to emphasize the importance of cells during resuscitation with hyperosmolar solutions, his model did not consider a detailed description of solute transport through the cellular membrane.

The goal of the present work, therefore, is to develop a more general mathematical model which describes the combined effect of cellular and transcapillary transport during non-traumatic perturbations such as infusions with varied resuscitants, and following traumatic perturbations such as hemorrhage. This work extends the previous compartmental MVES models developed by our group [e.g., Bert and Pinder, 1982; Bert et al., 1988; Bowen et al., 1989; Chapple et al., 1993; Xie et al., 1995]. Initially, the interpretation of the role of the MVES in the dynamics of whole body fluid and solute distribution was approached by providing a realistic characterization of the interstitial and plasma compartments and by establishing appropriate relationships for the interstitial and plasma compliances together with the capillary and lymphatic transports. These early MVES models were concerned with fluid and protein redistribution under normal conditions, and incorporated transport parameters that were estimated based on statistical comparison of the model predictions with experimental data that corresponded to this case. The
good predictions offered by these MVES models under normal circumstances made it possible to further increase their complexity to allow the investigation of traumatic conditions. For example, a model developed to simulate burn injuries [e.g., Ampratwum et al., 1995; Bert et al., 1997] considered the transport of fluid and proteins between three fluid compartments, namely, plasma, a normal interstitium and an injured (burned) interstitium. In the present work, the complexity of these previous MVES models is taken a step further to account for more fluid compartments (i.e., intracellular compartments), more species participating in transport (i.e., small ions), an independent description of the renal function, and new traumatic scenarios such as hemorrhagic shock.

The work undertaken here can be divided into three major sections that can be summarized as follows:

1) First, a generalized compartmental model that includes additional cellular sub-compartments is formulated and validated against experimental data. The newly introduced cellular components become important sites of exchange, for example, when attempting to describe the response of the body to external infusions of hypertonic saline solutions with or without added colloidal compounds. The specific objectives of this section are:
   • to introduce two intracellular compartments, namely the red blood cells and a generalized tissue cell compartment, and to establish their physical and transport characteristics,
   • to elucidate the governing principles of cell volume regulation,
   • to account for the presence of all small ions involved in exchanges across the cellular membranes and to describe their transport between the intra- and extracellular compartments as well as between the plasma and interstitium, and
   • to validate the model by comparing its predictions with animal and human data that involve infusions of different resuscitants and different resuscitation schemes in an otherwise non-traumatized body.

2) Second, a new 'kidney module', that is linked to and used in conjunction with the model of whole-body fluid and solute transport, is formulated. The two specific objectives targeted in this section are:
   • to propose a model formulation of kidney function that accounts for the renal excretion of fluid and all the small ions present in the system,
• to validate the whole-body model with the newly introduced kidney module against data involving infusions in normal animals and/or humans, and against data involving external blood losses through hemorrhage.

3) Third, the applicability of the model to the study of hemorrhage and the evolution of hemorrhagic shock is demonstrated. Hemorrhagic shock is a traumatic condition of considerable interest to physicians. However the succession of events leading to shock is not completely understood and, in some respects, is controversial. The highest priority of this section, therefore, is to assemble a summary of the available literature information on hemorrhagic shock and to clarify the succession and importance of all the pathophysiological mechanisms that can bring about an altered mass exchange within the body. As part of this section the model is used to provide insights into the dynamics of mass exchange that follows the hemorrhage for the following cases:

• in the absence of a hyperosmolar compensatory response,
• in the presence of an increased plasma osmolarity due to the release of glucose and/or other solutes, and
• during a severe shock state, in the presence of an impaired cellular function that causes disturbances in the transport of water and small ions across the cell membranes.

Additionally, based on the model-predicted results, this section attempts to identify if the cellular defect that occurs after a massive hemorrhage is the pathophysiological mechanism responsible for vascular collapse observed in the final stages of shock.

The rest of the thesis is organized as follows. Chapter 2 briefly introduces the physiological background required for understanding the mass-exchange behaviour of the compartments under study. Chapter 3 provides both a qualitative and quantitative description of the various exchanges that take place across the two important barriers within the body, namely, the capillary and cellular membranes. Chapter 4 presents the general model of microvascular and cellular exchange, while Chapter 5 describes the important numerical methods used in solving the model equations. Chapters 6 and 7 provide separate validations of the general model using experimental data obtained for animals and humans, respectively. Data obtained for the administration of resuscitant fluids that are of ongoing interest in clinical research (e.g., hypertonic saline solutions with or without added colloids) were included in these validation studies. Due to its central role in controlling the body's fluid and solute reserves, a kidney module is another important feature that was lacking in previous models. A new approach to
modeling the renal function, based on clinical concepts, is presented in Chapter 8. In Chapter 9, the complete model, which includes cellular compartments and a kidney module, is applied in a preliminary study of hemorrhagic shock. Finally, Chapter 10 summarizes the important conclusions resulting from this work and recommends several avenues for extending and further testing this model.
Chapter 2: PHYSIOLOGICAL OVERVIEW OF THE COMPARTMENTS OF STUDY

2.1 Introduction

On average the total body water (TBW) accounts for approximately 55 to 60% of the male body weight (BW) and around 50 to 55% of the female BW [e.g., Guyton, 1991]. This is the medium in which solutes (organic and inorganic) found in the human body are dissolved. One of the most important functional features of the body is to maintain constancy of the volume of water and its dissolved solutes. Under normal circumstances, over a reasonable period (e.g., during the course of a 24-h day), the amount of water and solutes taken into the body (by ingestion through the gastrointestinal tract and by metabolic production) is equal to the amount of water and solutes lost from the body (mainly through the kidneys but to some extent through lungs, skin, etc.).

Beyond this simple mass balance that imposes equality between the inputs and outputs of the body, are complex physiological processes such as capillary and cellular exchanges that act synergistically to bring about this task. These exchanges normally provide a sufficient turnover of water and solutes between the different compartments of the body that homeostasis is assured. In abnormal situations (e.g., overhydration, hemorrhage, burns, etc.), when these exchanges are altered, the volume and composition of all the fluid compartments that participate in exchange are compromised. In order to understand the exchange processes mentioned above, it is essential to first understand what compartments are involved in exchange and which solutes are exchanged between them. The objective of this chapter, therefore, is to give a general overview of the compartments that participate significantly in fluid and solute exchanges within the human body. The following discussion is primarily concerned with normal physiological conditions with only brief references made to traumatic conditions, where necessary.

2.2 Water Distribution in Fluid Compartments

The total body water of about 42 l in the average human can be divided into two compartments. The fluid found inside the cells is called the intracellular fluid. This is the medium in which all the biochemical reactions associated with cell metabolism occur. About 65 to 75% of the total body water (or about 28 to 30 l) is contained within the cellular compartment [Guyton, 1991; Berne and Levy, 1988]. All of the fluid found outside the cells is known as
extracellular fluid. The intracellular volume (ICV) and the extracellular volume (ECV) are separated by a semi-permeable cellular membrane. In normal humans, the ECV accounts for about 27 to 35% of the TBW (or about 12-15 l) [Guyton, 1991; Kleinman and Lorenz, 1984] and includes the plasma volume ($V_{PL}$) and the interstitial volume ($V_{IT}$). The interstitial fluid comprises by far the highest proportion of the ECV, i.e., about 67 to 74%, or the equivalent of 20 to 25% of TBW. The rest of the ECV (or about 7 to 9% of TBW) is plasma.

The percentages presented above can change significantly from one individual to another depending on age, weight, percentage of body fat, and status of hydration. Normally, for modeling studies, it is convenient to express the fluid distribution in the body as a percentage of body weight corresponding to the archetypal 'Reference man'. This hypothetical reference man is defined as a well-hydrated, healthy young man, in supine position who has a height of 170 cm and a weight of 70 kg [Reference Man ICRP 23, 1975]. A summary of the fluid distribution amongst the various compartments discussed above for this 70 kg man is presented in Fig. 2-1.

Figure 2-1. Distribution of body fluids in compartments as a percentage of body weight for a 70 kg man [Reference Man ICRP 23, 1975]. For discussion see main text. Adapted from Arfors and Buckley [1989].
Chapter 2: Physiological Overview

The ECV represents the real 'internal environment' of the body and can be envisaged as a transport system that circulates throughout the body between the vascular compartment and the tissue spaces (i.e., the interstitium). The partition of the ECV between plasma, as part of the vascular compartment, and the interstitial fluid, as part of the interstitium, is determined by the dynamic exchange of fluid and solutes across the capillary membrane and by the physical characteristics of the two fluid compartments. The partition of total body fluids between the intra- and extracellular spaces is governed by exchange across the cellular membranes. Both of these exchanges are very important in the model developed in this study and will be detailed separately in the following chapters.

2.3 Distribution of Solutes between Different Fluid Compartments

Table 2-1 presents a compilation of the literature data [Guyton, 1991; Greger and Windhorst, 1996; Ganong, 1991; Kleinman and Lorenz, 1984] on the distribution of small ions between the ECV and ICV in the human body. As exemplified in this table, although similar organic and inorganic solutes can be found in both compartments, the partition of these solutes between the two is quite different. Each pair of columns in Table 2-1 shows the normal concentration range as well as the normal average value for each solute.

As can be seen from Table 2-1, the principal cation that resides in the ICV is potassium. In normal humans, 98 to 99% of the total body potassium of about 60 mEq/(kg-BW), resides inside the cells and has an average concentration of about 150 mEq/l [Kleinman and Lorenz, 1984]. The remaining 1-2% can be found in the combined plasma and interstitial fluids. By contrast, of the total 27 mEq/(kg-BW) sodium found in the body fluids, approximately 85 to 87% is located in the ECV while the remainder yields an average ICV concentration of about 10 mEq/l [Kleinman and Lorenz, 1984]. The remaining positive species present in the body fluids are represented as a single entity in Table 2-1. These species consist mainly of calcium and magnesium, each having a positive charge of +2.

The negatively-charged chloride ion is the major anion in the ECV. The total chloride content normally found in the body fluids is about 20 mEq/(kg-BW) [Guyton, 1991], of which only about 8 to 9% is located in the ICV. Inside the cells, the other negatively-charged species are the proteins and smaller anions such as sulfates, phosphates and acidic amino-acids.
Table 2-1: Normal physiological concentration ranges and average values (in bold) for small ions and proteins showing their partition between the intra- and extracellular compartments [Guyton, 1991; Greger and Windhorst, 1996; Ganong, 1991; Kleinman and Lorenz, 1984]. The plasma and interstitial fluids are presented as distinct compartments of the ECV in order to provide clear evidence for the Donnan distribution between the two compartments. The values for the intracellular compartment (ICV) are representative average values of skeletal muscle cells.

Table 2-1 presents two separate entries for all anions other than chloride, one for proteins and one for the remaining negative species. The different distribution of proteins (mainly albumin), between the plasma and interstitial compartments, leads to an additional partition of small ions between these compartments due to the Donnan effect. This redistribution of small ions caused by the Donnan effect will be discussed in detail in later chapters.

Transport across the cellular membranes, due to both active and passive mechanisms, brings about the differences in small ion concentrations between the ICV and ECV. Of all the solutes present, Na\(^+\), K\(^+\) and Cl\(^-\) are the most important ions involved in transport across the cellular membrane. These species also play an important role in governing the electrical properties of cells. However, all the solutes presented in Table 2-1 contribute to the osmotic properties of the cells.

Despite the differences in distribution of the various solutes between the ECV and ICV, the total chemical activity of the cations and anions within each compartment must be equal, i.e.,
electrical neutrality must be respected. Additionally, the ICV is in osmotic equilibrium with the ECV, i.e., the osmolarity of the two compartments must be the same.

More about the specific solute compositions of the various fluid compartments will be presented and discussed in later chapters. The remainder of this chapter presents a brief physiological background describing some of the physical characteristics associated with the vascular and interstitial compartments.

2.4 Characterization of the Vascular Compartment

Blood is the medium of the vascular compartment. The circulation of blood throughout the vasculature is carried out by the circulatory system. A description of both the system for transport and the transported material is essential for understanding the role of the vascular compartment in promoting fluid and solute exchanges within the body.

2.4.1 Cardiovascular system and vascular segments

The circulatory system fulfils multiple functions, among which the delivery of nutrients (e.g., O₂, glucose, amino-acids, etc.) to the tissue cells, the removal of metabolic waste products from these cells and the regulation of the body temperature, are just a few. These tasks are achieved through the movement of blood in a close circuit from the heart to the different organs of the body and then, back to the heart. The circulatory system can be divided into the systemic circulation (or peripheral circulation) and the pulmonary circulation.

As depicted schematically in Fig. 2-2, the cardiovascular system is comprised of two components: the heart which acts as a variable pump for blood, and a system of vessels which are branching elastic tubes. The contraction of the right or left ventricles of the heart pumps blood to either the pulmonary or systemic circulation, respectively. In healthy humans at rest, the blood pumped by the heart through the vessels per unit time, i.e., the cardiac output, is around 5-6 l/min. After exiting the heart, the blood enters the aorta which, in turn, branches into arteries, arterioles and ultimately capillaries. The blood from the capillaries is returned to the right side of the heart via the venous segment of the circulation.

Except for the capillaries and venules, all the vessels of the circulation act as passive conduits for blood. The capillaries and to some extent the venules are the major sites of exchange between the blood and interstitium and are referred to as the microcirculation. A summary of the types, characteristics and functions of the various vessels that make up the cardiovascular system
is given in Table 2-2. Except for the large arteries and veins, in order to exemplify the relative sizes of different vessels, all values presented in Table 2-2 correspond to an arbitrary human tissue. It should be noted, however, that these values do differ from tissue to tissue and also with age, gender and species.

The volumetric flow rate of blood that is distributed to specific tissues varies greatly according to the need of the tissue to perform its specific function as well as with the level of metabolic activity within the tissue. The normal distribution of the cardiac output to different organs in the human body, under resting conditions, is depicted in Fig. 2-3. The values indicated are basal values adequate for survival. In both normal and abnormal physiological situations, the increase in the metabolic needs of an organ is paralleled to some extent by an increase in the cardiac output and, therefore, the flow to the organ is augmented. However, the maximal cardiac output in a normal human reaches a limiting value of about 25-28 l/min [Greger and Windhorst, 1996] (i.e., about 4-5 times the baseline value) which represents about half the amount that would be theoretically required by all the combined tissues, to maintain their maximal levels of activity. Therefore, when the blood flow to an organ is augmented, the remaining so-called 'inactive organs', that are not directly involved in a specific process at the time, are collaterally vasoconstricted and maintain blood flows at low basal levels [Greger and Windhorst, 1996]. Examples for different patterns of blood flow can be given for normal as well as traumatic scenarios. For instance, during normal physical exercise skeletal muscles require an increased amount of blood flow which is obtained at the expense of other organs. Alternatively, in a pathological scenario such as hemorrhage, a reduction in blood volume can cause a powerful vasoconstriction of the skeletal muscle, kidney and the splanchnic bed. This will result in a redistribution of blood, by as much as 50%, toward the vital organs (heart and brain).

It would appear from the above discussion (see also the values presented in Fig. 2-3) that, at the tissue level, the circulatory system displays specific blood flow characteristics. However, the functional features of the circulatory system can be generalized and averaged such that a common description can be provided for all parts of the circulatory system.
Figure 2-2. Distribution of blood volume in different segments of the circulatory system. The figure illustrates the two components of the circulatory system, i.e., the pulmonary and the systemic circulations. Although only four generic capillary beds are shown in this simplified diagram, it should be understood that each organ possesses its own microcirculatory site of exchange.

From Guyton [1991].
<table>
<thead>
<tr>
<th>Type of Vessel</th>
<th>Average values</th>
<th>Characteristics and function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (mm)</td>
<td>Number</td>
</tr>
</tbody>
</table>
| Aorta         | 10 - 25       | 1      | 2.5 - 4                           | (80-120)                        | - strong vascular walls  
|               |               |        |                                  |                                 | - conduits for blood under high pressure and high velocity  
|               |               |        |                                  |                                 | (e.g., aorta - 35 cm/sec)*  
| Large arteries| 3 - 10        | 40     | 20                               | (80-100)                        | - strong muscular walls  
|               |               |        |                                  |                                 | - represent the last branches of the arterial system  
|               |               |        |                                  |                                 | - act as control valves though their ability to open/close intermittently  
| Small arteries| 0.02          | 4x10⁷  | 50                               | (75-80)                         | - thin walls  
| Arterioles*   | 0.007         | 4x10⁸  | 700                              | (50-60)                         | - semi-permeable membrane (see also Chapter 3)  
|               |               |        |                                  |                                 | SITES OF FLUID AND SOLUTE EXCHANGE  
| Capillaries   | 0.004         | 2x10⁹  | 2500 - 4000                      | (23-27)                         | - thin walls  
|               |               |        |                                  |                                 | - collect blood and waste products from the capillaries  
|               |               |        |                                  |                                 | - highly distensible  
| Venules       | 0.02          | 10⁹    | 250 - 300                        | (4-10)                          | - thin vascular walls  
|               |               |        |                                  |                                 | - transport blood from the tissues to the heart  
|               |               |        |                                  |                                 | - act as reservoirs for blood (see Fig. 2-2)  
| Small veins   | 0.04          | 10⁸    | 100                              | (1-2)                           | |
| Vena cava     | 12.5          | 1      | 8 - 10                           | ~1                              | |

* the value corresponds to measurements (and/or estimations) under resting conditions

Table 2-2. Characteristics and function of different vessels of the circulation. The table indicates the average estimated diameter, number and cross-sectional areas as well as the average pressure in each of the different segments. Adapted from [Guyton, 1991; Schmid-Schönbein, 1996; Holtz, 1996]
Figure 2-3: Distribution of cardiac output to several organs in normal human at rest. Adapted from Holtz [1996].

2.4.2 Composition of blood

As previously stated, blood is the medium transported in the vasculature. It is a fluid with a viscosity three times higher than that of water [Guyton, 1991] and it accounts for about 5 to 8% of the total body weight [Berne and Levy, 1988; Guyton, 1991]. This fluid consists of a suspension of cellular elements, that occupies approximately 40-45% of the total volume, in an aqueous solution containing various solutes such as small ions, proteins, glucose, etc. [Berne and Levy, 1988]. Centrifugation of blood causes a separation of blood into two fractions: plasma and cells. Clotting of blood allows for a separation of serum from the blood. With the exception of one macromolecular species, fibrinogen, which is removed in the clot, plasma and serum have similar compositions and are both cell-free fluids.
2.4.2.1 Cellular fractions of blood

The cellular fractions of blood include red blood cells (or erythrocytes) (RBCs), white blood cells (leukocytes) and a variety of platelets or cell fragments. RBCs constitute by far the highest proportion of blood cells. The remaining cells contribute only to about $10^{-3}$ of the total cell fraction. Therefore, although they are functionally important, quantitatively they can be safely ignored from a modeling perspective.

By virtue of their accessibility and size, the RBCs have been the subject of numerous investigations. Among the metabolic functions performed by the RBCs, by far the most important is their role in the exchange of $O_2$ and $CO_2$. Some of the averaged physical characteristics of red blood cells are given in Table 2-3.

\[
\begin{array}{l|l}
\text{RBC} & \text{Average Dimensions} \\
\hline
\text{Diameter} & 8.5 - 9 \text{ (µm)} \\
\text{Surface Area} & 150-170 \text{ (µm}^2\text{)} \\
\text{Volume} & 80-90 \text{ (µm}^3\text{)} \\
\end{array}
\]

Table 2-3: Average geometric properties of normal red blood cells in humans [Burton, 1965; Altman and Dittmer, 1971].

The percentage of the total volume of blood occupied by cells is called hematocrit, Hct, and is expressed as,

\[
Hct(\%) = \frac{V_{\text{RBC}}}{(V_{\text{RBC}} + V_{\text{PL}})} \cdot 100 \tag{2-1}
\]

where $V_{\text{RBC}}$ and $V_{\text{PL}}$ are the volumes of red blood cells and plasma, respectively. The normal range of hematocrit values is around 37 to 44% for women and 38 to 49% for men [Ganong, 1991; Guyton, 1991; Reference Man ICRP 23, 1975]. Corresponding to an average hematocrit of 40%, if the intracellular volume of the RBCs is assumed to be all water, it will account for about 2.1 l or 5% of the total body water.

2.4.2.2 Plasma

As presented in Section 2.2 (see also Fig. 2-1), plasma averages between 45 to 50 ml/(kg-BW) of total body weight corresponding to a volume of about 3000 to 3500 ml for humans [Guyton, 1991; Altman and Dittmer, 1971]. By weight, slightly more than 90% of plasma is
plasma is water while plasma proteins account for about 7%. The remaining percentage is made up of organic and inorganic solutes [Berne and Levy, 1988].

The principal solutes normally found in plasma were presented in Table 2-1. The small ions present in plasma are mainly sodium and chloride, moderate concentrations of bicarbonates (about 30 mEq/l) and relatively low concentrations of potassium, calcium, magnesium, phosphates, sulfates and organic acids.

The organic and inorganic solutes of plasma listed in Table 2-1, contribute to a total osmotic pressure of about 6000 mmHg and generate a plasma osmolarity of ~ 300 mOsm/l. This osmolarity of plasma is equivalent to a physiological saline solution of 0.9% (by weight).

The majority of small solutes are transported relatively rapidly across the highly permeable capillary membrane; therefore, the plasma proteins are the primary contributors to the osmotic pressure governing the exchange of water between the plasma and the interstitial compartments. This pressure, referred to as the colloid osmotic pressure, is about 23-27 mmHg. Albumin as well as various globulins synthesized by the parenchymal cells of the liver make up the majority of the proteins found in plasma. Since the molecular weight of albumin is considerably less than that of the globulins (i.e., 69,000 vs. 90,000 - 160,000 g/mol), albumin is the single most important contributor to the plasma colloid osmotic pressure.

2.5 Characterization of the Interstitial Compartment

2.5.1 Structure of the interstitium

The interstitium is made up by the connective and supporting tissues of the body and is spatially located outside the vasculature, parenchymal cells (tissue cells) and the lymphatic vessels. The composition and structure of the interstitium has been the subject of several reviews [e.g., Aukland and Reed, 1993; Bert and Pearce, 1984]. Although the composition and structure of the interstitium varies from tissue to tissue, there are basic characteristics and functions that are representative of interstitia of most tissues. Therefore, as a generalized description, the interstitium can be thought of as a three-dimensional meshwork composed of a complex aggregation of protein fibers, glycoconjugates and carbohydrate polymers.

The main roles of the interstitium are to provide a three-dimensional mechanical support matrix for cells and different types of blood vessels and an adequate medium for transporting all the solutes exchanged between plasma, cells and the lymphatics. Based on these two functions,
most of the interstitia can be essentially divided into two phases: 1) the *interstitial fluid* and 2) the *structural molecules of the interstitial matrix*.

### 2.5.1.1 Interstitial fluid

The fluid found in the interstitium results from a balance between the fluid that is filtered from the vasculature across the capillary membrane and the fluid that is not drained by the lymphatic system. The dissolved species in the interstitial fluid, such as small ions, proteins, hormones, O₂, CO₂, etc., are similar to those found in plasma. However, as shown in Table 2-1, the protein concentration in the interstitium is lower than that of plasma. Additionally, due to the Donnan effect, the interstitial fluid has a slightly higher concentration of small anions and a lower concentration of small cations.

### 2.5.1.2 Structural molecules present in interstitium

The main interstitial structural molecules are collagen fibers, elastic fibers and glycosaminoglycans (GAGs).

The collagen fibers have a high tensile strength, especially when stressed longitudinally, [Aukland and Reed, 1993] and they thus provide the mechanical strength of the tissues. They are soft flexible fibers that have a collagen molecule as their functional unit. Collagen, a monomer with a rod-like shape having a length of about 300 μm and a diameter of 1.5 nm [Comper, 1984], is the most important structural molecule in the body. At physiological pH values, the collagen bears a slightly positive charge although the presence of amino and carboxylic groups within its structure provides this molecule with polyampholytic properties (i.e., it has both positive and negative charged regions). Several collagen monomers form collagen fibril aggregates through covalent bonds. These fibrils combine further to form collagen fibers. By means of covalent cross-linked bonds, the collagen fibers form a meshwork that tends to immobilize other structural molecules such as proteoglycans, and, consequently, confer further resistance to changes in the volume of tissues. The collagen fibers account for 70 to 75% of the dry weight of normal adult skin [Uitto et al., 1984].

The elastic fibers contain two types of structural components, elastin fibers and microfibrils. Elastin is an insoluble protein that accounts for about 70 to 90% of the mass of the elastic fiber. It can best be described as a rubber-like material that is responsible for the elasticity of the tissue. The hydrophobic chains of amino-acids that form the protein are cross-linked in
random configurations. The additional components of the elastic fibers - the microfibrils - are composed mainly of glycoproteins. They can be associated with the elastin fibers or, alternatively, can form independent aggregates. The elastic fibers account for only a small fraction, about 2-4%, of the weight of dry skin in normal adults [Uitto et al., 1984].

The glycosaminoglycans (GAGs) occur in the body as hyaluronan, the only GAG that is a free polymer in solution, or as proteoglycans, that are aggregates of GAGs covalently linked with a core protein. The GAGs are highly hydrophylic substances. The most common of the GAGs, hyaluronan (a high molecular weight, unbranched polymer), when placed in a solution, coils and absorbs water such that it will occupy a volume as high as 1000 to 10000 times its own unhydrated volume [Aukland and Reed, 1993].

2.5.2 Physical characteristics of the interstitium

2.5.2.1 Volume exclusion

The presence of numerous interstitial macromolecules, particularly hyaluronan and collagen-based species, creates a macromolecular crowding of the interstitial space. As mentioned above, these solutes generate expanded interstitial mesh-like structures that, under certain conditions, exceed the volume of polymers themselves. Consequently, the space available to other interstitial species is less than the total interstitial volume, i.e., a given interstitial solute will distribute itself only outside the meshwork or, alternatively, through those spaces of the meshwork that have dimensions larger than that of the solute. This phenomenon of geometrical *interstitial exclusion* was first described by Ogston and Phelps [1961] and refers to the fact that two solid structures cannot occupy the same confined volume at the same time. The magnitude of the exclusion effect depends on the geometrical configuration of not only the excluding species but also the excluded one. Thus, the geometrical exclusion phenomenon is relevant only for species with high molecular weight such as proteins and not for, e.g., small ions.

In addition to this geometrical exclusion, due to the fact that GAGs are negatively charged at normal pH values, electrostatic factors are also involved in selectively excluding other negatively charged solutes (e.g., proteins) found in interstitium.

For the reasons described above, the interstitial volume can be divided into two fractions, the *excluded volume*, $V_{IT,EX}$, and *available volume*, $V_{IT,AV}$, such that

$$V_{IT} = V_{IT,AV} + V_{IT,EX}$$

[2-2]
where $V_{IT}$ is the total interstitial volume.

The magnitude of the excluded volume has an important role to play in the dynamics of transcapillary exchange. Due to exclusion, the effective protein concentration in the interstitium is much higher than the value that would be estimated if it were assumed that all the fluid in the interstitium was available.

### 2.5.2.2 Interstitial compliance

The interstitial compliance reflects, to some extent, the elastic properties of the interstitial space. When the volume of the interstitium increases, the interstitial matrix attempts to limit this expansion, causing an increase in the interstitial pressure. The equation relating the interstitial hydrostatic pressure and the interstitial volume is known as the **interstitial compliance**. Generally, this equation assumes that the change in interstitial fluid volume, $\Delta V_{IT}$, is proportional to the change in interstitial fluid pressure, $\Delta P_{IT}$, i.e.,

$$\Delta V_{IT} = F_{COMPI} \cdot \Delta P_{IT} \quad [2-3]$$

where $F_{COMPI}$ is a proportionality constant called the interstitial compliance.

Most of the measurements of interstitial compliance come from animal studies [e.g., see Guyton, 1965; Reed and Wiig, 1981]. The compliance of human tissues has not been studied as extensively. A compliance curve is generated by the measurement of several pairs of interstitial pressure-volume data that correspond to specific conditions of tissue hydration. An example of such a curve obtained from experiments on dog skin is presented in Fig. 2-4. As shown in this figure, for the dehydration region (i.e., volume less than normal), the curve has a steep slope. As the interstitial volume increases, the slope decreases until the curve becomes almost flat. The reciprocal of different segments of this slope represents the interstitial compliance, $F_{COMPI}$.

For the region of normal to low interstitial volume (i.e., the dehydration region), the compliance is low. A low compliance implies that small changes in interstitial fluid volume produce large changes in interstitial pressure. Correspondingly, the high compliance observed in the overhydration region means that large changes in volume result in only small changes in interstitial pressure. Moderate compliance values are obtained for a moderately hydrated interstitium. Similar conclusions were drawn from compliance studies involving several other animals [Wiig and Reed, 1981; 1985; 1987].
2.6 Characterization of the Lymphatic System

Although the lymphatics are not accounted for as a distinct compartment in the model, their anatomical position and physiological role justifies their description immediately following the characterization of the interstitial compartment. The lymphatic system consists of a network of vessels that parallels the arterio-venous system. The lymphatic vessels drain excess fluid, proteins, bacteria, particulate material (e.g., in lungs) and immune cells from the interstitium back into the blood. Therefore, the lymphatic system has the dual role of preventing fluid and solute accumulations in the interstitial space (i.e., preventing edema formation) and acting as an 'immune-surveillance system' [Holtz, 1996]. The lymphatic vessels are classified as initial (terminal) lymphatics, lymphatic collectors or central lymphatic conduits.
The initial lymphatics are small vessels with diameters between 15 and 150 μm (depending on the anatomical location of the vessel) and are involved directly in the collection of interstitial fluid in the microvascular system. They are located near the capillary beds. A detailed review of the micro-anatomical characteristics of the initial lymphatics has been presented by Schmidt-Schönbeim [1990]. As shown schematically in Fig. 2-5, these vessels have a single layer of overlapping discontinuous endothelial cells. The majority of initial lymphatics (with the exception of those in the brain, spinal cord and ocular space) have open junctions between adjacent cells. It was observed that the endothelial lining is irregularly tethered to its surrounding tissue by collagen-anchoring filaments [Leak, 1987; 1968]. The unattached luminal side of the cells forms flaps that float freely above the junction [Schmidt-Schönbeim, 1990]. This structural arrangement allows the initial lymphatics to operate as microvalves between the interstitium and the lumen of the lymphatics. When the interstitial pressure exceeds that in the initial lymphatics, the valves open allowing the entry of fluid, small ions and macromolecules, but when the pressure in the lymphatic vessel becomes greater than that in the surrounding tissue, the flaps overlap preventing the outward movement of lymphatic fluid. Specific mechanisms that allow the movement of lymph into and/or through the initial lymphatics have never been observed microscopically. It has been proposed, however, that the compression of the initial lymphatics that are inherent when normal bodily activities are performed (e.g., during skeletal muscle contraction, respiratory movements, etc.) determine the uptake and transport of interstitial fluid in these vessels [Olszewski and Engeset, 1980; Aukland and Reed, 1993].

The collecting lymphatics drain the incoming fluid from several initial lymphatics. These vessels reach diameters up to 600 μm and are characterized by two types of segments. Segments similar to the initial lymphatics in that they have a discontinuous endothelial layer (and can uptake fluid directly from the interstitium) are often called lymphatic precollectors, while the other type of segments have walls that resemble those of small veins and are called lymphatic collectors. Both types of segments are endowed with bileaflet valves positioned in the direction of lymph flow such that the back flow of lymph is prevented (see the lymphatic segment on the far right in Fig. 2-5).

It has been proposed that the collecting lymphatics are organized as discrete contractile units surrounded by circular layers of smooth muscle and nerve endings [Olszewski and Engeset, 1980]. Each contractile unit is separated from the next one by a bileaflet valve and exhibits a
peristaltic contraction with a synchronized opening and closing of the valve. So far, this is the most accepted mechanism of unidirectional lymph propulsion.

Figure 2-5: Schematic diagram of the initial lymphatic and collecting vessels. From Holtz, [1996].

The large central lymphatic conduits, which are the final vessels in the lymphatic architecture, carry the lymphatic fluid back into the blood circulation via the jugular veins.

The composition of lymph undergoes some modification in its route from the initial lymphatics to the central conduits. The lymphatic nodes positioned throughout the lymphatic system, besides being involved in the formation and differentiation of lymphocytes, act as filters for bacteria and particles. From the perspective of this study, and based on other literature information [Aukland and Nicolaysen, 1981], the non-excluded interstitial fluid and the lymphatic fluid are considered identical in composition. Furthermore, the lymphatics are not approached as a distinct fluid compartment; rather they will be treated as passive conduits of interstitial fluid integrated into the microvascular exchange system.
Chapter 3: TRANSPORT ACROSS THE CAPILLARY AND CELLULAR MEMBRANES

3.1 Introduction

In order to provide a better understanding of the system being studied in this thesis, Chapter 2 presented a general overview of the characteristics of the different fluid compartments that are assumed to represent the body. The partition of fluid and solutes amongst these compartments is determined ultimately by the various exchange processes that occur between them. The purpose of the present chapter therefore is to discuss in detail the two primary sites of exchange within the body, namely the capillary and cellular membranes. In particular, generalized equations, representative of all tissues, as well as the governing driving forces that cause material exchange across both membranes, will be introduced. To guide the discussion that follows, the presentation will be conceptually separated into two distinct parts, one for each of the two types of membranes involved.

Part I: Transcapillary Exchange

3.2 Structure and Function of the Microcirculation

When a generalized description of the circulatory system was presented earlier, it was shown that the microcirculation is an integral part of this system. All the extracellular exchanges of materials between the circulating blood and the various types of interstitia of the body occur in the capillary beds that comprise the microvascular exchange system (MVES).

The morphological design of the microcirculation is such that it favors efficient exchanges of fluid and solutes between the blood and the surrounding tissues and organs. Accordingly, in these exchange vessels (e.g., capillaries and venules), the velocity of blood is slowest (< 1 mm/sec) while the surface area of exchange is highest [Renkin and Crone, 1996; Guyton, 1991].

Normally the blood flows intermittently through individual capillaries. This process is termed vasomotion and is due to an intermittent opening and closing of the precapillary sphincters. The on-off pattern of blood flow repeats itself every few seconds under normal circumstances, but can be severely disturbed during conditions such as hemorrhage (when the capillary circulation can shut off for as long as 15 minutes). In order to assure that the nutritive
demands of the tissues are met, each organ may auto-regulate to some extent its own blood supply by causing dilations or contractions of the smooth muscles of the metarterioles and the precapillary sphincters. This control is based on the specific local requirements (mainly oxygen demand, but also to some extent, the local concentration of nutrients, an excess of metabolic products, etc.) [Guyton, 1991]. A maximized transfer of nutrients is assured by the fact that, generally, the capillary-to-tissue cell distances, are extremely low, i.e., between 20-50 μm [Renkin and Crone, 1996; Guyton, 1991]. Additionally, the microcirculation of each organ is anatomically tailored to serve the organ's particular needs. Thus, in skeletal muscle, the capillaries are positioned along the muscle fibers [Renkin and Crone, 1996] while, as will be shown later in Chapter 8, typical renal microvessels surround the renal tubules as in interwoven arrangement [e.g., Guyton, 1991; Krieger and Sherrard, 1991]. Despite variations in the structural configuration of different capillary beds, similar arrangements serve the same exchange purposes. Therefore, a general characterization, in terms of pathways for transport or governing forces that cause mass exchange across individual capillaries, is possible.

3.3 The Capillary Wall and its Transport Pathways

The capillary wall is about 0.2 to 0.5 μm thick [Guyton, 1991; Renkin and Crone, 1996] and is composed of a unicellular layer of endothelial cells supported by a specialized region of collagenous fibers termed the basement membrane (or basal lamina) [Simionescu and Simionescu, 1984; Bert and Pierce, 1984]. The capillary wall acts as a semi-permeable membrane that separates the blood from the interstitium.

Transport of fluid and solutes between the blood and interstitium can take place as a consequence of local transmembrane differences in concentration and/or hydrostatic and colloid osmotic pressures or, by more specific mechanisms such as via plasmalemmal (endoplasmic) vesicles. The endoplasmic vesicles are believed to play a role in selectively shuffling substances (including macromolecules) through the capillary wall [Wissig and Charonis, 1984].

Generally, material can selectively permeate the capillary wall via three main routes: directly through the cell membranes, through channels within the cells, or through the spaces between adjacent cells [Renkin, 1977; Schneerberger, 1992]. The several transport pathways identified for the passage of fluid and small molecules [Renkin, 1985; Schneerberger, 1992] can be summarized as follows: intercellular clefts, fenestrae, plasmalemmal vesicles, discontinuous vessels and stable pores. These pathways are illustrated in Fig. 3-1.
Figure 3-1. Transport of different types of solutes (see below) through the multiple pathways identified for transcapillary exchange. The transport pathways in the capillary endothelium include: (1) transport through the cell membranes; (2) transport through intercellular clefts, across small or large pores; (3) transport across fenestrae that can be specialized pathways through the cell or areas where the two membranes of the endothelial cell are fused; and (4) continuous (4a) or discontinuous (4b) vesicular shuttling of substances. Modified from Curry [1984].

The intercellular clefts are openings present between two adjacent endothelial cells. Typically they are around 10-20 nm across [Fung, 1984] and, in most tissues, they provide the major pathway for the transport of water and macromolecules. Sometimes, these clefts are intermittently closed due to the small areas of contact between two adjacent cells. Moreover, in some microvascular beds, such as the microvasculature of the brain, these clefts are completely closed.

The fenestrae are formed by a reduction of the capillary wall to a small thickness of 6-8 nm as a result of the fusion of the membranes of two endothelial cells. In some exchange sites, such as the glomerular capillaries of the kidney, the fenestrae are completely open.

The spherical bodies of the plasmalemmal vesicles belong to the cytoplasm of the endothelial cells. These vesicles are believed to shuttle back and forth between opposing cell surfaces, thus delivering their fluid and solute contents to the opposite surface. A temporary fusion of a number of vesicles, such that an open water channel through the capillary wall is created, has also been described [Wissig and Charonis, 1984].

The discontinuous vessels are referred to as sinusoids and are most commonly found in liver or spleen. They are characterized by large discontinuities in the endothelium and its basement membrane and thus confer the entire organ with an increased permeability for solutes.
As a consequence, the reflection coefficient for proteins in the liver microvasculature is almost zero, compared to a characteristic value of about 0.9 in skeletal muscle.

The degree to which several types of transport pathways occur is dependent on the location and function of the microvascular bed in question. Continuous vessels are commonly found in the microvascular beds of lung, nervous system, skeletal muscles and skin [Simionescu and Simionescu, 1984]. Fenestrated vessels are predominant within the microvasculature of pancreas, endocrine glands and the gastrointestinal tract. As previously mentioned, the sinusoid vessels are typical of liver.

3.4 Transcapillary Transport Equations

Owing to the multitude of the capillaries present in the body, their overall function must be averaged. Therefore, in all subsequent quantitative analyses of transport in the MVES, only average values for capillary pressure, blood flow rate, material transport rates, area available for transport, etc., will be used.

Throughout most of the medical literature, discussions about the different pathways available for capillary exchange, as presented in the previous section, remain largely qualitative. The most accepted methods of quantifying the transcapillary mass exchange still use expressions derived for transport across porous membranes. Thus, the capillary membrane is treated either as a homoporous or as a heteroporous barrier [e.g., Rippe, 1989; McNamee and Wolf, 1991]. Mass exchange rates for fluid, small ions and macromolecules (i.e., proteins) are therefore assumed to be given by the ratio of a lumped driving force divided by the transport resistance of the transcapillary barrier.

3.4.1 Fluid transport across the capillary wall

The principal driving forces governing fluid flow across the capillary membrane between the vascular space and the neighboring interstitium are the hydrostatic and colloid osmotic pressure differences. These driving forces are known as Starling forces and are depicted in Fig. 3-2. The hydrostatic pressure in the capillary along with the interstitial colloid osmotic pressure promote fluid movement from the capillary (i.e., fluid filtration), while the hydrostatic pressure in the interstitium together with the plasma colloid osmotic pressure drive fluid into the capillary (i.e., fluid absorption).
**Osmotic and colloid osmotic pressures**

Any of the solutes found in the plasma and interstitium that cannot freely cross the endothelial barrier can, by means of exerting a transcapillary osmotic pressure, influence fluid transport across the capillary membrane. The degree to which a solute affects transcapillary fluid flow depends in part upon the permeability of the capillary wall for that solute. The fraction of the total osmotic pressure exerted by a species upon the capillary membrane is given by the *reflection coefficient*, $\sigma$.

Thus the osmotic pressure difference, $\Delta\pi_S$, exerted by a solute $S$ having a reflection coefficient $\sigma_S$ across a semi-permeable membrane is given by the following relationship derived from the van't Hoff law:

$$\Delta\pi_S = RT \sigma_S (C_{S,1} - C_{S,2})$$  \[3-1\]

where $(C_{S,1} - C_{S,2})$ is the concentration difference of solute $S$ across the membrane while $R$ and $T$ are the absolute temperature and the universal gas constant, respectively.

---

**Figure 3-2.** The Starling forces that contribute to fluid exchange across the capillary membrane.
A reflection coefficient of 1 denotes complete impermeability of the capillary membrane for a given solute. A value of 0 indicates a solute permeability across the capillary equal to that of water, i.e., the solute moves freely through the capillary pores [Parker et al., 1984]. Most lipid-soluble gases such as O₂ and CO₂ have reflection coefficients near zero [Renkin and Crone, 1996], while the values for proteins, except in some microvascular beds such as liver, are found to be nearly unity. The capillaries of skeletal muscle have reflection coefficients for proteins around 0.95 to 1 [e.g., Renkin, 1988; Renkin and Crone, 1996]. The values for the small lipid-insoluble solutes, such as Na⁺, K⁺ and glucose, are somewhat more uncertain. Traditionally, it was assumed that most of the latter species have low reflection coefficients (i.e., σ < 0.1) and therefore any differences in their concentrations between plasma and interstitium are dissipated rather quickly [Michel, 1984; Wolf, 1980]. More recent reports, however, suggest reflection coefficient values for small ions to be as high as 0.3 to 0.5 [Wolf and Watson, 1989].

Theoretically, all the osmolites typically found in plasma or interstitial fluid could exert a total osmotic pressure of about 6000 mmHg across a membrane permeable only to water [Kleinman and Lorentz, 1984]. Of this value, only about 25 to 30 mmHg are provided by the presence of proteins. Owing to the high permeability of the capillary for most solutes other than plasma proteins, under normal conditions these latter species are the predominant contributors to the transcapillary osmotic driving force. To differentiate it from the total osmotic pressure exerted by the extracellular solutes, the osmotic pressure due to the plasma proteins is termed the colloid osmotic pressure.

**Gibbs-Donnan effect**

The contribution of plasma proteins to the colloid osmotic pressure difference is actually greater than would be predicted if only the number of dissolved protein molecules in plasma is considered. This added pressure difference is due to the *Gibbs-Donnan effect* and occurs as a result of the retention in the capillary of various protein species (mainly albumin), which, at physiological plasma pH values, are negatively charged. In order to neutralize these charges, a large number of plasma cations (mainly Na⁺ by virtue of its abundance in plasma, but to some extent also K⁺, Ca²⁺ and Mg²⁺) surround the proteins. These cations are not chemically bound to the macromolecular species, but are held in their proximity by electrostatic forces. As a result, the number of osmotically active species prevented from crossing the endothelial barrier is
higher and consequently the colloid osmotic pressure exerted in the presence of these proteins must also be higher.

In light of the above discussion, the Donnan effect therefore has the following implications: 1) it contributes to an additional osmotic pressure difference between the plasma and interstitium, and 2) it causes an asymmetric distribution of ions between the two compartments. The consequence of this statement is the partition of small ions shown in Fig. 3-3, which depicts a higher concentration of non-diffusible positive small ions in the plasma compartment, and a correspondingly higher accumulation of anions in the interstitium. This partition is directly related to the concentration of proteins in plasma; e.g., it is expected that, during hemodilution when the concentration of plasma proteins decreases, the Donnan effect will decrease in importance. Traditionally, the asymmetric distribution of charges across the capillary membrane has been determined by accounting for the Na\(^+\), K\(^+\) and Cl\(^-\) ions, and the plasma proteins. Such a description is not useful if other positive and negative ions are considered to take part in transcapillary transport. A detailed description on how the Donnan effect was accounted for in the current model, where multiple ionic species are present, is given in Chapter 4. For now it will be assumed that the Donnan contribution to the colloid osmotic pressure generated by plasma proteins is incorporated in the term \(\pi_{P,PL}\) of Fig. 3-2.

![Figure 3-3. Schematic representation of the Donnan partition of small ions across the capillary. The size of the symbols, reflect a higher concentration of non-diffusible positive charges, Cat\(^+\), on the plasma side and the corresponding higher accumulation of non-diffusible negative charges, An\(^-\), on the interstitial side. For more discussion see text.](image-url)
Based on the overview given above regarding the various driving forces that act upon the capillary membrane, the rate of fluid movement across this membrane is given by the classic equation based on Starling's hypothesis (Starling, 1890). Thus, if a generic protein species (representative of the multitude of proteins found in plasma) has a reflection coefficient $\sigma$ and if the effect of small solutes can be neglected, then the fluid flow rate across the capillary membrane, $J_{IT}$, can be written as:

$$J_{IT} = L_{cap} S \cdot [(P_c - P_{IT}) - \sigma(\pi_{P, PL} - \pi_{P, IT})]$$  \[3-2\]

Equation 3-2 delineates the balance between the hydrostatic and colloid osmotic forces in governing transcapillary fluid movement. The symbols that appear in Eq. [3-2] are defined as follows: $L_{cap}$ and $S$ are the hydraulic conductivity and the exchange surface area of the capillary, respectively; $P_c$ and $P_{IT}$ are the capillary and interstitial hydrostatic pressures, respectively; while $\pi_{P, PL}$ and $\pi_{P, IT}$ represent the plasma and interstitial colloid osmotic pressures, respectively. The subscript IT used for the transcapillary fluid flow indicates that, under normal conditions, there is a positive filtration of fluid from plasma toward the interstitium.

The hydraulic conductivity, $L_{cap}$, is an intensive parameter of the capillary wall that is functionally connected to the variable surface area available for transport, $S$. Usually, transport equations that have the form of Eq. [3-2] take into account only the product of the above two terms; that product is expressed as $k_T$, the capillary filtration coefficient.

### 3.4.2 Protein transport across the capillary wall

Because proteins are the primary contributors to compartmental osmotic pressures, the rate of protein transport across the capillary membrane has an important role in determining the partition of the extracellular volume between plasma and interstitium. There are several equations available to describe the transcapillary transport of macromolecules depending on the assumptions made with respect to the number of pathways available for transport.

The Kedem and Katchalsky [1958] equation assumes that the diffusion and convection of proteins from plasma to interstitium occurs as two independent processes. This equation does not make any assumptions with respect to the geometry of the channels within the porous membrane.

Thus, the transcapillary transport of protein, $Q_{IT}$, from plasma toward the interstitium is given by,
\[ Q_{IT} = J_{IT} (1 - \sigma) \cdot \left( \frac{c_{P,PL} + c_{P,IT,AV}}{2} \right) + PS \cdot (c_{P,PL} - c_{P,IT,AV}) \]  

where the first r.h.s. term is the convective contribution and the second term, the diffusive contribution. The terms \( c_{P,PL} \) and \( c_{P,IT,AV} \) are the protein concentrations in plasma and in the available interstitial volume, respectively. Recall from Chapter 2 that only a certain fraction of the interstitial volume, i.e., about 25%, is available for protein distribution. In the convective term, it is assumed that the appropriate protein concentration being carried by the fluid flow, \( J_{IT} \), is the average value for the two compartments. \( PS \) in the diffusive term of Eq. [3-3] represents the permeability-surface area product for proteins; i.e., it is the product of the protein permeability through the capillary, \( P \), and the total capillary area that is available for exchange, \( S \).

An alternative equation for the transport of proteins (and generally for the transport of any neutral species), was proposed by Patlak et al. [1963] as follows:

\[ Q_{IT} = J_{IT} (1 - \sigma) \cdot \frac{c_{P,PL} - c_{P,IT,AV} \cdot \exp\left(-\frac{J_{IT} (1 - \sigma)}{PS}\right)}{1 - \exp\left(-\frac{J_{IT} (1 - \sigma)}{PS}\right)} \]  

where all the symbols in this equation were defined previously. Equation [3-4] is obtained by solving the one-dimensional convection-diffusion equation for a uniform cross-sectional channel. This equation can be rearranged into the following form proposed by Bresler and Groome [1981]:

\[ Q_{IT} = J_{IT} (1 - \sigma) \cdot c_{PL} + \frac{J_{IT} (1 - \sigma)}{1 - \exp\left(-\frac{J_{IT} (1 - \sigma)}{PS}\right)} (c_{P,PL} - c_{P,IT,AV}) \]  

Equation [3-5] is not equivalent to the sum of uncoupled diffusion and convection proposed by Kedem and Katchalsky [1958] (i.e., Eq. 3-6), although it gives similar predictions at high flow rates [Parker et al., 1984].

### 3.4.3 Transport of small ions across the capillary

To this author's knowledge, the Kedem and Katchalsky equation is the only expression that has been proposed and used for the transcapillary movement of small ions. Considering a generic species, ION, that represents one of the multitude of cations and anions found in plasma and interstitium, the transcapillary movement of each ionic species can be expressed as,
\[ M_{\text{ION,IT}} = J_{\text{IT}} (1 - \sigma_{\text{ION}}) \left( \frac{[\text{ION}]_{\text{PL}} + [\text{ION}]_{\text{IT}}}{2} \right) + \text{PS}_{\text{ION}} \left( [\text{ION}]_{\text{PL}} - [\text{ION}]_{\text{IT}} \right) \]  

where \([\text{ION}]_{\text{PL}}\) and \([\text{ION}]_{\text{IT}}\) are the ionic concentrations in the plasma and interstitium, respectively; while \(\sigma_{\text{ION}}\) and \(\text{PS}_{\text{ION}}\) are the reflection coefficient and the permeability-surface area product, respectively, for this small solute.

Several slightly modified forms of Eq. [3-6] can be found in the literature, where the average ionic concentration inside the channel (see the first term of this equation) is replaced by either the logarithmic average or simply by the ionic concentration at the plasma interface, i.e., \([\text{ION}]_{\text{PL}}\). Based on experimental studies, it was concluded that the algebraic average provides a better approximation of the experimental ionic transport data in the MVES [Parker et al., 1984].

The above description completes the quantitative analysis of the contributions of the capillary membrane to fluid and solute redistribution in the extracellular environment. The governing equations for transcapillary fluid and solute transport were provided in relation to the two extracellular compartments: plasma and interstitium. At steady state, there are also continuous exchanges of fluid and solutes across the cellular membrane between the red blood cells and the surrounding plasma as well as between the tissue cells and the interstitial fluid. The next part of this chapter deals with an analysis of this exchange.

**Part II: Cellular Exchange**

**3.5 Theoretical Considerations**

The discussion that follows will be primarily concerned with the characterization of the transport between a cellular compartment (ICV) and its extracellular environment (ECV). Due to the fact that the ECV and ICV are in direct contact, any disturbance in the extracellular volume or composition will have repercussions on the intracellular fluid and solute properties.

The cells in the body possess unique mechanisms for transporting fluid and small solutes across the cellular membrane. These transport mechanisms are central to the maintenance of a constant cellular volume and, generally speaking, body homeostasis. Regulation of cellular volume implies a close control of intracellular water activity. Mammalian cells have a water content of somewhere between 75-85% of their total weight [MacKnight, 1984(b)]; therefore, the control of cellular water implies a control of cellular volume. Even though the cellular membrane
is highly permeable to water, under normal physiological conditions, the water content and, implicitly, the cellular volume are maintained within very narrow limits.

3.5.1 Cell membrane

The average difference in fluid composition between the ICV and ECV compartments was presented previously in Table 2-1 (see Chapter 2). The partition of solutes between the two environments is brought about by the existence of an outer cellular membrane called the plasma membrane of the cell, or simply the cell membrane. Although the presence of the cellular membrane as a distinct physical barrier between the two environments is generally accepted, its role in cell volume regulation is still under debate. Most researchers agree, however, that the membrane not only assures a physical separation between the ECV and ICV fluids, but it also provides a controlled communication between the interior and exterior of the cells [e.g., MacKnight, 1994; Greger and Windhorst, 1996]. To attain the ionic partition illustrated in Table 2-1, the cell membrane must maintain very large ionic gradients and, implicitly, must develop a membrane potential. Additionally, the ions have to be transported across the membrane in a controlled fashion in agreement with the cellular requirements. As will be described in this section, some of the control mechanisms for differentiating the cytosol from the extracellular environment reside within the membrane itself.

From a structural point of view, the cell membrane consists of a lipidic bilayer arrangement [see e.g., Matthews, 1998]. The phospholipids of the membrane arrange themselves in a bilayer sandwich-type structure, with the polar (hydrophylic) regions oriented toward the interior or exterior of the cell, while the non-polar hydrophobic tails are pointing toward each other. The proteins that form complex aqueous pores and/or channels through the membrane protrude through the lipidic layer. By weight, about 30% of the membrane is composed of lipids while the remainder is primarily proteins [Greger and Windhorst, 1996, Matthews, 1998]. However, the lipidic portion offers a higher available area for transport [Jensen, 1989].

3.5.2 General characteristics of transport through the cellular membrane

Transport through the cellular membrane has been the topic of several extensive reviews [e.g., Tosteson, 1981; MacKnight, 1994]. It is currently accepted that the solutes and water can permeate the cellular membrane by either passing through the lipid bilayer or, alternatively, directly through the protein pathways.
There are two basic types of transport across the cell membrane: 1) *passive transport* and, 2) *active transport*.

Passive transport represents the simple physical diffusion of a solute down its concentration and/or electrochemical gradient and can, in turn, be sub-divided into the following three categories:

a) Solubility-diffusion (or simple diffusion) - the net movement of a species down a concentration or chemical potential gradient (e.g., movement of water)

b) Electrodiffusion - the movement of ionic species driven by both concentration and electrical potential differences (e.g., transport of Na$^{+}$, K$^{+}$ and Cl$^{-}$)

c) Facilitated diffusion - involves a specific interaction between the transported solute and another solute that crosses the cellular membrane. A particular subset of facilitated diffusion can occur when two solutes move simultaneously across the membrane in either the same direction (*cotransport*), or the opposite direction (*countertransport*).

Active transport requires the presence of a membrane component that transports ions through the membrane against their concentration (or electrochemical) differences. In order to achieve this type of transport, the cell must expend energy. The most common type of active transport is the one associated with the electrogenic Na$^{+}$-K$^{+}$-pump.

Generally, the literature dealing with the structure and function of the cellular membrane describes the protein channels as highly selective for the passage of a given small ion. This implies that the ionic permeability is at least partially determined by the specific function of the membrane proteins that make up the channels. It would be expected therefore that some protein macromolecular aggregates embedded in the lipidic bilayer might constitute elaborate 'membrane transport devices'. So far, however, very few such devices have been observed and none have been analyzed to the point where their function can be quantitatively described.

### 3.5.3 Cell membrane permeability

An important consideration in the transport of solutes across the cell membrane is its permeability to various solutes as well as to water.

For porous membranes, the net flow of water across the membrane is due mostly to the osmotic pressure difference between the intra- and extracellular compartments [Weiss, 1998]. Consequently, the water permeability is characterized by the so-called osmotic permeability coefficient, $p_w$, expressed in cm/s and defined as:
Chapter 3: Transcapillary and Cellular Exchange

\[ P_w = \frac{RT \cdot L_v}{V_{m,w}} \]  

[3-7]

were \( L_v \) is the hydraulic conductivity (cm/(mmHg-s)) and \( V_{m,w} \) is the partial molar volume of water (cm\(^3\)/mole).

For ions that are passively transported across the lipidic membrane, the ionic permeability refers to an electro-diffusive mechanism, characterized by the diffusive permeability coefficient, \( p_{ION} \), which is given by

\[ p_{ION} = k_{ION} \frac{D_{ION}}{d_m} \]  

[3-8]

where \( D_{ION} \) (cm\(^2\)/s) is the diffusion coefficient of the ionic species, ION; \( d_m \) is the membrane thickness (cm) and \( k_{ION} \) is the partition coefficient for that ion between the lipidic phase of the membrane and the aqueous phase of the intra- or extracellular medium. This permeability is determined by the relative lipid-water solubility of the diffusing solute, the size of the membrane pores and the electric charge of the membrane, as well as the dimensions of and the charge carried by the diffusing solutes.

The reported membrane permeability values for the solutes of primary interest to this work are presented in Table 3-1 for RBCs and skeletal muscle cells. As is apparent from the discrepancies seen in this table, the true values of these membrane permeabilities are somewhat uncertain. The permeability of water is usually estimated from transient osmotic data. There are basically two approaches used to determine the membrane permeability of ions: the flux method in which the net rate of movement of a radioactive tracer through the cell membrane is measured, and the electrical method in which the electric currents carried by ions moving through the membrane are measured. Both of these methods have some significant disadvantages. The flux method has a low time resolution and sensitivity, while in the electrical method, the ion flow affects the membrane potential, which, in turn, can alter the permeability value. Usually, both of these methods can be applied to either intact, relatively large cells such as RBC and skeletal muscle cells, or, as is often the case, to 'artificial membranes', i.e., cell-membrane lipid layers which are subjected to measurements after the proteins have been extracted.

The solute permeability values presented in Table 3-1 are, in general, extremely low if one thinks in terms of the diffusion of the same solutes through aqueous layers having thicknesses comparable to those of the cell membranes. This includes the water permeability, which has the highest value reported in this table. However, the rate at which water movement,
for instance, occurs through the cell membrane is extremely rapid when the dimensions of real
cells are considered. A sample calculation for a RBC, whose typical volume is about 90 µm³ and
surface area is 160 µm² [Burton, 1965], shows that the total volume of water that can diffuse in
and out of the cell per second is about 80 times greater than the cell volume itself. (The
calculation was performed based on the permeability value for water reported by Dick [1970]
which is presented in Table 3-1.)

<table>
<thead>
<tr>
<th>Species</th>
<th>RBC Permeability (cm-s⁻¹)</th>
<th>Skeletal Muscle Cell Permeability (cm-s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>5 x 10⁻³ [House, 1974]</td>
<td>1 x 10⁻² [Jensen, 1989]</td>
</tr>
<tr>
<td></td>
<td>3 x 10⁻² [Dick, 1970]</td>
<td></td>
</tr>
<tr>
<td>Sodium, Na⁺</td>
<td>4 x 10⁻¹⁰ [Hoffman, 1987]</td>
<td>4.5 x 10⁻⁷ [Jakobsson, 1980]</td>
</tr>
<tr>
<td></td>
<td>(1-4) x 10⁻¹⁰ [Altman and Dittmer, 1971]</td>
<td>2 x 10⁻⁸ [Jensen, 1989]</td>
</tr>
<tr>
<td>Potassium, K⁺</td>
<td>10⁻¹⁰ [Hoffman, 1987]</td>
<td>5 x 10⁻⁵ [Jakobsson, 1980]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 x 10⁻⁵ [Fromkin, 1973]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 10⁻⁶ [Jensen, 1989]</td>
</tr>
<tr>
<td>Chloride, Cl⁻</td>
<td>10⁻⁴ [Altman and Dittmer, 1971]</td>
<td>5 x 10⁻⁵ [Jakobsson, 1980]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 x 10⁻⁶ [Jensen, 1989]</td>
</tr>
<tr>
<td>Proteins and Sucrose</td>
<td>0.0 [Greger and Windhorst, 1996]</td>
<td>0.0 [Altman and Dittmer, 1971]</td>
</tr>
</tbody>
</table>

Table 3-1: Reported permeabilities for red blood cells and skeletal muscle cells (see table entries for references)
3.6 Exchanges of Ions and Water across the Cellular Membrane

All the previously mentioned transport mechanisms operate concurrently to assure the normal distribution of ions across the cellular membrane. It is not possible to present a complete account of all these mechanisms here. The present work therefore will rely on a simplified, but still reasonable, model of cell volume regulation (CVR). The full CVR model is given as a part of the model formulation in Chapter 4. The intention of this section is to present only the ion transport equations needed to provide a basic understanding of cellular exchange. The final goal of this section is to build a reasonable picture of a typical cell, which is able to control its volume while actively exchanging solutes across its enclosing membrane.

First, the characteristics of a typical cell, the partition of ions across its membrane and the accepted transport mechanisms that bring about this partition will be discussed. Following this, the transmembrane ionic transport for such a cell will be detailed in a hierarchical fashion, i.e., from the simple electro-diffusional transport of two ions across the membrane, up to a more complex case where three ions participate in both active and passive processes.

3.6.1 Mechanistic description of a typical cell

Figure 3-4 illustrates the typical distribution of ions and their transport directions across the cell membrane of muscle cells. Of all the solutes present in the intra- and extracellular fluids, Na\(^+\), K\(^+\) and Cl\(^-\) are the primary species involved in transport across the cellular membrane. Additionally, they govern the electrical properties of cells, i.e., the generation of a membrane potential. Similar to the approach of other authors [e.g., Wolf, 1980; Jakobsson, 1980], it is therefore assumed that only these three species are transported across the cellular barrier. The permeabilities, which control the passive transmembrane transport of these ions are \(p_{Na}\), \(p_{K}\) and \(p_{Cl}\), respectively. The remaining solutes are considered to be fixed and mobile anions, (FA) and \(A\), respectively, or fixed and mobile cations, (FC) and \(C\), respectively, confined to either the interior (ICV) or exterior (ECV) of the cell. None of these other solutes can cross the cellular membrane; however, they contribute to both the ICV and ECV total osmolarities and thus are important contributors to the osmolarities of the intra- and extracellular environments.

As a result of differences in the distribution of these charged species between the ICV and ECV, a resting cellular membrane potential, \(V_m\), is generated. By convention, the cellular membrane potential is measured with reference to the potential outside the cell; therefore, \(V_m\) for
the type of cell described in Fig. 3-4 (i.e., skeletal muscle cells) is somewhere between -85 and -90 mV, with the interior of the cell being negatively charged.

The simplest and most accepted cell membrane transport mechanisms for Na\(^+\), K\(^+\) and Cl\(^-\) ions are 1) electrodiffusion due to concentration and electrical potential differences and 2) active transport. Based on these two primary mechanisms, the three major ions are transported across membrane as follows. The sodium ion diffuses passively from the ECV to the ICV as a consequence of concentration and electric driving forces (the dashed line of Fig. 3-4 indicates the electrodiffusional transport). This ion is also transported back out of the cell through active transport, by means of a Na\(^+\)-K\(^+\)-pump (indicated by the solid line of Fig. 3-4).

The permeability of the membrane to potassium is about two orders of magnitude higher than for sodium. K\(^+\) is transported out of the cell by electrodiffusion down a concentration gradient and against an electrical potential difference. The potassium ion is pumped back into the cell by active transport. The chloride ion, with its relatively high permeability (similar to that of potassium), is the only negative species transported across the cell membrane. This ion is transported passively from the ECV to the ICV because of a concentration difference, and from the ICV to the ECV due to a potential difference. The net rate of exchange of Cl\(^-\) should be just sufficient to maintain an electrically neutral transport of all ions across the membrane.

Water moves freely across the cell membrane. The instantaneous shift of water is determined only by changes in the ICV or ECV osmolarity. The total osmolarity in the extracellular or intracellular medium is given by the sum of all the osmolites present in the ECV or the ICV, respectively, at any given time.

The equations that govern the transport of ions across the cell membrane are presented next.

### 3.6.2 Transport of ions due to electrical and diffusional driving forces

As part of a model of cellular exchange, quantitative expressions for passive and active ionic transports are required. In order to detail these transports, it will be assumed that the partition of ions between the ECV and ICV is equivalent to that given in Fig. 3-4, which reflects the normally measured concentrations of these ions in the body (see also Table 2-1). Furthermore, due to their concentration differences across the membrane, a transmembrane potential difference, \(V_m\), is generated.
Chapter 3: Transcapillary and Cellular Exchange

Intracellular fluid (ICV)

- 10 mEq/l Na

Extracellular fluid (ECV)

- 139 mEq/l Na

3 Na

- 150 mEq/l K

- 4 mEq/l Cl

- 107 mEq/l Cl

FA

FC

Figure 3-4: Distribution of ionic species across the cellular membrane as a consequence of the different types of transport mechanisms. Representative average concentrations and membrane permeabilities are presented for the transferring species. Na⁺, K⁺ and Cl⁻ are the only ions that participate in solute exchange across the membrane. The 'fixed charges' FA, FC, A and C cannot pass through the membrane; they contribute, however, to the total osmolarity of the ICV and the ECV. The dashed and solid lines indicate the electrodiffusional and active transport mechanisms, respectively. The Na⁺-K⁺-pump, represented by the closed circle in the membrane, is an electrogeneric pump, i.e., it extrudes 3 ions of Na⁺ out of the cell for every two K⁺ ions brought into the cell. The function of this pump requires consumption of energy by the cell. The dotted line used for the transport of Cl⁻ indicates that this ion moves back and forth across the membrane at a rate dependent on Na⁺ and K⁺ movement. Water is assumed to move freely across the cell membrane. More details are given in the main text.
Chapter 3: Transcapillary and Cellular Exchange

The transport rate of potassium by electrodiffusion, \( T_{dK} \), that takes place from the ICV to the ECV, is given by the following expression:

\[
T_{dK} = \frac{F \cdot V_m}{RT} \cdot P_K \cdot \left[ \frac{[K]_{ECV} \cdot e^{\left(\frac{FV_m}{RT}\right)} - [K]_{ICV}}{e^{\left(\frac{FV_m}{RT}\right)} - 1} \right]
\]

Similarly, the electro-diffusional flux of chloride from the ECV to ICV, \( T_{dCl} \), is given by:

\[
T_{dCl} = \frac{F \cdot V_m}{RT} \cdot P_{Cl} \cdot \left[ \frac{[Cl]_{ECV} - [Cl]_{ICV} \cdot e^{\left(\frac{FV_m}{RT}\right)}}{1 - e^{\left(\frac{FV_m}{RT}\right)}} \right]
\]

In Eqs. [3-9] and [3-10], the ionic concentrations for potassium, \([K]\), and chloride ion, \([Cl]\), in the extracellular (ECV) and intracellular (ICV) media are expressed in \((\text{mEq/l})\), the permeabilities for potassium and chloride are \(P_K\) and \(P_{Cl}\) respectively, expressed in \((\text{cm/s})\); while \(F\), \(R\) and \(T\) are Faraday's constant, the gas constant and the absolute temperature, respectively. As written, Eqs. [3-9] and [3-10] account for a charge of -1 for the chloride ion and +1 for the potassium ion. These equations are based on the Goldman-Hodgkin-Katz [Goldman, 1943; Hodgkin and Katz, 1949] 'constant field' formulation of the ionic current that passes through the membrane, which in turn is derived from Nernst-Plank electrodiffusion theory subject to assumptions that the membrane is homogenous, the electric field within the membrane is constant, and the individual ions move through the solution independently. A detailed derivation of the general form of the above equations is presented in Appendix A.

If \(K^+\) and \(Cl^-\) ions were the only species transported across the cell membrane, a steady-state distribution of the two ionic species, \(K^+\) and \(Cl^-\), could exist across the cellular membrane when their electrical gradient driven transports counterbalance exactly their concentration gradient driven transports, i.e., when \(T_{dK} \equiv T_{dCl} = 0\). Furthermore, this dynamic equilibrium condition can be characterized by a specific transmembrane potential called the Nernst potential. Both ionic species, \(K^+\) and \(Cl^-\), are at equilibrium if their Nernst potentials are the same. If \(E_K\) is the Nernst equilibrium potential for \(K^+\) while \(E_{Cl}\) is the corresponding potential for \(Cl^-\), equilibrium is attained when the transmembrane potential equals the Nernst potentials of both ions as follows:
Chapter 3: Transcapillary and Cellular Exchange

\[ V_m = E_K = \frac{RT}{F} \ln \left( \frac{[K]_{ECV}}{[K]_{ICV}} \right) = E_{CI} = \frac{RT}{F} \ln \left( \frac{[Cl]_{ECV}}{[Cl]_{ICV}} \right) \]  

[3-11]

The hypothetical result obtained above is not representative, however, of the cells in the body. As discussed in the previous section, the cellular membrane is also permeable to the sodium ion. Thus, sodium ions are also transported into the intracellular environment by both concentration and electrical difference driving forces according to

\[ T_{d,Na} = \frac{F \cdot V_m}{RT} p_{Na} \left[ \frac{[Na]_{ICV} \cdot e^{\left( \frac{FV_m}{RT} \right)} - [Na]_{ECV}}{e^{\left( \frac{FV_m}{RT} \right)} - 1} \right] \]  

[3-12]

If nothing prevents the movement of this ion from the ECV to the ICV, its intracellular accumulation will cause swelling and eventual bursting of the cell. The presence of this third transportable species, i.e. Na\(^+\), has the following consequences [Matthews, 1998]:

1) the species that participate in transport (i.e., Na\(^+\), K\(^+\) or Cl\(^-\)) can no longer achieve an electro-diffusional equilibrium, i.e., a Nernst equilibrium does not exist for this case,

2) the resting membrane potential, \( V_m \), must now incorporate a contribution due to sodium transport across the cell membrane, and

3) an expenditure of cellular energy is required to actively extrude sodium from the intracellular environment.

In order to prevent the intracellular accumulation of Na\(^+\) as it diffuses down its concentration and electrical gradients, a mechanism, other than simple electrodiffusion has to actively extrude this ion from the cell at a rate high enough to counterbalance the inward electrodiffusion. Stated otherwise, the resulting osmotic disturbance and accompanying cellular swelling caused by the Na\(^+\) ions must be prevented by achieving an apparent impermeability of the cellular membrane to this ion. This continuous extrusion of Na\(^+\) from the cell is provided by what is known as the Na\(^+\)-K\(^+\)-pump which utilizes a high energy phosphate compound (ATP) as a source of metabolic energy. This mechanism is referred to as the pump-leak hypothesis and was first described by Wilson [1954] and further elaborated by Leaf [1956] and Tosteson and Hoffman [1960]. Cumulative evidence from researchers ascribing to the pump-leak hypothesis [Post, 1957; Skou, 1957; Tosteson, 1984] shows that the pump extrudes Na\(^+\) in exchange for K\(^+\) via Na\(^+\)-K\(^+\)-ATPase in the ratio 3Na\(^+\): 2K\(^+\).
The pump is characterized therefore by:

a) a *pumping rate* (or the 'velocity' of the pump) symbolized by $RP$, which is a proportionality constant between the active transport flux of sodium and the intracellular $\text{Na}^+$ concentration, and

b) a fixed *pumping ratio* (the 'transporting capacity' of the pump) symbolized by $\rho$ which represents the number of sodium ions extruded from the cell per number of potassium ions introduced into the cell at each cycle performed by the pump, i.e., $\rho = 3/2$ [Post, 1957].

For the sodium ion, the flux due to active transport, $T_{a,\text{Na}}$ (which alternatively can be called simply the *pump flux*) is given by

$$T_{a,\text{Na}} = RP [\text{Na}]_{IC \text{V}} \tag{3-13}$$

Hence, the active transport flux of potassium ions, $T_{a,\text{K}}$, can be obtained from

$$T_{a,\text{K}} = RP [\text{Na}]_{IC \text{V}} / \rho \tag{3-14}$$

One of the requirements for a cell to achieve a steady-state ionic distribution, therefore, is that the total net flux, $T_{\text{ION}}$, of both $\text{Na}^+$ and $\text{K}^+$ across the cell membrane must be zero. Taking the cellular environment as the reference these conditions are respected if

$$T_{\text{Na}} = T_{d,\text{Na}} - T_{a,\text{Na}} = 0 \tag{3-15}$$

and

$$T_{\text{K}} = T_{a,\text{K}} - T_{d,\text{K}} = 0 \tag{3-16}$$

where the flux of a given ion due to electrodiffusion, $T_d$, and the flux due to active transport, $T_a$, are expressed in mmol/(s-cm$^2$).

### 3.6.3 The resting membrane potential

As previously mentioned, the unequal distribution between the extra- and intracellular media of all three ions (i.e., $\text{Na}^+$, $\text{K}^+$ and $\text{Cl}^-$) assumed to cross the cellular membrane, determines the value of the cellular membrane potential which, at steady state, is called the *resting membrane potential*, $V_m$. The value of $V_m$ reflects a balance between the competing influences of the equilibrium Nernst membrane potentials for $\text{Na}^+$, $\text{K}^+$, and $\text{Cl}^-$. The *actual membrane potential*, $V_m$, assumes an intermediate value between these extreme equilibrium potentials having the electrical driving forces for the transport of the potassium, sodium and chloride ions given by $(V_m - E_K)$, $(V_m - E_{\text{Na}})$ and $(V_m - E_{\text{Cl}})$, respectively.
The following three factors determine the value of the resting potential, $V_m$ [Matthews, 1998]:

1) the ionic concentrations inside and outside the cells, which determine the equilibrium potential for each small ion,

2) the cellular membrane permeability for a given small ion, which will establish the relative importance of that ion in determining the value of $V_m$, and

3) the presence (or absence) of an electrogenic pump that actively transports certain ions into or out of the cell.

Since the potassium permeability through the cellular membrane is about two orders of magnitude higher than that of sodium, it is expected that the resting membrane potential will be close to the Nernst equilibrium potential for $K^+$. This is the reality for skeletal muscle cells (and generally most animal cells) that bear characteristics similar to the cell schematically depicted in Fig. 3-4, where it is shown that $V_m$ is in the range of -85 to -90 mV [Matthews, 1998].

The first quantitative relationship between the transmembrane potential, ion concentrations and ion permeabilities was developed by Goldman [1943] for the case where electrodiffusion is the only mechanism of transmembrane transport. His constant field equation is known simply as the Goldman equation. When only three species (i.e., $Na^+$, $K^+$ and $Cl^-$) cross the cellular membrane, the equation can be written as

$$V_m = \frac{RT}{F} \ln \left( \frac{P_{Na} \cdot [Na]_{ECV} + P_{K} \cdot [K]_{ECV} + P_{Cl} \cdot [Cl]_{ECV}}{P_{Na} \cdot [Na]_{ICV} + P_{K} \cdot [K]_{ICV} + P_{Cl} \cdot [Cl]_{ICV}} \right)$$  \[3-17\]

where $V_m$ is the cellular transmembrane potential expressed in mV. All the other symbols are as previously described. The derivation of this equation is presented in Appendix A.

Conversely, if other species in addition to the three ions previously considered are also transported across the cell membrane, Eq. [3-17] can be extended to incorporate the contributions of these ions as follows:

$$V_m = \frac{RT}{F} \ln \left( \frac{P_{Na} \cdot [Na]_{ECV} + P_{K} \cdot [K]_{ECV} + P_{Cl} \cdot [Cl]_{ECV} + \sum P_{Cat} \cdot [Cat]_{ECV} + \sum P_{An} \cdot [An]_{ECV}}{P_{Na} \cdot [Na]_{ICV} + P_{K} \cdot [K]_{ICV} + P_{Cl} \cdot [Cl]_{ICV} + \sum P_{Cat} \cdot [Cat]_{ICV} + \sum P_{An} \cdot [An]_{ICV}} \right)$$  \[3-17a\]

where the symbols Cat and An represent all other diffusible cations and anions, respectively. Equation [3-17a] is seldom used to establish the membrane potential of a cell due to the unknown permeability values of the many other possible ions that could participate in cellular transport.
The Goldman equation, Eq. [3-17], was extended, first by Moreton [1969] and then by Jakobsson [1980], to incorporate the contribution of the Na\(^+\)-K\(^+\)-pump. This extended equation, which is implicit in \(V_m\), has the following form:

\[
V_m = \frac{RT}{F} \ln \left( \frac{P \cdot [\text{Na}]_{ECV} + p_K \cdot [\text{K}]_{ECV} + p_{Cl} \cdot [\text{Cl}]_{ECV} + RP \cdot [\text{Na}]_{ECV} \cdot \frac{(1-1/p)}{(F \cdot V_m / RT)} (1-1/p)}{(1-1/p)} \right) \tag{3-18}
\]

where all the terms were previously defined. A detailed derivation of this equation in its final form, starting from the Goldman equation, is also provided in Appendix A.

Equations [3-9], [3-10], [3-12] and [3-18] presented above, can be used together to quantitatively determine the exchange rates of various small ions across the cell membrane in the presence of a cell membrane potential. However, in order to define the steady state for the cell schematically depicted in Fig. 3-4, there are two basic constraints that must be imposed on the bulk intra- and extracellular environments: 1) the electroneutrality condition and 2) the isotonicity condition.

3.6.4 Electroneutrality condition

The electroneutrality condition imposes an additional constraint on the movement and distribution of ions across the cell membrane. It states that, despite a continuous movement of ions across the cellular membrane, the intracellular and the extracellular environments must be electrically neutral, i.e.,

\[
\sum_{\text{ION}} (z_\text{ION} \cdot [\text{ION}])_{ICV} = \sum_{\text{ION}} (z_\text{ION} \cdot [\text{ION}])_{ECV} = 0 \tag{3-19}
\]

where \([\text{ION}]\) and \(z_\text{ION}\) are, respectively, the molar concentration and charge of the ionic species \(\text{ION}\) (including the non-diffusing FA, FC, A and C species).

Equation [3-19] should, more accurately, be based on the activities of the ions in the solution and not their concentrations such that the chemical interactions between the species are accounted for. The choice of using the concentrations instead of the activities of the ions is a mathematical simplification employed by several authors [MacKnight, 1994; Jakobsson, 1980; Wolf, 1980] to avoid the uncertainties related to the type of interactions found in the intracellular environment. Moreover, for relatively large cells such as red blood cells there is experimental
Chapter 3: Transcapillary and Cellular Exchange

46

evidence that the activities of Na\(^+\), K\(^+\) and Cl\(^-\) ions are essentially the same as their molar concentrations (i.e., the intracellular medium of the RBC behaves as an infinitely dilute solution) [Hoffman, 1987; Edzes and Berendsen, 1975].

Based on the above discussion, it can be fairly reasonably assumed that, under normal physiological conditions, the bulk intracellular concentration of cations is equal the bulk intracellular concentration of anions.

3.6.5 Osmotic pressure, osmolarity and isotonicity condition

Generally, the osmotic and hydrostatic pressures are important factors in determining the distribution of water among the body compartments. In the majority of cells within the body (with a few exceptions such as some epithelial cells of the renal tubules), the cellular membrane cannot withstand a difference in hydrostatic pressure and, therefore, the movement of water across the cell membrane occurs only by osmosis. By definition, osmosis is the passive movement of water across a semi-permeable membrane from a solution with a higher water concentration (i.e., a lower concentration of solutes) to a solution with a lower water concentration (i.e., a higher concentration of solutes).

The theoretical osmotic pressure of a solution assumes that the membrane is impermeable to the dissolved solutes and, hence, is proportional to the number of osmoles (Osm) found in that solution. Very often, in the dilute solutions normally found in the human body, the osmolality of the solution (i.e., the number of solute particles per unit weight of solution) and the osmolarity (i.e., the number of solute particles per unit volume of solution) are assumed to be equal.

The effective osmotic pressure of a solution is dependent on both the osmolarity of the solution and the permeability of the membrane for the solutes. In general, the higher the permeability of a membrane to a given solute, the lower will be the effective osmotic pressure of the solution containing that solute. The experimental methods available for the measurement of osmolarity (e.g., solution freezing point) determine the theoretical, not the effective, osmotic pressure.

In order to differentiate between the effective and theoretical osmolarity in cell physiology, it is customary to use the term tonicity. By definition, a solution is isotonic with a cell if it has no effect on the cell volume when the cell is placed in that solution. If the solution causes the cell to shrink (i.e., if water is lost from the cells), it is considered to be hypertonic; if
the solution causes the cells to swell (i.e., if incoming water increases the volume of the cell), it is said to be hypotonic.

As previously discussed, the cellular membrane of most cells is highly permeable to water, but has a much reduced permeability to all other non-lipid species such as ions. This differential permeability of the cellular membrane is the cause of the rapid movement of water between the intra- and extracellular solutions, when the two media have different osmolarities. It is reasonably assumed that, under the conditions which occur within the body (dilute solutions, constant temperature, etc.), the rate of osmosis is directly proportional to the concentration (or osmolarity) difference between the non-diffusible species on the intra- and extracellular sides of the cellular membrane.

The isotonicity condition imposes a constraint for osmotic equilibrium between the cells and their environment and, implicitly, for the distribution of water across the cell membrane. This condition states that water will be at equilibrium if the total concentration of impermeant intracellular solute is the same as the total concentration of impermeant extracellular solute, i.e., ICV tonicity equals ECV tonicity, or

\[ \sum_{\text{ION}} (\sigma_{\text{ION}} \cdot [\text{ION}])_{\text{ICV}} = \sum_{\text{ION}} (\sigma_{\text{ION}} \cdot [\text{ION}])_{\text{ECV}} \]  \[3-20\]

where \( \sigma_{\text{ION}} \) is the reflection coefficient of species ION.

The left hand side of Eq. [3-20] represents the intracellular osmolarity, while the right hand side is the extracellular osmolarity.

If the volumes of the ICV and ECV are \( V_{\text{ICV}} \) and \( V_{\text{ECV}} \), respectively, we can determine the amount of osmotically active species in the two media as

\[ \text{Osm}_{\text{ICV}} = V_{\text{ICV}} \cdot \sum_{\text{ION}} [\text{ION}]_{\text{ICV}} \]  \[3-21\]

and

\[ \text{Osm}_{\text{ECV}} = V_{\text{ECV}} \cdot \sum_{\text{ION}} [\text{ION}]_{\text{ECV}} \]  \[3-22\]

where \( \text{Osm}_{\text{ICV}} \) and \( \text{Osm}_{\text{ECV}} \) are the number of milliosmoles in the intra- and extracellular compartments, respectively.

The volume of water shifted across the cellular membrane can be obtained from the isotonicity condition, i.e., Eq. [3-20], by assuming that any disturbance in the extracellular or
intracellular osmolarity will cause an instantaneous shift of water across the cellular membrane. This assumption is based on experimental evidence showing that erythrocytes and skeletal muscle cells behave as perfect osmometers for a wide range of hypertonic and hypotonic external media (for reviews, see House, 1974; Weiss, 1998). The algebraic equation describing the volume of water, \( V_w \), shifted into or out of the tissue cells, at any instant, has the following form:

\[
V_w = \frac{Osm_{ECV} \cdot V_{ICV} - Osm_{ICV} \cdot V_{ECV}}{(Osm_{ICV} + Osm_{ECV})}
\]  

[3-23]

According to Eq. [3-23], the volume of water shifted across the cellular membrane, \( V_w \), is positive when the extracellular osmolarity increases thereby causing the cells to shrink. Alternatively, when the extracellular osmolarity decreases, the term \( V_w \) is negative causing the cells to swell.

The equations presented above summarize the simplest, most accepted mathematical descriptions for the transport of ions and water across the cell membrane. The equations are representative of typical mammalian, spherical, non-excitable cells. It is expected that the relative magnitude of the different types of transport vary amongst the multitude of cells found in the human body.

This chapter summarized the transport of water, small ions and/or proteins across the two important transport barriers found in the body, i.e., the capillary and cellular membranes. As part of this work, the mathematical descriptions of cellular and transcapillary exchanges presented in this chapter will be incorporated into a compartmental model that is intended to predict fluid and solute movements in the human and/or animal body. A description of this model is the objective of the next chapter.
4.1 Introduction

As a complement to experimental studies of resuscitation protocols, there is a need to develop, as well as validate, mathematical models capable of predicting the fluid, protein and small ion shifts that take place between the vascular, interstitial and cellular compartments following the administration of a given type of solution. A number of mathematical models have emerged over the past few years that address the issue of body fluid distribution and transport with the particular goal of predicting blood volume changes following hemorrhage. Significant modeling effort has been directed toward describing the mechanisms of blood restitution and, most importantly, the requirements of different resuscitation schemes [Carlson et al., 1996; Mazzoni et al., 1988]. However, prior to modeling mass transport in a highly perturbed state such as burn or hemorrhage, it would be prudent to create and validate an initial model based on the normal physiological behavior of the system, including the estimation of parameters which are difficult to measure or unavailable from the literature.

The present chapter presents the formulation of a compartmental model that describes fluid, protein and small ion exchanges in the human body. This model is concerned with the two main sites of exchange in the body namely, the microvascular exchange system (MVES) and the cellular exchange. The necessary physiological background required for understanding the model was provided in Chapters 2 and 3.

A mathematical description of microvascular exchange is a subject of ongoing interest to our group. In an attempt to accurately describe the human MVES, compartmental as well as distributed models have been considered [e.g., Bert et al., 1988; Taylor et al., 1990]. Initially these models were formulated for different animals; but, as more information became available, they have gradually been modified to accommodate transport in humans. Historically, these models have been developed in a hierarchical fashion; from simple to complex. New fluid compartments and/or new species and their corresponding transport equations were introduced when the availability of new physiological information allowed an increase in complexity without comprising the accuracy of these models. To simulate traumatic conditions such as burns required a further increase in model complexity [e.g., Ampratwum et al., 1995]. The addition of
cellular exchange as a subset to the MVES compartmental models is the primary new feature introduced in this work.

The present chapter is concerned primarily with the following two topics:

- a general review of previous models involving transport of fluid and solutes within the MVES and/or between the intra- and extracellular fluids.
- a description of the mass balances, transport equations and constitutive relationships of the human model proposed in this work.

4.2 Literature Review

4.2.1 Compartmental MVES models

Guyton was among the first physiologists to emphasize an analytical approach to the understanding of physiological control. His most complex system analysis of body fluid dynamics and its control [e.g., Guyton et al., 1973], involved not only the circulatory dynamics, but also many other aspects including the dynamics of free and gel fluids in tissues, the intracellular fluid and the endocrine control of body fluid volume. Useful simulations with this model and the predicted results for fluid distribution and transport in the human body are available at http://phys-main.edu/WORKSHOP/MODELS/ [Coleman, 2000]. Subsequent to Guyton's initial formulation, Wiederhielm [1979] developed a MVES model that had the following important feature: in addition to plasma proteins, it considered that the interstitium also contains other osmotically active substances, which exhibit unusual volume exclusion characteristics. This model was employed to simulate the steady-state and transient responses to perturbations in arterial and venous pressures, plasma oncotic pressure and interstitial mucopolysacharide content as well as to disruptions of the lymphatic system.

As a follow-up to the Wiederhielm model, Bert and Pinder [1982] introduced a constant value for interstitial volume exclusion based on the observation that the volume from which albumin is excluded remains unchanged, even during tissue swelling. The Bert and Pinder model was used for studying both the steady-state and transient responses to different forms of edema. Parts of this model are found incorporated in all other models subsequently developed by this group.

Using the same basic approach, Bert et al. [1988] developed a dynamic mathematical model to describe the distribution and transport of fluid and plasma proteins between the circulation, the interstitial space of skin and muscle, and the lymphatics in the rat. Two theories
of transcapillary transport were investigated and tested, i.e., a homoporous 'Starling Model' and a heteroporous 'Plasma Leak Model'. It was concluded, based on statistical fitting of the model predictions to the available experimental data, that the 'Plasma Leak Model' gave a slightly better description of microvascular exchange. However, due to the fact that the 'Plasma Leak Model' requires a larger number of transport parameters which are usually not available experimentally, it was abandoned in future studies in favor of the Starling-type models.

Chappie et al. [1993] employed a 'Coupled Starling' or 'Patlak' model of transcapillary exchange to describe the distribution and transport of fluid and albumin in the circulation, interstitium and lymphatics of humans. Xie et al. [1995] also used the 'Coupled Starling Model' and a statistical fitting procedure involving clinical data to determine the transport parameters during normal human microvascular exchange. This model was successful in simulating the transient responses of the normal MVES to cases where saline and albumin solutions were infused.

In all previous models of microvascular transport formulated by our group [Bert et al., 1988; Chappie et al., 1993], it was assumed and reasonably justified that, under specific conditions, the cellular compartments did not play a significant role in the exchange process, i.e., the small ions and fluid in the cells were considered at all times to be at equilibrium with those in the plasma and interstitium. Nevertheless, important clinical situations, such as hemorrhage with its altered cellular activity and use of hypertonic resuscitants, require the consideration of a cellular component which plays an active role in exchange involving fluids and ions during short-term fluid regulation. The electrophysiological basis for this requirement (e.g., cell membrane depolarization, cellular edema) is well established now by studies pioneered by Shires et al. [1972] and Nakayama et al. [1985].

4.2.2 Cellular volume regulation (CVR) models

There are only a few mathematical models available that allow a quantitative analysis of cellular volume regulation. This is partly due to the experimental difficulty in obtaining sufficient information to both formulate and validate any mathematical description of cellular behavior. Thus, the predictions provided by these models are only speculative, at best. Some modeling progress has been made in describing the steady-state behavior of a typical mammalian cell bathed in an infinite extracellular volume [e.g., Jakobsson, 1980]. However, there have been only a few successful attempts to formulate models that predict the transient behavior of a
cellular compartment embedded in a finite extracellular volume [e.g., Carlson et al., 1996], as is required in the present study.

The steady-state models described below refer to a single generalized cell (i.e., cellular compartment) characterized by a constant area of transport and defined cellular membrane permeabilities. The cell is bathed by an infinite extracellular medium with a constant composition.

Tosteson and Hoffman [1960] pioneered the first steady-state model of a red blood cell that controls its cation composition and volume by the action of a Na\(^+\)-K\(^+\)-pump. Their model was intended to establish the processes responsible for counterbalancing the osmotic forces that produce a natural, constant tendency of cells to swell. Simple diffusion equations were written and solved, one at a time, for Na\(^+\), K\(^+\) and Cl\(^-\). The equilibrium Nernst potential for Cl\(^-\) was considered to provide the constant transcellular membrane potential. The contribution of sodium or potassium to the cell membrane potential was not specifically taken into account in any of their equations. The Na\(^+\)-K\(^+\)-pump was considered to exchange one sodium ion from inside the cell for one potassium from outside, i.e., a non-electrogenic pump with \(\rho = 1\).

The mathematical models that followed extended the Tosteson and Hoffman model to include more precise descriptions of membrane permeabilities, ion fluxes and the Na\(^+\)-K\(^+\)-pump [Stein, 1967; Fromkin, 1973]. Wolf [1980] developed a mathematical model for a red blood cell in which he incorporated net steady-state fluxes for Na\(^+\) and K\(^+\) derived by integrating the Nernst-Planck equation under constant field conditions [Plonsey, 1969], together with a mathematical expression for the Na\(^+\)-K\(^+\)-pump [Tosteson, 1964]. He also assumed a variable osmotic coefficient for hemoglobin. A computer program associated with this model allowed the prediction of the steady-state water and electrolyte distributions between the interior and exterior of the cell. Although it represented a major step forward in the understanding of cell volume regulation, his model had several weaknesses. For example, it assumed that extracellular electroneutrality and osmolarity are due only to contributions from the Na\(^+\), K\(^+\) and Cl\(^-\) ions; it is well established, however, that other ions present inside or outside the cells are important determinants of water movement across the cellular membranes. Although the values chosen for these ions respect external electroneutrality, the isotonicity condition between the exterior and interior of the cell was not satisfied. Also, only discrete values were ascribed to the membrane potential (as imposed by the chloride equilibrium condition). As was standard practice at the time, the model equations were not solved simultaneously.
One of the most detailed models of cellular volume regulation is the one proposed by Jakobsson [1980]. His model consists of transport equations for each of the permeant species assumed to cross the cellular membrane, as well as equations for bulk internal electroneutrality within the cell and for the condition of isotonicity between the intracellular and extracellular fluid. This was the first model where the transport equations together with the electroneutrality and isotonicity conditions were solved simultaneously. By using this approach, Jakobsson was able to determine the steady-state cellular transport parameters. Modifications of his initial steady-state model allowed Jakobsson to further follow the transient behavior of the cellular volume under a variety of postulated conditions. His analysis stressed the interdependence of passive and active ionic fluxes, membrane potential, cellular composition and cellular volume, as well as the need to quantitate all of these variables in order to interpret experimentally induced changes in cellular volume. One important feature of Jakobsson's model was his extension of the steady-state equations to account for the dynamics of cell volume regulation. Jakobsson's model will be extensively used in this work; therefore, a complete development of both his steady-state and time-dependent equations are presented in Appendix B.

The models enumerated so far rely on known and/or estimated values of the cellular membrane permeability in order to describe the transport of small ions through a cellular membrane. Due to inherent experimental difficulty in determining the cellular membrane permeabilities (see Table 3-1), there is some concern about the accuracy of the literature values.

As an alternative to the previous modeling approach, several mathematical models have appeared over the past decade that describe the volume regulation of the epithelial cells of renal tubules by using the so called 'equivalent circuit' approach [Civan and Bookman, 1982; Duszyk and French, 1991; Gordon and Macknight, 1991; Strieter et al., 1992]. These models have the ability to incorporate a very detailed description of both the primary and secondary transports (i.e., transmembrane transport by simple electrodiffusion and facilitated co-transport of two different ions, respectively). The contributions of $K^+\text{-Cl}^-$ and $Na^+\text{-Cl}^-$ co-transport as well as $Cl^-$ counter-transport to the steady-state cellular membrane potential were discussed extensively in a paper by Gordon and Macknight [1991]. These models are based on the assumption that the net electric current carried by an ion across a polarized barrier is given by the sum of a constant field electric term and a contribution from a specialized transfer function within that barrier. The cellular membrane therefore is essentially represented as an electric capacitor. The only appreciable currents are considered to be those due to the transmembrane passage of $Na^+$, $K^+$ and
4.2.3 Mathematical models of whole-body fluid and solute exchange

In some early models of fluid volume regulation, Wolf [1982] and Mazzoni et al. [1988] described the movement of water, protein and crystalloids between plasma and interstitial fluid compartments and took into consideration only the fluid exchange with cells. The exchange of solutes between the intracellular and extracellular compartments was not accounted for. The more complex model of Carlson et al. [1996] added several perturbations hypothetically descriptive of hemorrhage to a precursor model. Although this latter model describes fluid, protein and small ion exchanges between the extra- and intracellular compartments, no true validation was attempted. Additionally, the magnitudes of the imposed perturbations were adjusted to fit their specific set of experimental data. Although novel in concept, because the Carlson et al. model lacks validation, the reliability of its predictions are unknown.

The primary goal of the present study is to extend our previous modeling efforts [Bert et al., 1988; Bowen et al., 1989; Chappie et al., 1993], as well as those of other authors [Jakobsson, 1980; Mazzoni et al., 1988; Wolf, 1982] to develop a compartmental model which describes fluid and solute exchanges between the plasma, interstitium and cells. Furthermore, to have confidence in our description of the underlying physiological mechanisms which might contribute to effective plasma replacement, we will first test this model against experimental data which take into account solely the effects of different types of infusions, i.e., in the absence of any type of trauma such as hemorrhage or burn injury.

4.3 Model Description

The purpose of this section is to develop a conceptually up-to-date mathematical model which describes the fluid and solute (i.e., protein and small ions) exchanges between circulating blood and interstitium, taking into account the exchanges occurring with the cells associated with these spaces.
4.3.1 General considerations

In the present study, the fluid and solute exchanges in the body are described in terms of a compartmental model. Hence, all variables are considered to be spatially invariant, and the dynamic behavior of the system can be represented by a set of ordinary differential equations.

The transcapillary fluid exchange is based on Starling's hypothesis presented in Chapter 3, i.e., hydrostatic and osmotic pressures govern fluid flow across the capillary membrane. The transcapillary transport of proteins is described by the Patlak equation for the transport of a neutral solute through a homoporous membrane. The transport of small ions is assumed to take place through uncoupled diffusion and convection and is described by the Kedem-Katchalsky equation. Based on their molecular weight, the solutes introduced to the body by infusion will be considered to be macromolecules or small ions. Hence, their transcapillary transport is also described by either the Patlak or Kedem-Katchalsky equations. The Donnan effect exerted across the capillary membrane is accounted for in both the fluid transport and small ion transport equations.

The cellular exchange portion of the model assumes an instantaneous shift of water between the cells and their corresponding extracellular compartments based on rapid osmotic equilibration. The movement of ions across the cell membrane described by the Nernst-Planck equation, is influenced by changes in the concentration difference driving forces and in the cell membrane potential. Active transport of Na\(^+\) and K\(^+\) ions is also accounted for by the inclusion of an electrogenic Na\(^+\)-K\(^+\)-pump.

4.3.2 Model compartments

The model consists of the set of compartments presented schematically in Fig. 4-1. Two interconnected homogenous compartments, namely the plasma compartment and the generalized interstitial compartment, constitute the extracellular fluid. The two compartments exchange fluid, proteins and small ions across a semi-permeable membrane, the capillary wall, as well as, via the lymphatics. As was discussed in Chapter 2, although there may be an anatomical barrier between the interstitium and the lymphatic vessels, due to an incomplete elucidation of the mechanisms responsible for lymphatic transport, the lymphatics are treated as membraneless, passive conduits for fluid and solute transport from the interstitium to plasma.
Figure 4-1: Schematic of a general model of fluid, protein, small ion and additional solute (introduced through infusion) exchange between plasma, interstitium and cells. Arrows indicate fluid (J), protein (Q), ion (M) and solute (R) transport rates in relation to the compartments participating in mass exchange. The subscripts are defined in the main text as well as in the Nomenclature section found near the end of the thesis.
Each of the two main fluid compartments, plasma or interstitium, contains an embedded cellular fluid compartment. As a consequence, the interstitial compartment is composed of interstitial fluid having a volume $V_{IT}$, and tissue cells, whose fluid volume is $V_{TC}$. The generic term 'tissue cells' describes cells with lumped properties, which represent the diversity of cells included in the generalized interstitium. Additionally, for a first formulation of this model, the volume of endothelial cells is also lumped into the generalized 'tissue cell' compartment. Since about 60 to 70% [Koushanpour, 1976] of the interstitial intracellular water is found in the skeletal muscle cells of the body, it is assumed in this work that the tissue cells have the same properties as skeletal muscle cells. The vascular compartment is made up of plasma having a volume $V_{PL}$, and red blood cells (RBC) with a fluid volume $V_{RBC}$. The RBC volume is related to the plasma volume via the systemic hematocrit, Hct, given by

$$Hct = \frac{V_{RBC}}{(V_{RBC} + V_{PL})} \cdot 100\%$$  \hspace{1cm} [4-1]

### 4.4 Model Assumptions

All models that purport to describe biological systems involve varying degrees of simplification. Compartmental models, especially, are often criticized for excessively idealizing the system under study. Usually they are formulated such that a complex, real system is reduced to a set of sub-systems or compartments where the spatial heterogeneity of material properties is largely ignored while the physical boundaries between different compartments are described in terms of lumped parameters. Nevertheless, owing to their relative mathematical simplicity and minimal parameter requirements, such models are the only reasonable choice for simulating whole body fluid and solute exchanges during normal and pathophysiological states (e.g., hemorrhage or burns, followed or not by fluid resuscitation). In order to minimize such criticisms, it is important to clearly specify the assumptions upon which the model is formulated and, furthermore, to base such assumptions on reliable physiological information.

The set of assumptions that form the basis of this model, as well as their justifications, are listed below.

- All compartments, including the cellular sub-compartments, are considered to be well-mixed with spatially constant descriptive parameters, i.e., mean values for descriptive properties (e.g., transport parameters) and dependent variables (e.g., concentrations of specific solutes) are used at any given time.
This assumption is consistent with the type of data normally measured experimentally for both intra- and extracellular fluid compartments, [e.g., Vick, 1970; Houser, 1980]. Additionally, the use of spatially-invariant compartments has proven to be successful in several previous modeling studies [e.g., Wiederhielm, 1979; Wolf, 1980; Bert et al., 1988; Carlson et al., 1996].

- All proteins in the system are assumed to have the same properties as albumin with an average molecular weight, $MW_p = 67000$ g/mol. These species generate oncotic pressures and are exchanged only between plasma and interstitium (i.e., there is no protein exchange across the cell membrane). Additionally, they are responsible for generating a Donnan effect across the capillary membrane.

In all of the MVES models previously developed by this group [e.g., Chappie et al., 1993], albumin was used as a surrogate protein to represent the collective influence of all plasma proteins. This assumption was justified by the fact that albumin accounts for more than 50% of the total plasma protein mass in humans, and generates approximately 65% of the total plasma colloid osmotic pressure. In the present work, however, similar to what was done in other more recent models [e.g. Carlson et al., 1996], the model accounts for the concentration and amount of total proteins present in the extracellular fluid.

- The same conductive pathways in the capillary membrane serve as sites for both fluid and protein transport between plasma and interstitium.

This assumption implies the use of the Patlak [Patlak et al., 1963] equation for coupled transcapillary transport of fluid and macromolecular species which is valid over a wide range of hydrostatic and colloid osmotic pressure differences across the membrane. The formulation applies to homogenous membranes in which convective and diffusive transport occur within identical channels that exhibit a constant reflection coefficient for the protein. Chappie et al., [1993] found that Patlak's representation of transcapillary fluid and protein transport was the most suitable for compartmental models.

- Five types of mobile ions are accounted for the in the two extracellular compartments: $Na^+$, $K^+$, $C^2+$ (a species representative of all other positive ions), $Cl^-$ and $A^-$ (representative of all other anions).

Sodium, potassium and chloride represent the bulk of ions in the human body. These ions are the primary contributors to the total osmolarity of the body fluids; of the total 300 mOsm/l typically reported for the fluids of the body, approximately 280 mOsm/l are provided by the combined
contribution of these three ions [e.g., Kleinman and Lorentz, 1984]. Additionally, they are the main species transported across the capillary and cellular membranes. The cationic species $C^{2+}$ and the anionic species $A^-$ were introduced in order that the electrical neutrality requirements of both the plasma and interstitium were respected. Since most of the additional positive charge in these two fluid compartments is due to $Mg^{2+}$ and $Ca^{2+}$ [Kleinman and Lorenz, 1984], a charge of +2 was attributed to the $C^{2+}$ species. The negative species $A^-$ represents other anions such as acetates, carbonates, lactates, etc. Hence, a negative charge of -1 was assumed for this species.

All of these ions participate in transcapillary exchange. Additionally, they are distributed on either side of the capillary membrane according to a Donnan equilibrium that will be discussed later in this chapter.

- The transport of small ions across the capillary membrane takes place through uncoupled convection and diffusion.

This assumption implies the use of the equation for diffusive-convective transport of fluid and small solutes proposed by Kedem and Katchalsky [1958]. Although the capillary channels are not well defined structurally, an increasing number of researchers [e.g., Wolf, 1996; Meinild et al., 1998] report the presence of additional (either diffusive or convective) transcapillary pathways for solutes and/or water other than the typical inter-endothelial junctions. Such an assumption, therefore, is not unreasonable and seems to be increasingly employed in modeling studies [e.g., Carlson et al., 1996].

- Five types of ions are also accounted for in the two intracellular compartments: $Na^+$, $K^+$, $FC^{2+}$, $Cl^-$ and $FA^-$. The only small ions transported across the cellular membrane between the intra- and extracellular compartments are sodium, potassium and chloride. All other intracellular cations besides $Na^+$ and $K^+$ are denoted as $FC^{2+}$, and all anions other than $Cl^-$ are denoted as $FA^-$. These species are considered to be present in fixed amounts, i.e., they do not cross the cell membrane. Thus, any changes in the concentrations of these species are due solely to cellular swelling or shrinking. Such an assumption is in agreement with other modeling studies [e.g., Wolf, 1980; Jakobsson, 1980].

- The transport of $Na^+$ and $K^+$ across the cell membrane occurs by both electrodiffusion and active transport (i.e., by means of a $Na^+-K^+$-pump).
This type of cellular transport, was described in some detail in the previous chapter, and reflects our current understanding of cellular transport. As in a previously reported model [Jakobsson, 1980], the Na\(^+\)-K\(^+\)-pump is characterized by a constant ratio of Na\(^+\) to K\(^+\) transport, \(\rho = ([Na^+]_{\text{out}}/[K^+]_{\text{in}}) = 3/2\), and a constant rate, RP. The flux of Na\(^+\) or K\(^+\) due to active transport is dependent on the pump rate and the intracellular sodium and extracellular potassium concentrations.

- Chloride is not transported across the cellular membrane by electrodiffusion in response to its electrochemical gradient and no active transport is ascribed to this ion. Since Cl\(^-\) is the only negative ion considered to cross the cell membrane, the transport of this ion is correlated to Na\(^+\) and K\(^+\) exchanges through electroneutrality considerations, i.e., the rate of Cl\(^-\) transfer is equal to the net rate of Na\(^+\) and K\(^+\) exchange.

- Changes in the cell transmembrane potential, \(V_m\), take place instantaneously as a direct consequence of the redistribution of ions across the cell membrane. Several authors who modeled cellular transport [e.g., Wolf, 1980; Jakobsson, 1980], have used a similar approach. This assumption is based on the fact that, compared with the amount of ions normally present inside and outside the cells, only a minute separation of charges is required to produce a change in the membrane potential [Matthews, 1998]. Additionally, changes in membrane voltage due to the redistribution of charges on either side of the cell membrane occur within milliseconds, while changes in intracellular concentrations take place in the order of seconds to minutes. Thus, at every instant, the transmembrane potential is in a quasi-steady state dictated by the ionic concentration differences across the cell membrane, the membrane permeabilities for small ions, and the magnitude of the active transport term.

- Changes in cell volume are directly related to the change in cellular water content and are assumed to occur instantaneously (i.e., the cellular membrane is assumed to be freely permeable to water). Such an assumption has as its basis the high water content of most mammalian cells, i.e., on the order of 80-90% [MacKnight and Leaf, 1977; MacKnight, 1994]. As in other models [Jakobsson, 1980], the water shifts across the cell membrane are imposed by the isotonicity condition between the extra- and intracellular environments. Since, in most cells, the cellular membrane permeability for water is about three to five orders of magnitude higher than for small ions [Jakobsson, 1980], these shifts are assumed to take place instantaneously [MacKnight and Leaf, 1977; MacKnight, 1994]. One consequence of this justifiable assumption is that the model does
not predict the dynamics associated with the movement of cellular fluid based on differences in
tonicity.

- The model accounts for a basic requirement neglected in previous models [e.g., Tosteson
and Hoffman, 1960; Wolf, 1980], namely that the electroneutrality of the bulk intracellular
solution must be respected.

- The cellular characteristics are taken to be different for red blood cells and tissue cells
according to experimental information [Hoffman, 1987; Kleinman and Lorenz, 1984]. These
include the intracellular concentrations of small ions as well as such cellular transport
parameters as the ion permeabilities.

In addition to the above statement, it was also considered that the tissue cells have the same
characteristics as skeletal muscle cells. This assumption is based on the fact that 65% of the total
tissue mass available for capillary exchange is attributed to skeletal muscle [Landis and
Pappenheimer, 1963]. Additionally, the muscle cells represent about 30% of the total normal
body weight or alternatively about 70% of the ICV [Guyton, 1991; Koushanpour, 1976]. It is
expected, therefore, that the skeletal muscle cells are an important source of water mobilization
when the interstitial fluid is osmotically disturbed.

- The properties of the cellular and capillary membranes, e.g., the transport parameters, are
unaffected by the type of perturbation (e.g., different types of non-traumatic infusions)
simulated in this study.

Unfortunately, there is not enough experimental information to either confirm or contradict the
above statement. However, this assumption will be reconsidered appropriately, if validation of
the model proves to be unsatisfactory.

- All species introduced by infusion that are not already constituents of the system will have
transport characteristics similar to either proteins or small ions depending on their molecular
weight.

According to the above assumption, the following arbitrary classification of solutes has been
made:
- solutes with molecular weights greater than or equal to 1000 g/mol (e.g., dextrans) are
transported in the MVES via the same mechanisms as the ones described for proteins.
- solutes with molecular weights less than 1000 g/mol (e.g., glucose) are transported in a
similar fashion as the small ions.
In both cases, however, the specific transport parameters of the solute (e.g., reflection coefficient, permeability-surface area product, etc.) will be obtained from the literature.

- For simplicity and due to lack of sufficient data, the model does not account for the complex endocrinal and neural effects that might take place during the type of perturbation simulated in this work (i.e., fluid overload and/or external loss of blood through hemorrhage).

4.5 Model Equations

To predict the interdependent fluid, protein and small ion distribution and transport in the vascular, interstitial and intracellular compartments, the model requires, as a next step, mass balance equations for each species and compartment considered, as well as descriptions of the transcellular and transcapillary membrane exchanges. The discussion that follows is given in reference to the schematic diagram of the four fluid compartments illustrated in Fig. 4-1.

4.5.1 Mass balance equations for extracellular compartments

The dynamic behavior of the two extracellular compartments, plasma (PL) and interstitium (IT), is based on the following mass balance equations. All the symbols used are defined at the end of this section and also in the Nomenclature section near the end of the thesis.

\[ \frac{dV_{PL}}{dt} = J_{RES} - J_{IT} + J_{L} - J_{UR} \]  \hspace{1cm} [4-2]

\[ \frac{dV_{IT}}{dt} = J_{IT} - J_{L} - J_{PER} - J_{ISL} \]  \hspace{1cm} [4-3]

**Protein balances:**

\[ \frac{dQ_{PL}}{dt} = \dot{Q}_{RES} - \dot{Q}_{IT} + \dot{Q}_{L} \]  \hspace{1cm} [4-4]

\[ \frac{dQ_{IT}}{dt} = \dot{Q}_{IT} - \dot{Q}_{L} \]  \hspace{1cm} [4-5]

**Infused macromolecular species (e.g., dextran) balances:**

\[ \frac{dR_{PL}}{dt} = \dot{R}_{RES} - \dot{R}_{IT} + \dot{R}_{L} - \dot{R}_{UR} \]  \hspace{1cm} [4-6]

\[ \frac{dR_{IT}}{dt} = \dot{R}_{IT} - \dot{R}_{L} \]  \hspace{1cm} [4-7]
A total of ten additional mass balance equations, five for each extracellular compartment, describe the transport of the small ions, Na\(^+\), K\(^+\), C\(^2+\), Cl\(^-\) and A\(^-\). Since the balances of all ions in a given compartment, either PL or IT, have a similar form, in order to avoid repetition, the generic subscript ION is used.

**Small ion balances:**

\[
\frac{dM_{\text{ION,PL}}}{dt} = M_{\text{ION,RES}} - M_{\text{ION,IT}} + M_{\text{ION,L}} - M_{\text{ION,RBC}} - M_{\text{ION,UR}} \tag{4-8}
\]

\[
\frac{dM_{\text{ION,IT}}}{dt} = M_{\text{ION,IT}} - M_{\text{ION,L}} - M_{\text{ION,TC}} - M_{\text{ION,PER}} \tag{4-9}
\]

In Eqs. [4-2] - [4-9], V, Q, R and M represent the compartmental fluid volume, protein, infused macromolecular species (e.g., dextran) and ion contents, respectively; while J, Q, R and M represent the rates of transport of fluid, protein, infused species and small ions, respectively, into or out of the compartment. The subscripts PL, IT, RBC and TC denote plasma, interstitial, red blood cell and tissue cell compartments, respectively; L indicates lymph; while ION is a generic term used to describe any of the ionic species (i.e., ION = Na\(^+\), K\(^+\), C\(^2+\), Cl\(^-\) or A\(^-\)). The subscript RES stands for resuscitation when a time-dependent resuscitation rate constitutes an input to the model. PER indicate the loss of fluid through perspiration, while ISL denotes the insensible losses (e.g., losses of solute-free water from lungs or through skin), for specific cases where these losses are considered, i.e., when time-dependent rates of these latter fluids constitute known or predictable outputs from the interstitial compartment (see also Fig. 4-1). UR represents the urine production rate.

### 4.5.2 Mass balance equations for intracellular compartments

The behavior of the two intracellular compartments is described by a set of six time-dependent ordinary differential equations corresponding to each of the three ions participating in cellular transport (i.e., Na\(^+\), K\(^+\) and Cl\(^-\)) in both compartments. Two additional algebraic equations account for water shifts to and from the cells, in response to external changes in osmolarity. This approach of describing cellular exchange follows that previously formulated by Jakobsson [1980]. Given below are the mass balances describing the tissue cell compartment, TC.
Chapter 4: Model Formulation

Intracellular sodium balance:

\[
\frac{dM_{Na,TC}}{dt} = A_{TC} \left[ p_{Na,TC} \Delta \phi_{TC} \cdot ([Na]_{IT} - [Na]_{TC} \cdot \exp(\Delta \phi_{TC})) \right] \frac{(\exp(\Delta \phi_{TC}) - 1)}{-RP_{TC} \cdot [Na]_{TC}} \]  \hspace{1cm} [4-10]

Intracellular potassium balance:

\[
\frac{dM_{K,TC}}{dt} = A_{TC} \left[ p_{K,TC} \Delta \phi_{TC} \cdot ([K]_{IT} - [K]_{TC} \cdot \exp(\Delta \phi_{TC})) \right] \frac{(\exp(\Delta \phi_{TC}) - 1)}{+RP_{TC} \cdot \frac{[Na]_{TC}}{\rho_{TC}}} \]  \hspace{1cm} [4-11]

Intracellular chloride balance:

\[
\frac{dM_{Cl,TC}}{dt} = \frac{dM_{Na,TC}}{dt} + \frac{dM_{K,TC}}{dt} \]  \hspace{1cm} [4-12]

In Eqs. [4-10]-[4-12], \( M_{Na,TC}, M_{K,TC} \) and \( M_{Cl,TC} \) represent the sodium, potassium and chloride ion contents of the tissue cell compartment; \( A_{TC} \) is the membrane surface area of this compartment; \( p_{Na} \) and \( p_{K} \) denote the permeabilities for \( Na^+ \) and \( K^+ \), respectively; \( RP \) is the rate of the \( Na^+ - K^+ \)-pump; while \( \rho \) is the pump ratio. \([Na], [K] \) and \([Cl]\) are, respectively, the sodium, potassium and chloride concentrations for either the intracellular medium (i.e., tissue cells), subscript TC, or the extracellular medium (i.e., interstitium), subscript IT. \( \Delta \phi \) is the dimensionless cell membrane potential, defined as \( \Delta \phi = FV_m/RT \), where the ratio \( F/RT \) is 26.7 mV for mammalian cells at body temperature, while \( V_m \) is the dimensional cell membrane potential.

The first term on the right hand side of Eqs. [4-10] and [4-11] represents transport due to electrodiffusion and establishes the interdependence between the ion permeabilities, the intracellular and extracellular concentrations, and the transmembrane potential. The second right-hand-side term of these two equations describes active transport and states that the transport rate associated with the \( Na^+ - K^+ \)-pump is a linear function of the intracellular sodium concentration. The area term in Eqs. [4-10] and [4-11] represents the membrane surface area of the entire cellular compartment which is available for transport. This area is assumed to be constant, i.e., an increase or decrease in cellular volume would stretch or compress the cellular membrane without affecting the area (i.e., the number of pores or exchange sites) available for exchange (R.K. Reed, personal communication).
Equation [4-12] states that the chloride ion crosses the membrane at a rate sufficient to maintain intracellular electroneutrality (assuming that the compartment is initially electroneutral). Therefore, the Cl⁻ mass balance equation implicitly includes the electroneutrality condition assumed for the internal environment of either type of cell.

According to the assumptions mentioned in the previous section, the other positive, FC²⁺, and negative, FA⁻, species are not transported across the cellular membrane. Consequently, the transport rates across the cell membrane for these two fixed species are zero, their intracellular contents are constant and their concentrations are determined solely by the changes in cellular volume.

The algebraic equation describing the volume of water shifted at any instant into or out of the tissue cells, $V_{w, TC}$, has the following form:

$$V_{w, TC} = \frac{Osm_{IT} V_{TC} - Osm_{TC} V_{IT}}{(Osm_{IT} + Osm_{TC})}$$  \[4-13\]

where $Osm_{IT}$ and $Osm_{TC}$ are the total osmolarities of the interstitial and tissue cell compartments, respectively. This equation is a direct consequence of the isotonicity condition, i.e., the external osmolarity equals the intracellular osmolarity, and shows that any disturbance in the extracellular osmolarity will cause an instantaneous water shift across the cellular membrane. With each water shift, the interstitial volume, $V_{IT}$, is updated instantly to $(V_{IT} + V_{w, TC})$, independent of the terms on the right hand side of Eq. [4-3].

The tissue cell transmembrane potential, $\Delta \phi_{TC}$, is described by a non-linear algebraic equation employed initially by Jakobsson [1980]. As presented in Chapter 3, this equation is a modified form of the Moreton equation for the transmembrane potential [Moreton, 1969] and can be written as:

$$\Delta \phi_{TC} = \ln \left[ \frac{1 - \frac{1}{\rho_{TC} \Delta \phi_{TC}}} {\frac{1}{\rho_{TC} \Delta \phi_{TC}}} \right]$$

$$= \ln \left[ \frac{\left[ p_{Na, TC} [Na]_{IT} + p_{K, TC} [K]_{IT} + p_{Cl, TC} [Cl]_{TC} + RP_{TC} [Na]_{TC} \right]} {\left[ p_{Na, TC} [Na]_{TC} + p_{K, TC} [K]_{TC} + p_{Cl, TC} [Cl]_{IT} + RP_{TC} [Na]_{TC} \right]} \right]$$  \[4-14\]

All of the symbols used in Eq. [4-14] were defined earlier.
A similar set of five equations describes the behavior of the RBC compartment.

In summary, a total of 22 ordinary differential equations for fluid, protein, infused species, and five type of small ions, Eqs. [4-2] to [4-12], together with two algebraic water-shift equations of the type of Eq. [4-13], and two non-linear membrane potential equations, similar to Eq. [4-14], describe the interdependent fluid, protein, and small ion distribution and transport in the vascular, interstitial and intracellular compartments. These equations are solved simultaneously in order to obtain a complete description of the time-dependent behavior this system. The numerical and computational procedure for solving the above system of equations is given in the next chapter.

4.5.3 Transport equations

The mass balance equations presented above require a number of transport equations that characterize fluid, protein and small ion exchanges across the capillary and cell membranes as well as through the lymphatics. A generalized discussion of these transport equations was presented in Chapter 3. However, the formulation of the model, which includes contributions of small ions to extracellular osmotic pressures as well as Donnan considerations, requires revision of some of these equations.

4.5.3.1 Transport across the capillary membrane

Fluid, protein and small ions are transported across the capillary wall by filtration, diffusion and convection. The magnitude and direction of the transcapillary fluid movement is determined by differences in hydrostatic pressure, colloid osmotic pressure exerted by proteins and infused macromolecules, and osmotic pressure provided by the small ions. Thus, the rate of fluid filtration, \( J_{IT} \) (ml/h), from the plasma to the interstitial compartment is given by the extended form of the Starling equation:

\[
J_{IT} = k_F [P_C - P_{IT} - \sigma (\pi_{P,PL} - \pi_{P,IT}) - \sum_{ION} \sigma_{ION} (\pi_{ION,PL} - \pi_{ION,IT} - \pi_{ION,D}) - \sigma_R (\pi_{R,PL} - \pi_{R,IT})]
\]

[4-15]

where \( k_F \) (ml/mmHg^-1-h^-1) is the fluid filtration coefficient representing the hydraulic conductivity of the capillary membrane; \( P_C \) and \( P_{IT} \) (mmHg) are the hydrostatic pressures in the capillary and interstitium respectively; \( \pi_{P,PL} \), \( \pi_{R,PL} \) and \( \pi_{P,IT} \), \( \pi_{R,IT} \) (mmHg) are the colloid
osmotic pressures exerted by the proteins and infused dextran in the plasma and interstitial compartments, respectively; $\pi_{\text{ION,PL}}$ and $\pi_{\text{ION,IT}}$ (mmHg) represent the osmotic pressures exerted by the small ions in plasma and interstitium, respectively; while $\pi_{\text{ION,D}}$ (mmHg) indicates the osmotic contribution of the small ions restricted in their movement due to Donnan constraints. A more detailed description of the small ion contribution to the Donnan effect and the way in which this effect was accounted for in the model is given in Section 4.5.4.4. $\sigma$, $\sigma_R$, and $\sigma_{\text{ION}}$ are the average reflection coefficients for proteins (i.e., albumin), dextran and small ions, respectively.

The rate of albumin transport across the capillary membrane, $Q_{\text{IT}}$ (g/h), is governed by the Patlak equation [Patlak et al., 1963; Bresler and Groom, 1981], which non-linearly couples the protein transport from the circulation to the interstitium with the fluid exchange:

$$ Q_{\text{IT}} = J_{\text{IT}} \cdot (1 - \sigma) \cdot \frac{c_{P,\text{PL}} - c_{P,\text{IT,AV}} \cdot \exp(-\frac{J_{\text{IT}} \cdot (1 - \sigma)}{PS})}{1 - \exp(-\frac{J_{\text{IT}} \cdot (1 - \sigma)}{PS})} $$

In Eq. [4-16], $c_{P,\text{PL}}$ and $c_{P,\text{IT,AV}}$ (g/ml) are the protein concentrations in plasma and available interstitial volume. As discussed in more detail in Section 4.5.4.3, $c_{P,\text{IT,AV}}$ is the effective interstitial protein concentration calculated as the interstitial protein content divided by the interstitial volume available to macromolecular species (see also Section 2.5.2.1). $PS$ (ml/h) is the protein permeability-surface area product of the capillary. The argument of the exponential operator represents a modified Pécel number given as the ratio of convective to diffusive protein transport, i.e., $Pe = J_{\text{IT}} \cdot (1 - \sigma) / PS$.

A similar expression is used to describe the rate of transport of the macromolecular species, $R_{\text{IT}}$ (g/h), e.g., dextran, introduced through infusion:

$$ R_{\text{IT}} = J_{\text{IT}} \cdot (1 - \sigma_R) \cdot \frac{c_{R,\text{PL}} - c_{R,\text{IT,AV}} \cdot \exp(-\frac{J_{\text{IT}} \cdot (1 - \sigma_R)}{PS_R})}{1 - \exp(-\frac{J_{\text{IT}} \cdot (1 - \sigma_R)}{PS_R})} $$

where the subscript $R$ stands for dextran while all the other symbols were defined for Eq. [4-16].
Chapter 4: Model Formulation

It was assumed in the present study that the transport rate of small ions across the capillary membrane, \( M_{\text{ION,IT}} \) (mmol/h), occurs through separate convective and diffusive pathways [Kedem and Katchalsky, 1958] and therefore is described as:

\[
M_{\text{ION,IT}} = J_{\text{IT}} \cdot (1 - \sigma_{\text{ION}}) \cdot \left( \frac{[\text{ION}]_{\text{PL}} + [\text{ION}]_{\text{IT}}}{2} \right) + P_{\text{ION}} \cdot ([\text{ION}]_{\text{PL}} - [\text{ION}]_{\text{IT}} - \Delta\text{ION}_{D}) \tag{4-18}
\]

where \([\text{ION}]_{\text{PL}}\) and \([\text{ION}]_{\text{IT}}\) (mmol/ml), are the ion concentrations in the plasma and interstitium, respectively; and \( P_{\text{ION}} \) (ml/h) is the capillary permeability-surface area product for small ions. For a given ion, \( \Delta\text{ION}_{D} \) (mmol/ml), represents the concentration difference across the capillary membrane caused by the Donnan effect which is described in Section 4.5.4.4. Equation [4-18] is used to represent the transcapillary transport of all the small ions present in the system except for \( A^{-} \). It was assumed that the transport of species \( A^{-} \) occurs at a rate that is just sufficient to maintain an overall electroneutral transport across the capillary wall. Therefore,

\[
M_{\text{A,IT}} = -\left( \frac{Z_{\text{Na}} \cdot M_{\text{Na,IT}} + Z_{K} \cdot M_{\text{K,IT}} + Z_{C} \cdot M_{\text{C,IT}} + Z_{\text{Cl}} \cdot M_{\text{Cl,IT}}}{Z_{A}} \right) \tag{4-19}
\]

where \( z \) is the charge of the ionic species.

According to the measurements reported by Crone and Christensen [1981], the capillary endothelium has a very low electrical resistance. If no active transport is considered across the endothelial cells, the magnitude of the Donnan potential difference can be calculated as being 1-2 mV, which is not expected to influence the ionic transport significantly. Hence, for simplicity, the transcapillary membrane potential was not accounted for, and the contribution of an electrical term in Eq. [4-18], describing the electrodiffusion of small ions, was ignored.

4.5.3.2 Transport through the lymphatics

Tissue fluid, proteins, ions, and infused macromolecules that accumulate as a result of transcapillary exchanges are drained back into the circulation by the lymphatic system, at a fluid flow rate \( J_{L} \) (ml/h), protein rate \( Q_{L} \) (g/h), ion rate \( M_{L} \) (mmol/h), and dextran rate \( R_{L} \) (g/h) respectively. It is assumed that no accumulation of material occurs in the lymphatics; consequently, the transport of fluid and solutes toward the vascular compartment by this mechanism takes place instantaneously. At steady state, the filtration flow rate equals the
lymphatic flow rate while the net rate at which each individual solute is exchanged across the capillary membrane equals the rate at which it is returned by the lymphatic fluid.

The equations for lymph flow used in the model are based on previous work done by this group on modeling the MVES exchange in both animals [Bert et al., 1988] and humans [Chapple et al., 1993; Xie et al., 1995]. Under normal conditions, the lymph flow rate, $J_L$, is always positive. Thus, lymph always flows from the interstitium toward the terminal lymphatics.

The equations describing lymphatic transport assume that the changes in lymph flow rate from its baseline value depend on the hydration status of the interstitium. Therefore, the lymph flow rate varies linearly with the interstitial hydrostatic pressure under both overhydrated and slightly dehydrated conditions but ceases when the interstitial pressure becomes equal to or falls below that corresponding to the excluded volume of the interstitium.

For the case of an overhydrated interstitium, i.e., when $V_{IT} \geq V_{IT, NL}$, $J_L$ is described by the following relationship:

$$J_L = J_{L,NL} + LS \cdot [P_{IT} - P_{IT, NL}], \quad P_{IT} \geq P_{IT, NL} \tag{4-20}$$

Equation (4-20) shows that $J_L$ increases from the normal steady-state value $J_{L,NL}$ proportionally with the change in the interstitial hydrostatic pressure by a factor $LS$, where $LS$ (ml/mmHg-h) is referred to as the lymph flow sensitivity. $J_{L,NL}$, the lymph flow rate under normal steady-state conditions corresponds to the interstitial hydrostatic pressure, $P_{IT, NL}$. $P_{IT}$ is the actual interstitial hydrostatic pressure.

For the dehydrated case, where $V_{IT, NL} \geq V_{IT} > V_{IT, EX}$, $J_L$ is given by,

$$J_L = J_{L,NL} \left[ \frac{P_{IT} - P_{IT, EX}}{P_{IT, NL} - P_{IT, EX}} \right], \quad P_{IT, NL} \geq P_{IT} > P_{IT, EX} \tag{4-21}$$

where $P_{IT, EX}$ is the hydrostatic pressure when the interstitial volume reaches the excluded volume $V_{IT, EX}$ (i.e., $V_{IT, AV} = 0$).

Once the interstitial volume reaches the dehydration limit, $V_{IT, EX}$, the lymph flow ceases completely. Thus, for any $V_{IT} \leq V_{IT, EX}$:

$$J_L = 0, \quad P_{IT} \leq P_{IT, EX} \tag{4-22}$$
The composition of the initial lymph is always the same as that of the corresponding interstitial fluid. Because of the non-sieving character of the lymphatic wall, the reflection coefficient \( \sigma \) for protein (and ions) is zero and therefore there is no colloid osmotic pressure difference exerted between the interstitium and lymph. Thus, the lymphatic transport of proteins from the interstitium into the main circulation, \( Q_L \) (g/h), occurs only by convection, i.e.:

\[
Q_L = J_{L} \cdot c_{P,IT} \tag{4-23}
\]

A similar approach is taken when describing the lymphatic transport of an infused species such as dextran as well as the small ions, i.e., \( R_L \) and \( M_{ION,L} \), respectively. Thus,

\[
R_L = J_{L} \cdot c_{R,IT} \tag{4-24}
\]

\[
M_{ION,L} = J_{L} \cdot [ION]_{IT} \tag{4-25}
\]

where all of the symbols were defined previously. Equation [4-25] is employed to represent the lymphatic transport of all small ions present in the system except for \( A^- \). As previously assumed for transcapillary transport (see Eq. [4-19]), it was also assumed that the transport of \( A^- \) via the lymphatics occurs at a rate that is just sufficient to maintain an overall electroneutral lymph return. Therefore,

\[
M_{A,L} = -(\frac{z_{Na} \cdot M_{Na,L} + z_{K} \cdot M_{K,L} + z_{Cl} \cdot M_{Cl,L}}{z_{A}}) \tag{4-26}
\]

where all the symbols were previously defined.

In order to complete the model description, the next section presents the additional algebraic equations needed to define certain compartmental variables (e.g., concentrations, colloid osmotic pressures, etc.), or the constitutive relationships that exist between these variables (e.g., compartmental compliance, Donnan partition, etc.).

### 4.5.4 Constitutive relationships

#### 4.5.4.1 Capillary hydrostatic pressure and circulatory compliance

As discussed in Chapter 2, all the vessels of the circulatory system, including the capillaries, are, to some extent, distensible. An increase in hydrostatic pressure in any segment of the circulation dilates the vessel and decreases its resistance to blood flow. Hence, the blood flow rate as well as the quantity blood that is stored in the vessel is increased. In order to avoid
the inherent complexity associated with the process for controlling capillary pressure (i.e., the vasomotor tone, capillary recruitment, etc.), the model makes the assumption that any changes in plasma volume will affect the capillary pressure proportionally. This assumption implies the existence of a vascular compliance. The term vascular (circulatory) compliance means, literally, the ratio of the change in vascular volume to a change in the vascular filling pressure. Hence, the circulatory compliance, FCOMPC, can be expressed as:

$$\text{FCOMPC} = \frac{\Delta V_{\text{PL}}}{\Delta P_c}$$ \[4-27\]

where $\Delta V_{\text{PL}}$ and $\Delta P_c$ are the changes, relative to their normal values, of the plasma volume and capillary pressure, respectively.

The reciprocal of the compliance, $P_{c,\text{COMP}}$, can then be used to determine the capillary pressure from:

$$P_c = P_{c,\text{NL}} + P_{c,\text{COMP}} (V_{\text{PL}} - V_{\text{PL,NL}})$$ \[4-28\]

where the subscript NL indicates the normal, steady-state value. The normal values of all variables are discussed in Section 4.6. There is insufficient literature data to allow a direct determination of $P_{c,\text{COMP}}$ for humans. Thus Chappie et al. [1993] used data from rats [see also Bert et al., 1988] to estimate an approximate human $P_{c,\text{COMP}}$ value of 0.0096 mmHg/l. When the plasma value falls below 2367 ml, the $V_{\text{PL}}$ value at which $P_c = 3$ mmHg, it is assumed that the capillary pressure remains at 3 mmHg, i.e., $P_c$ cannot take a value lower than this arbitrarily imposed minimum.

### 4.5.4.2 Interstitial pressure and interstitial compliance

The notion of interstitial compliance was briefly explained in Section 2.6.2.2 in relation to the physical characteristics of the interstitium. Similar to the vascular compliance, the interstitial compliance FCOMPI relates the changes in interstitial volume, $\Delta V_{\text{IT}}$ with the changes in interstitial pressure, $\Delta P_{\text{IT}}$. However, unlike the vascular compliance, data from several animal studies [Wiig and Reed, 1981; 1985; 1987] indicate that there is no unique constant FCOMPI for the entire range of interstitial volume changes. This conclusion is corroborated by one human study [Stranden and Myhre, 1981]. Stranden and Myhre's data, however, are too scattered to establish a compliance curve for the human interstitium. Therefore, as a compromise, Chappie et
al. [1993] used animal data to develop a 'most-likely' relationship for the interstitial compliance of humans as follows.

Based on pressure-volume experimental data from rats, Bert et al. [1988] established separate compliance relationships for the skin and muscle of these laboratory animals. These compliance relationships determined for the rat were used in conjunction with the available human data. The experimental data showed that an increase in the interstitial volume by 210% from normal, yielded an increase in the interstitial hydrostatic pressure of 2.9 mmHg in humans [Stranden and Myhre, 1981] and a corresponding increase of 2.4 mmHg in rats [Reed and Wiig, 1981]. The human compliance relationship was, therefore, developed by using the rat-to-human scaling ratio of (2.9/2.4). Hence, each pair of \((P_{IT,RAT} - V_{IT,RAT})\) data can be scaled to the appropriate human values as,

\[
P_{IT,HUMAN} - P_{IT,HUMAN,NL} = (P_{IT,RAT} - P_{IT,RAT,NL}) \cdot \left(\frac{2.9}{2.4}\right)
\]  [4-29]

and

\[
V_{IT,HUMAN} = V_{IT,RAT} \cdot \left(\frac{V_{IT,HUMAN,NL}}{V_{IT,RAT,NL}}\right)
\]  [4-30]

where all the symbols have been previously defined.

The compliance relationship is separated arbitrarily into three regions: a 'dehydration segment', an 'intermediate segment', and an 'overhydration segment' as shown in Fig. 4-2. The range of interstitial volume values and the compliance relationships corresponding to each of the above-mentioned segments are as follows:

**Dehydration segment:**

\[
P_{IT} = -0.7 + 1.96 \times 10^{-3} \cdot [V_{IT} - 8.4 \times 10^3], \quad V_{IT} \leq 8.4 \times 10^3 \text{ ml}
\]  [4-31]

**Overhydration segment:**

\[
P_{IT} = 1.88 + 1.05 \times 10^{-4} \cdot [V_{IT} - 12.6 \times 10^3], \quad V_{IT} \geq 12.6 \times 10^3 \text{ ml}
\]  [4-32]

**Intermediate segment:**

\(8.4 \times 10^3 \leq V_{IT} \leq 12.6 \times 10^3 \text{ ml}\)

For interstitial volumes in the range of \(8.4 \times 10^3 \leq V_{IT} \leq 12.6 \times 10^3 \text{ ml}\), the interstitial pressure is obtained by interpolating the discrete \(P_{IT}\) and \(V_{IT}\) data generated from Eqs. [4-29] and [4-30] above (see Section 5.3).

In all of the above relationships, \(P_{IT}\) is expressed in mmHg and \(V_{IT}\) is expressed in ml.
Figure 4-2. The 'most-likely' human compliance curve (solid line) with its three distinct segments for dehydration, moderate hydration and overhydration. The symbol (filled circle) indicates the reference pair of $P_{IT}-V_{IT}$ data that correspond to the normal values of $V_{IT}$ and $P_{IT}$ (i.e., 8.4 l and -0.7 mmHg, respectively). At a minimal dehydration state (i.e., the value at which $V_{IT}$ becomes equal to the excluded volume, $V_{IT,EX}$) the lymph flow equals 0.0. The starting value for overhydration is $V_{IT} = 12.6$ l. Data correspond to normal humans as reported by Chapple [1993].
4.5.4.3 Compartmental concentrations and colloid osmotic pressure relationships

The concentrations of various solutes within a given compartment are expressed by simple algebraic equations that relate the amount of the solute and the compartmental volume. Thus, for example, the concentration of plasma proteins, \( c_{P,PL} \), expressed in g/l, is given by

\[
\frac{Q_{PL}}{V_{PL}}
\]  

[4-33]

Similar simple equations can be written for the plasma concentrations of dextran, \( c_{R,PL} \), and small ions, \([ION]_{PL}\), expressed in g/l and mmol/l, respectively.

Correspondingly, for the interstitial fluid compartment, the interstitial protein concentration, \( c_{P,IT} \) (g/l), is given as

\[
\frac{Q_{IT}}{V_{IT}}
\]  

[4-34]

and similar relationships describe the dextran, \( c_{R,IT} \), and small ion, \([ION]_{IT}\), concentrations in the interstitium, expressed in g/l and mmol/l, respectively.

As described in Chapter 2, it is the effective interstitial concentration of proteins (and any other high molecular weight solute) that governs exchange across the capillary. For the two types of macromolecular species, i.e., proteins and dextran, these effective concentrations are given by the distribution of their respective amounts in the available interstitial volume, \( V_{IT,AV} \), as

\[
\frac{Q_{IT}}{V_{IT,AV}}
\]  

[4-35]

and

\[
\frac{R_{IT}}{V_{IT,AV}}
\]  

[4-36]

respectively, where \( V_{IT,AV} = V_{IT} - V_{IT,EX} \).

The subscripts AV and EX indicate the available and excluded interstitial volumes, respectively. According to Bell et al. [1980] and Bert and Pinder [1982], the excluded interstitial volume accounts for about 25% of the total interstitial volume. The effective concentrations described by Eqs. [4-35] and [4-36] must be taken into account when determining the interstitial colloid osmotic pressure.
Several empirical relationships between the plasma concentration of albumin (or total protein) and the colloid osmotic pressure exerted by these macromolecules have been proposed. Landis and Pappenheimer [1963] established separate relationships for the colloid osmotic pressures exerted by either albumin, or alternatively, by the total plasma proteins. By fitting available experimental data, Chappie [1993] established a simple relationship between the plasma albumin concentration and the colloid osmotic pressure exerted by all plasma proteins.

Similar to Chappie's approach the following simple relationship was found in this work to correlate the total plasma protein concentration and the total colloid osmotic pressure measured in plasma:

\[ c_{P,PL} = 2.7 \cdot \pi_{P,PL} \]  
\[ [4-37] \]
where the units of colloid osmotic pressure are mmHg and those of protein concentration are g/l. For a normal plasma protein concentration of about 70 g/l, Eq. [4-37] predicts a total plasma colloid osmotic pressure of 25.9 mmHg, which compares well with the value of 25.6 mmHg predicted by the corresponding Landis and Pappenheimer [1963] equation. Hence Eq. [4-37] is adopted here.

Due to lack of sufficient information, the same correlation will be used to relate the total interstitial colloid osmotic pressure and the available interstitial protein concentration, i.e.,

\[ c_{P,IT,AV} = 2.7 \cdot \pi_{P,IT} \]  
\[ [4-38] \]

The colloid osmotic pressures exerted in both extracellular compartments by the infused dextran are given by the following empirical relationships proposed by Webb [1992]:

\[ \pi_{R,PL} = 0.2688 \cdot c_{R,PL} + 1.101 \times 10^{-2} c_{R,PL}^2 \]  
\[ [4-39] \]
and

\[ \pi_{R,IT} = 0.2688 \cdot c_{R,IT,AV} + 1.101 \times 10^{-2} c_{R,IT,AV}^2 \]  
\[ [4-40] \]
where \( \pi_{R,PL} \) and \( \pi_{R,IT} \) are expressed in mmHg, while the dextran concentration in plasma, \( c_{R,PL} \), and the available interstitium, \( c_{R,IT,AV} \), are expressed in g/l. From Eq. [4-39] it can be calculated that, in agreement with other literature reports, a solution of 38 g/l of Dextran 70 is iso-oncotic with plasma [Tøllofsrud, 1997] (i.e., generates the same colloid osmotic pressure as the normal concentration of plasma proteins).
When the dextran species is introduced into plasma by infusion, the total plasma colloid osmotic pressure, $\Pi_{PL}$, is given by the sum of the individual colloid osmotic pressures exerted by the two plasma macromolecules (proteins and dextran), i.e.,

$$\Pi_{PL} = \pi_{P,PL} + \pi_{R,PL} \quad [4-41]$$

The osmotic pressures exerted by the small ions in both plasma and interstitium are assumed to respect the van't Hoff law of ideal solutions such that,

$$\pi_{ION,PL} = RT \cdot [ION]_{PL} \quad [4-42]$$

and

$$\pi_{ION,IT} = RT \cdot [ION]_{IT} \quad [4-43]$$

where the $R$ is the universal gas constant and $T$ the absolute temperature. Equations [4-43] and [4-42] therefore imply that interactions between the solutes in the two fluid compartments can be ignored and, furthermore, that each mole of a given small ion exerts an osmotic pressure of 19.3 mmHg.

4.5.4.4 Mathematical representation of the Donnan distribution

As described in Chapter 3, because the capillary barrier is only semipermeable to proteins, the plasma proteins are preferentially retained in the capillary lumen. Therefore, at any time, there will exist a protein concentration difference, $\Delta(Prot)$, between the plasma and the interstitium. The negatively-charged proteins on each side of the membrane will associate with an electrically equivalent number of positively-charged species, namely $Na^+$, $K^+$ and $C^{2+}$. An excess of these cations thus becomes effectively immobilized on one side of the membrane, thereby establishing a concentration difference of all the small ions across the capillary wall. There is no mathematical description of the Donnan partition for cases that involve the presence of more than sodium, potassium and chloride ions. A procedure that accounts for the Donnan effect in the presence of all five extracellular ionic species considered in this model is outlined below.

If $\Delta(Cat)$ represents the sum of all the differences in cation concentrations across the capillary membrane, then, at steady state,

$$\Delta(Cat) = \Delta(Na)_D + \Delta(K)_D + \Delta(C)_D \quad [4-44]$$

where
Chapter 4: Model Formulation

where

\[ \Delta(\text{Na})_D = (\text{Na})_{PL} - (\text{Na})_{IT} \] \[ \Delta(\text{K})_D = (\text{K})_{PL} - (\text{K})_{IT} \] \[ \Delta(\text{C})_D = (\text{C})_{PL} - (\text{C})_{IT} \] \[ \text{[4-45]} \]

In Eqs. [4-44] – [4-47], \( \Delta(\text{Na})_D, \Delta(\text{K})_D \) and \( \Delta(\text{C})_D \) are the Donnan transcapillary differences in the concentrations of sodium, potassium and other cations, respectively, where all the concentrations are expressed in mEq/l.

Since it is the presence of total plasma proteins that creates a \( \Delta(\text{Cat}) \) distribution across the capillary, it is assumed here that a proportionality constant, \( k_{\text{Prot}} \), relates the total cations concentration difference with the total protein concentration difference, \( \Delta(\text{Prot}) \), as follows:

\[ \Delta(\text{Cat}) = k_{\text{Prot}} \Delta(\text{Prot}) \] \[ \text{[4-48]} \]

where \( \Delta(\text{Prot}) \) also expressed in mEq/l, is

\[ \Delta(\text{Prot}) = \frac{c_{P,PL} - c_{P,IT,AV}}{z_p \cdot MW_p} = (c_{P,PL}) - (c_{P,IT,AV}) \]

where \( z_p \) and \( MW_p \) are the charge and molecular weight, respectively, of the proteins, while \( (c_{P,PL}) \) and \( (c_{P,IT,AV}) \) represent the total protein concentrations in plasma and interstitium, respectively, expressed in mEq/l. Note that, the charge \( z_p \) for the extracellular proteins was assumed to be the same as that of albumin, i.e., \( z_p = -17 \).

Based on the relationships [4-44] and [4-48], Eqs. [4-45], [4-46] and [4-47] can be rewritten for the non-steady-state as follows:

**Donnan distribution for Na\(^+\):**

\[ \Delta(\text{Na})_D = k_{\text{Prot}} \left[ \frac{(c_{P,PL})}{(Na)_{PL} + (K)_{PL} + (C)_{PL}} - \frac{(Na)_{IT}}{(Na)_{IT} + (K)_{IT} + (C)_{IT}} \right] \] \[ \text{[4-49]} \]

**Donnan distribution for K\(^+\):**

\[ \Delta(\text{K})_D = k_{\text{Prot}} \left[ \frac{(K)_{PL}}{(Na)_{PL} + (K)_{PL} + (C)_{PL}} - \frac{(K)_{IT}}{(Na)_{IT} + (K)_{IT} + (C)_{IT}} \right] \] \[ \text{[4-50]} \]

**Donnan distribution for C\(^{2+}\):**

\[ \Delta(\text{C})_D = k_{\text{Prot}} \left[ \frac{(C)_{PL}}{(Na)_{PL} + (K)_{PL} + (C)_{PL}} - \frac{(C)_{IT}}{(Na)_{IT} + (K)_{IT} + (C)_{IT}} \right] \] \[ \text{[4-51]} \]
Equations [4-49] – [4-51] assume that the ability of the proteins to associate with a given type of cation is proportional to the mass fraction of that cation in the plasma or interstitium.

At steady state, the condition of electroneutrality requires that the differences in positive charges across the capillary membrane be equal to the differences in negative charges, i.e.:

\[ \Delta(Cat) = \Delta(Prot) + \Delta(Cl)_D + \Delta(A)_D \]  

where \( \Delta(Cl)_D \) and \( \Delta(A)_D \) are, respectively, the transcapillary concentration differences for the chloride ion and the anionic species \( A^- \), expressed as mEq/l. By substituting Eq. [4-48] into Eq. [4-52], the following relationship, which relates the transcapillary anionic concentration differences to the protein concentration difference, is obtained:

\[ \Delta(Prot) (k_{Prot} - 1) = \Delta(Cl)_D + \Delta(A)_D \]

Consequently, the Donnan distributions for the negative charges can be written as:

**Donnan distribution for Cl⁻**:

\[ \Delta(Cl)_D = (k_{Prot} - 1) \left[ (c_{P,PL}) \frac{(Cl)_{PL}}{(Cl)_{PL} + (A)_{PL}} - (c_{P,IT,AV}) \frac{(Cl)_{IT}}{(Cl)_{IT} + (A)_{IT}} \right] \]

**Donnan distribution for A⁻**:

\[ \Delta(A)_D = (k_{Prot} - 1) \left[ (c_{P,PL}) \frac{(A)_{PL}}{(Cl)_{PL} + (A)_{PL}} - (c_{P,IT,AV}) \frac{(A)_{IT}}{(Cl)_{IT} + (A)_{IT}} \right] \]

Equations [4-49] – [4-51], [4-54] and [4-55] express the contribution of the Donnan effect to the distribution of small ions across the capillary membrane; these were taken into account in describing the transcapillary fluxes of fluid and small ions in the model (see Eqs. [4-15] and [4-18]).

### 4.5.5 Fluid and solute inputs and outputs

Figure 4-1 shows a number of fluid and solute rates that are either introduced into the body by infusion or lost from the body through renal output and sensible/insensible losses. The plasma compartment is the recipient of infusate and the source of renal elimination. The sensible and insensible losses, i.e., PER and ISL, respectively, occur from the interstitial compartment.
Inputs

The infusion rates are normally obtained from experimental or clinical data. Depending on the specific needs of the patient (in case of a trauma) or the experimental protocol employed (in the case of testing an infusate), the composition, volume and rate of infusion are varied during the resuscitation procedure. These inputs are accounted for in the model by simple time-dependent source terms in the appropriate mass balance equations. Thus the rate of fluid input by infusion can be written as

\[ J_{\text{RES}} = f(t) \]  \hspace{1cm} [4-56]

This fluid may contain species that already exist in the body such as albumin and small ions, that are infused at the rates of \( Q_{\text{RES}} \) and \( M_{\text{RES}} \), respectively. In some instances, resuscitation with alternative colloidal compounds, which are foreign to but well tolerated by the body, is desired. The rate of infusion of such materials is symbolized as \( R_{\text{RES}} \). The infusate \( R \) can be any macromolecular species such as Dextran 40, Dextran 70 and HES (hydroxyethyl starch), etc., but because of its importance as a colloidal resuscitant, a brief background about the characteristics and use of Dextran 70 is given in Section 4.5.6.

Outputs

Under normal conditions, fluid is lost from the body as urine at a rate \( J_{\text{UR}} \), through perspiration (sensible loss) at a rate \( J_{\text{PER}} \), and through insensible losses at a rate \( J_{\text{ISL}} \).

The solutes eliminated in urine include all five species of small ions found in plasma, but not proteins. Although Dextran 70 is eliminated in urine after breakdown in the body, these losses occur only after 3 to 4 h post-infusion [Haljamäe, 1993; Arffors and Buckley, 1989]. Such times are beyond those normally simulated by the model. For initial model validation, the urinary rate, \( J_{\text{UR}} \), as well as the rate of small ion output, \( M_{\text{ION,UR}} \), are obtained from experimental data. A new kidney model that can predict both fluid and solute renal outputs will be presented later in Chapter 8.

Data for fluid and ion rates lost through perspiration were obtained from the literature. The only ions involved are \( \text{Na}^+ \), \( \text{K}^+ \) and \( \text{Cl}^- \). For a normal, 70 kg human, the perspiration rate is usually very small, i.e., about 2 ml/h of fluid and contains sodium, potassium and chloride at concentrations of 45, 4 and 49 mEq/l, respectively [e.g., Krieger and Sherrard, 1991; Kleinman
Chapter 4: Model Formulation

and Lorenz, 1984]. The 'insensible losses' represent the combined losses of solute free water mainly through respiration but also, to some extent, through skin. In humans, \( J_{\text{isl}} \) occurs at a normal rate of about 40 ml/h.

4.5.6 Characterization of Dextrans, Dextran 70

Dextrans are plasma substitutes used for intravenous infusion. By virtue of their relatively high molecular weight, they have good plasma retention. When infused they exert a colloid osmotic pressure across the capillary membrane and thus they induce a transcapillary shift of water that results in plasma expansion, hemodilution and improved microcirculatory flow. They are used therefore for hypovolemic conditions (e.g., burn, hemorrhage, trauma). For reviews on the use of these colloidal resuscitants, see Arrfors and Buckley [1989], Webb [1992] and Haljamae [1993]. The two types of dextrans most commonly used in clinical practice are Dextran 70 (commercial name Macrodex) and Dextran 40 (commercial name Rheomacrodex). These natural colloids are composed of fractions with a wide variety of molecular weights. The number in their nomenclature represents the average molecular weight. Thus, Dextran 70 is a compound whose average molecular weight of about 70 kDa is very similar to that of albumin, 67 kDa.

The typical concentration of Dextran 70 in solutions used for infusion is 60 g/l [Tølløfsrud, 1997]. The colloid osmotic pressure exerted by 60 mg/ml of Dextran 70 is about 65 mmHg, i.e., slightly more than two times the normal value exerted by the plasma proteins. With time, Dextran 70 is completely degraded to smaller fractions that are eliminated from the body. Between 4 and 6 h post-infusion, about 60% of the dextran infused is still above the normal renal threshold for elimination [Arrfors and Buckley, 1989].

4.6 Steady state conditions

The normal steady-state conditions are those corresponding to a 'Reference man' [Reference Man ICRP 1975] defined as a healthy male in supine position who that has a weight of 70 kg and a height of 170 cm. These values are summarized in Table 4-1 and briefly described below.

The plasma and interstitial volumes as well as the hematocrit value are specified in the available literature [Wiederhielm, 1974; Noddeland et al., 1984; Fauchald et al., 1985(a); Fauchald et al., 1985(b)].
<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, BW</td>
<td>70 kg</td>
<td></td>
</tr>
<tr>
<td>Plasma volume, $V_{PL}$</td>
<td>3200 ml</td>
<td>[Fauchauld et al., 1985(a,b)], [Wiederhielm, 1979]</td>
</tr>
<tr>
<td>Red blood cell volume, $V_{RBC}$</td>
<td>2133 ml</td>
<td>calculated from Hct data</td>
</tr>
<tr>
<td>Hematocrit, Hct</td>
<td>40.0 %</td>
<td>[Kleinman and Lorenz, 1984]</td>
</tr>
<tr>
<td>Interstitial volume, $V_{IT}$</td>
<td>8400 ml</td>
<td>[Fauchauld et al., 1985(a,b)], [Wiederhielm, 1979]</td>
</tr>
<tr>
<td>Excluded interstitial volume, $V_{IT,EX}$</td>
<td>2100 ml</td>
<td>assumed based on [Reed et al., 1989]</td>
</tr>
<tr>
<td>Tissue cell volume, $V_{TC}$</td>
<td>28000 ml</td>
<td>calculated as 40% BW</td>
</tr>
<tr>
<td>Plasma hydrostatic pressure, $P_c$</td>
<td>11 mmHg</td>
<td>[Chapple et al., 1993]</td>
</tr>
<tr>
<td>Plasma colloid osmotic pressure, $\pi_{P,PL}$</td>
<td>25.9 mmHg</td>
<td>[Fadnes et al., 1986], [Xie et al., 1995]</td>
</tr>
<tr>
<td>Plasma protein concentration, $c_{P,PL}$</td>
<td>70 g/l</td>
<td>[Carlson et al., 1996], [Kleinman and Lorenz, 1984]</td>
</tr>
<tr>
<td>Interstitial hydrostatic pressure, $P_{IT}$</td>
<td>-0.7 mmHg</td>
<td>[Wiig and Reed, 1987], [Xie et al., 1995]</td>
</tr>
<tr>
<td>Interstitial colloid osmotic pressure, $\pi_{P,IT}$</td>
<td>14.7 mmHg</td>
<td>[Fadnes et al., 1986]</td>
</tr>
<tr>
<td>Interstitial protein concentration, $c_{P,IT}$</td>
<td>29.8 g/l</td>
<td>[Fauchauld et al., 1985(a,b)]</td>
</tr>
<tr>
<td>Protein concentration in the available volume, $c_{P,IT,AV}$</td>
<td>39.7 g/l</td>
<td>calculated as per Section 4.5.4.3</td>
</tr>
<tr>
<td>Protein reflection coefficient, $\sigma$</td>
<td>0.988</td>
<td>[Xie et al., 1995]</td>
</tr>
<tr>
<td>Permeability-surface area product for protein, $PS$</td>
<td>73.0 ml/h</td>
<td>[Xie et al., 1995]</td>
</tr>
<tr>
<td>Dextran 70 reflection coefficient, $\sigma_{DEX}$</td>
<td>0.5</td>
<td>[Haraldson et al., 1982], [Curry, 1984]</td>
</tr>
<tr>
<td>Permeability-surface area product for Dextran 70, $PS_{DEX}$</td>
<td>18.25 ml/h</td>
<td>[Garlick et al., 1970], [Altman and Dittmer, 1971]</td>
</tr>
<tr>
<td>Reflection coefficient for small ions, $\sigma_{ION}$</td>
<td>0.05 – 0.5</td>
<td>[Curry, 1979], [Wolf and Watson, 1989]</td>
</tr>
<tr>
<td>Permeability-surface area product for small ions, $PS_{ION}$</td>
<td>73,000 – 365,000 ml/h</td>
<td>assumed based on [Yudilevich et al., 1968]</td>
</tr>
<tr>
<td>Fluid filtration coefficient, $k_F$</td>
<td>121.1 ml/mmHg-h</td>
<td>[Xie et al., 1995]</td>
</tr>
<tr>
<td>Lymph flow sensitivity, $LS$</td>
<td>43.1 ml/mmHg-h</td>
<td>[Xie et al., 1995]</td>
</tr>
<tr>
<td>Lymph flow rate, $J_L$</td>
<td>75.7 ml/h</td>
<td>[Xie et al., 1995]</td>
</tr>
</tbody>
</table>

Table 4-1. Initial steady-state compartmental values for the 70 kg 'Reference man' (see text for a more detailed discussion).
As in previous modeling studies [Bert and Pinder, 1982; Bert et al., 1988; Reed et al., 1989], it was assumed that proteins are excluded from 25% of the total interstitial fluid volume. The volume of the red blood cell compartment was calculated based on the hematocrit. For the 'lumped' tissue cell compartment, it was considered that all these cells bear the properties and characteristics of skeletal muscle cells and constitute about 40% of the total body weight or approximately 28 l [Landis and Pappenheimer, 1963; Arfors and Buckley, 1989].

The normal compartmental hydrostatic and colloid osmotic pressures for plasma and interstitium employed by Xie et al. [1995] and Chappie et al. [1993] in formulating the human microvascular exchange model, are also given in Table 4-1. All these compartmental pressure values are obtained from the available literature and correspond to a combined tissue compartment and the general circulation. The same table shows a typical value of the plasma protein concentration available from literature sources [Kleinman and Lorenz, 1984] as well as the calculated interstitial protein concentrations (see Eqs. (4-33) - (4-35)).

The transport properties of the capillary wall, that separates the plasma and interstitial compartments, include the capillary filtration coefficient, \( k_F \), the capillary permeability-surface area products for proteins, Dextran 70 and small ions, PS, PS$_{DEX}$ and PS$_{ION}$, respectively, and the reflection coefficients for proteins, Dextran 70 and small ions, \( \sigma \), PS$_{DEX}$ and PS$_{ION}$, respectively. The fluid and protein transport coefficients together with the normal lymph flow, \( J_L \), and the lymph flow sensitivity, LS, were estimated by Xie et al. [1995] by statistical comparison of model predictions with clinical data available for humans. The literature value for PS$_{DEX}$ is about 0.20 to 0.25 times the PS for albumin [Garlick et al., 1970; Altman and Dittmer, 1971]. A value of 0.25 · PS was assumed for the present study. According to Haraldson et al. [1982] and Curry [1984], PS$_{DEX}$ ranges from 0.30 to 0.55 depending on the Dextran 70 concentration. For the concentration range commonly used in clinical practice (i.e., 6% Dextran 70 solution), PS$_{DEX}$ is at the higher end of this range. Thus, we assumed a value of PS$_{DEX}$ = 0.5.

There is little reliable information about the two transport parameters associated with the transport of small ions across the capillary. Based on the studies reported by Yudilevich [1963], it can be estimated that the value for PS$_{ION}$ is between 1000 and 5000 times higher than the corresponding permeability-surface area product for proteins, PS. Most physiological textbooks state that the small ions have a very low transcapillary reflection coefficient, i.e., less than 0.1. However, Wolf and Watson [1989] measured values of PS$_{ION}$ ranging from 0.1 to 0.5 for cat hindlimb, while Curry [1979] obtained an average reflection coefficient for small ions of 0.05 for
frog mesentery. In their hemorrhage model, Carlson et al. [1996] assumed \( \sigma_{\text{ION}} \) values of 0.045 for Na\(^+\) and Cl\(^-\) and a value of 0.1 for all other small solutes. Thus, it is anticipated that \( \sigma_{\text{ION}} \) should lie in the range of 0.05 – 0.5 as listed in Table 4-1. Since both \( \text{PS}_{\text{ION}} \) and \( \sigma_{\text{ION}} \) are imprecisely known, one of the objectives of the Chapter 6 will be to describe their estimation using available experimental data.

Table 4-2 shows how the small ions are normally partitioned between the intra- and extracellular compartments. The values selected for these ion concentrations as well as the properties ascribed to these cells are approximate, representative of a fairly large number of cells, but do not exactly characterize any of them. They are the result of combining experimental information with additional calculations. Table 4-2 was assembled based on the following considerations.

**Extracellular concentrations:**
The extracellular concentrations for Na\(^+\), K\(^+\) and Cl\(^-\) ions in plasma are obtained from experimental measurements in rats [Onarheim, 1995]. This reference was chosen in order to maintain the same source for initial conditions and for comparison with model predictions as will be discussed in Chapter 6. Similar concentrations have been reported for rabbits [Kunze, 1977], dogs [Vick et al., 1970] and humans [Kleinman and Lorenz, 1984].

The extracellular values for C\(^{2+}\) and A\(^-\) in plasma were calculated by solving simultaneously the following two algebraic equations:

\[
\sum_{\text{ION,PL}} (z_{\text{ION,PL}} \cdot [\text{ION}]_{\text{PL}}) + \frac{Z_p \cdot c_{p,\text{PL}}}{\text{MW}_p} = 0 \quad [4-57]
\]

\[
\Phi \cdot \sum_{\text{ION,PL}} ([\text{ION}]_{\text{PL}}) + \frac{c_{p,\text{PL}}}{\text{MW}_p} = S_{\text{PL}} \quad [4-58]
\]

where \([\text{ION}]_{\text{PL}}\) and \(z_{\text{ION,PL}}\) are the molar concentration and charge, respectively, of the ionic species ION (i.e., ION = Na\(^+\), K\(^+\), C\(^{2+}\), Cl\(^-\), A\(^-\)) present in plasma; while \(c_{p,\text{PL}}, z_p\) and MW\(_p\) are the molar concentration, charge and molecular weight of the proteins (i.e., albumin) in plasma. \(\Phi\) and \(S_{\text{PL}}\) are the osmotic coefficient and plasma osmolarity, respectively. Eq. [4-57] represents the condition of electroneutrality for the plasma compartment while Eq. [4-58] expresses the relationship for total plasma osmolarity. The unknowns in the above equations are the molar concentrations for the A\(^-\) and C\(^{2+}\) species.
<table>
<thead>
<tr>
<th>Charged Species</th>
<th>Red Blood Cells (RBC)</th>
<th>Plasma (PL)</th>
<th>Interstitium (IT)</th>
<th>Tissue Cells (TC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[z] [mEq/l] [mM] Ref.</td>
<td>[z] [mEq/l] [mM] Ref.</td>
<td>[z] [mEq/l] [mM] Ref.</td>
<td>[z] [mEq/l] [mM] Ref.</td>
</tr>
<tr>
<td>Positive Charges</td>
<td>Na</td>
<td>+1</td>
<td>11.0</td>
<td>140.0</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>+1</td>
<td>140.0</td>
<td>140.0</td>
</tr>
<tr>
<td></td>
<td>C or FC</td>
<td>+2</td>
<td>27.0</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>178</td>
<td>164.5</td>
<td>165.1</td>
</tr>
<tr>
<td>Negative Charges</td>
<td>Cl</td>
<td>-1</td>
<td>73.2</td>
<td>105.7</td>
</tr>
<tr>
<td></td>
<td>A or FA</td>
<td>-1.6</td>
<td>104.8</td>
<td>65.5</td>
</tr>
<tr>
<td></td>
<td>Proteins</td>
<td>---</td>
<td>---</td>
<td>-17</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>178</td>
<td>138.7</td>
<td>165.1</td>
</tr>
<tr>
<td>TOTAL CHARGES</td>
<td>356.0</td>
<td>303.2</td>
<td>330.2</td>
<td>303.2</td>
</tr>
</tbody>
</table>

Table 4-2. Steady-state values for small ions and proteins showing their partition between intra- and extracellular compartments. The values are obtained from: R - literature references; R1-[Hoffman, 1987], R2-[Kleinman and Lorenz, 1984], R3-[Onarheim, 1995], and C - calculations.
The value for the plasma osmolarity was obtained from experimental measurements for rats [Onarheim, 1995]. Following the modeling practice of previous authors [Wolf, 1982], both the intra- and extracellular media were assumed to be ideal solutions for which the van’t Hoff law applies; therefore, an osmotic coefficient, $\Phi = 1$ was assumed for all solutes, i.e., ions and proteins, in plasma. The molar and normal protein concentrations given in Table 4-2 were calculated based on the known mass concentration of protein in plasma and by considering for this species an average charge of $z_p = -17$ and a molecular weight of $M_{w,p} = 67000$ g/mol [Kleinman and Lorenz, 1984]. As previously mentioned, a charge of +2 was assumed for all positive ions other than Na$^+$ and K$^+$ and a charge of -1 was attributed to the A$^-$ species.

The concentrations of small ions in the interstitium were calculated from Donnan considerations across the capillary membrane based on the relationships presented in Section 4.5.4.4. All of the extracellular ion and protein concentrations presented in Table 4-2 are in good agreement with corresponding values reported in the literature, [Kleinman and Lorenz, 1984].

*Intracellular concentrations:*

Values for the intracellular concentrations of Na$^+$, K$^+$ and the intracellular non-diffusible positive species FC$^{2+}$, were available in the literature [Hoffman, 1987; Kleinman and Lorenz, 1984] for tissue and red blood cells. The intracellular chloride concentration for red blood cells was calculated using the ratio $[Cl^-]_{RBC}/[Cl^-]_{PL} = 0.69$ previously reported by Hoffman [1987].

Numerous negative species, mainly proteins, constitute the internal environment of the cells. The generic term FA$^-$ was used to describe these non-diffusible species. Its concentration and average charge were calculated by simultaneously solving two algebraic equations. The first equation is based on the bulk internal electroneutrality condition:

$$\sum_{ION,RBC} (Z_{ION,RBC} \cdot [ION]_{RBC}) + Z_{FA,RBC} \cdot [FA]_{RBC} = 0$$ \hspace{1cm} [4-58]$$

where $Z_{ION,RBC}$ and $[ION]_{RBC}$ have been defined previously, while $Z_{FA,RBC}$ and $[FA]_{RBC}$ are the unknown average charge and molar concentration of the non-diffusible species FA$. The second equation imposes the isotonicity condition between the cells and their surroundings, i.e.,

$$S_{PL} = S_{RBC}$$ \hspace{1cm} [4-59]$$

where $S_{RBC}$ represents the total osmolarity inside the red blood cells, i.e.,
\[ S_{RBC} = \sum_{ION,RBC} ([ION]_{RBC}) + [FA]_{RBC} \]  

A similar approach as for red blood cells was adopted to calculate the intracellular concentration and charge of FA\(^+\) for the tissue cells. The only exception made was with respect to the intracellular chloride ion, whose internal concentration was obtained from literature reports on skeletal muscle cells [Kleinman and Lorenz, 1984].

The above steps complete a simplified picture of the small ion partition between the intra- and extracellular media as well as between the plasma and interstitium. They provide an initial set of numerical values that are in a reasonable physiological range when compared to experimental data [Kleinman and Lorenz, 1984].

For each type of cellular compartment, four other cellular membrane parameters are required in order to completely describe the cellular exchange process. These parameters are the three membrane permeabilities, one for each of the ions transported across the cell membrane, i.e., \( p_{Na} \), \( p_{K} \) and \( p_{Cl} \), and the rate, \( R_P \), of the Na\(^+-\)K\(^+-\)-pump.

Based on known intra- and extracellular concentrations for a particular type of cell, i.e., RBC or tissue cell, the system of membrane transport equations given by Eqs. [4-10] and [4-11] coupled with the non-linear equation giving the membrane potential (Eq. [4-14]) were solved for steady-state conditions (i.e., the left-hand-side accumulation terms of Eqs. [4-10] and [4-11] were set to zero). Initial literature value for \( p_{Cl} \) and one of the other permeabilities, \( p_{Na} \) or \( p_{K} \), together with the intracellular and extracellular concentrations presented in Tables 4-2, were assumed known. The unknowns were then the other permeability \( p_{Na} \) or \( p_{K} \), \( R_P \) and \( \Delta \phi \) (where \( \Delta \phi = F V_m/RT \)). The membrane parameters obtained by this procedure are presented in Table 4-3.

All the compartmental values and parameters presented above represent the steady-state values of the system. This requirement was verified by running the computer program associated with the transient model for an extended period of time and in the absence of any perturbations to the system in order to evaluate whether there were significant changes in any of the dependent variables. Additionally, when perturbations to the system are considered, if all the inputs exactly equal the outputs from the system, the model predicts a return of all the compartmental variables to the normal steady-state.

As part of the previous work, most the transport parameters descriptive of mass exchange in the MVES (with the exception of those related with the transport of small ions and of the infused macromolecular species) were subjected to a detailed sensitivity analysis. In this work,
although results are not presented, similar sensitivity analysis studies were done for the transcapillary parameters related to the Dextran 70 transport (i.e., $P_{S_{DEX}}$ and $\sigma_{DEX}$). Additionally, the variation of the parameters describing the transport across the cell membrane (i.e., the cell membrane permeabilities and $RP$) for a wide range outside the values presented in Table 4-3, were also investigated. From this modeling exercise it was concluded that, the model predictions are relatively insensitive to changes in the above-mentioned transport parameters. Thus, all the values presented in Tables 4-1 to 4.3, are reasonable choices for the parameters described.

<table>
<thead>
<tr>
<th>Membrane Parameter</th>
<th>Red Blood Cells</th>
<th>Muscle Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{Na}$ (cm/sec)</td>
<td>$4 \times 10^{-10}$</td>
<td>$5.95 \times 10^{-7}$</td>
</tr>
<tr>
<td>$P_{K}$ (cm/sec)</td>
<td>$10^{-10}$</td>
<td>$5 \times 10^{-5}$</td>
</tr>
<tr>
<td>$P_{CL}$ (cm/sec)</td>
<td>$3.82 \times 10^{-10}$</td>
<td>$5 \times 10^{-5}$</td>
</tr>
<tr>
<td>$RP$</td>
<td>15.03 (C)</td>
<td>0.548 (C)</td>
</tr>
<tr>
<td>$RP$ $P_{Na}$</td>
<td>14.35 (C)</td>
<td>46.05 (C)</td>
</tr>
<tr>
<td>$V_{m}$ (mV)</td>
<td>-9 (C)</td>
<td>-84 [Nakayama et al., 1984]</td>
</tr>
<tr>
<td></td>
<td>-9.78 (C)</td>
<td>-90 [Shires et al., 1972]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-87.8 (C)</td>
</tr>
</tbody>
</table>

Table 4-3: Calculated (C) and literature values for steady-state cellular membrane parameters. Where available comparative literature values are provided in addition to the calculated values.

4.7 Normal conditions for subjects other than the 'Reference man'

a) Humans

In studies that involve humans having a body weight different than that of the generic 'Reference man', all the extensive properties considered in the model are scaled by a weight ratio, $WR$ [Ampratwum, 1995], given as

$$WR = \frac{\text{Weight of Human}}{\text{Weight of 'Reference man'}}$$  \[4-61\]
The variables that are subjected to scaling based on weight include the compartmental fluid volumes, amounts of proteins and ions, interstitial compliances as well as all the global transport parameters with the exception of the capillary reflection coefficient.

b) Animals

The scaling approach employed when the model is used for animal studies can be summarized as follows:

1. The animal plasma volume together with the intensive physiological properties of the vascular and interstitial compartments are considered to be the same as those of the normal 'Reference man' (e.g., $V_{\text{PL, HUMAN}} = V_{\text{PL, SCALED ANIMAL}}$; $\pi_{\text{P,PL,HUMAN}} = \pi_{\text{P,PL, SCALED ANIMAL}}$; $[\text{ION}]_{\text{PL, HUMAN}} = [\text{ION}]_{\text{PL, SCALED ANIMAL}}$, etc.).

2. All the extensive physiological properties of plasma, interstitium and tissue cells are scaled by the ratio ($V_{\text{PL,HUMAN}} / V_{\text{PL,ANIMAL}}$). Thus, for example, the value of the interstitial volume of the scaled animal is calculated as,

$$V_{\text{IT, SCALED ANIMAL}} = \frac{V_{\text{PL,HUMAN}}}{V_{\text{PL,ANIMAL}}} \cdot V_{\text{IT,ANIMAL}} \quad [4-62]$$

Note that the subscript 'ANIMAL' refers to the actual compartmental value of a real animal obtained usually from experimental information. The subscript 'SCALED ANIMAL' refers to the animal variable that is scaled to the appropriate human value.

3. The interstitial compliance and all the transport parameters, other than $\sigma$ values, are scaled based on the interstitial volume ratio of scaling, $R_{\text{Scaling}}$, given by,

$$R_{\text{Scaling}} = \frac{V_{\text{IT, SCALED ANIMAL}}}{V_{\text{IT,HUMAN}}} \quad [4-63]$$

Thus, for instance, the new values for the transport parameters can be obtained from an equation having the following form:

$$\text{Transport Parameter}_{\text{SCALED ANIMAL}} = R_{\text{Scaling}} \cdot \text{Transport Parameter}_{\text{HUMAN}} \quad [4-64]$$

An explicit example of the scaling procedure is provided in the Appendix C, where it is applied to determine compartmental values representative of dogs.
Chapter 5: NUMERICAL METHODS AND COMPUTATIONAL PROCEDURES

5.1 General Aspects

From a mathematical point of view, the overall model describing the four homogenous compartments (see Section 4.5), consists of twenty-two ordinary time-dependent differential equations (accounting for balances of fluid volumes, ions, proteins and macromolecules) coupled with two implicit non-linear algebraic equations (corresponding to the cellular transmembrane potentials) and two explicit algebraic equations (describing the changes in cellular volume). These equations are linked via several auxiliary algebraic equations such as compliance relationships and equations for osmotic pressures.

To solve the overall mathematical model, a computer program was developed using the Fortran-77 language as applied by the Microsoft Fortran PowerStation® compiler and programming environment [Microsoft, 1993]. The output of the Fortran program was connected to the Microsoft Excel® spreadsheet software for convenient handling and processing of the large data files. The calculations were performed in double precision.

5.2 Conceptual Design of the Program

The main objective in designing the program was to achieve a high degree of adaptability and robustness for the various simulation scenarios of interest, such as different fluid infusions, inclusion of kidney function and hemorrhage. Adaptability was assured by incorporating into the main program, easy to modify, ‘specific task oriented’ subroutines for input and output data files, calculation of the normal physiological values, managing the numerical solution of the different types of model equations, calculation of the normal physiological values, etc. The robustness of the program is provided by the numerical techniques employed. The complete computer code is listed in Appendix H.

The subroutines in order of appearance in the program, and their functions are:

CONS Determines the various constants used in the equations of the model, such as scaling ratios, parameters required for calculating the cellular area available for transport, etc. This subroutine also determines the
compartmental fluid volumes for cases involving humans with different body weights.

FIT  Establishes the compliance relationship (Chapter 4), by using cubic splines (via subroutine SPLINE) to fit the experimental pressure-volume data for the region of moderate overhydration (see Section 5.3 for a brief description of the method of splines).

INIT  Based on the compartmental volumes established above, assigns/calculates the initial values for all the model variables and parameters describing the extracellular compartments (i.e., calculates the normal unperturbed values for e.g., glucose, albumin and dextran concentrations, their osmotic pressures, etc., in plasma or interstitium).

PUMP  Supplies the cellular membrane parameters, such as ion permeabilities, pump rate and ratio.

MODEL  Defines the complete set of algebraic model equations for all species and for both the intra and extra-cellular compartments.

MEMBRP  Solves the non-linear membrane potential equation for the red blood and interstitial cells, using Brent's method via the external functions PFUNC, ZBRENT and the root bracketing subroutine ZBRAK (see Section 5.3 for a description of Brent's method).

HEM  This subroutine is used only in the case of hemorrhage simulations, to calculate the plasma and RBC volumes lost during hemorrhage.

ODEINT  Implements the integration of the system of twenty two ordinary differential equations (ODEs). The subroutine is based on the Runge-Kutta-Fehlberg method with adaptive error control but uses an alternate set of coefficients given by Cash and Karp [Press et al., 1992] (see also
subroutines RKCK, RKQS and DERIVS called by ODEINT). Section 5.3 provides an overview of this ODE solution technique.

**BALANCE** Calculates the water shift across the cellular membrane caused by disturbances in extracellular osmolarity. It also updates accordingly all the compartmental fluid volumes.

**ASSIGN; OUTPUT** These two subroutines handle the output data files by storing the variables in a matrix (ASSIGN) and setting up the proper format for the output as an Excel spreadsheet (OUTPUT).

To obtain an accurate solution, the non-linear membrane potential equations as well as the algebraic equations for cell volume adjustment were solved simultaneously with the system of differential equations. This was achieved by calling the non-linear equation solver (MEMBRP) as well as the subroutine handling the cellular volume updates (BALANCE), for each local time step of the Runge-Kutta-Fehlberg method (ODEINT). Thus the membrane potential together with the ion concentrations, albumin contents, fluid fluxes and cellular volumes were continuously updated until the integration converged over the imposed local time step.

### 5.3 Numerical Methods

Three numerical techniques are used in the present work, i.e., spline interpolation, non-linear equation solving via Brent's method and a variant of the Runge-Kutta-Fehlberg method to solve the system of ODEs. An overview of these methods is given next.

a) **Cubic Spline Interpolation**

Given a set of data values \((x_i, y_i; i=1,\ldots,N)\), cubic spline interpolation makes use of the second derivative, \(y''\), to fit a function \(y\) of the form [Press et al., 1992]:

\[
y = Ay_i + By_{i+1} + Cy'_i + Dy'_{i+1},
\]

[5-1]

to the data set, where the coefficients \(A, B, C\) and \(D\) are given by:
Equations [5-1] and [5-2] show that \( y \) has a cubic dependence on \( x \) through the coefficients \( C \) and \( D \).

In order to use Eq. [5-1], the second derivatives must be evaluated. Using the continuity condition imposed on the first derivative \( y' \), at a given point \( x_i \) situated at the boundary of two adjacent intervals \([x_{i-1}, x_i]\) and \([x_i, x_{i+1}]\), yields the following system of equations for the interior points \( i=2,\ldots,N-1, \)

\[
\frac{x_i-x_{i+1}}{6} y'_{i-1} + \frac{x_{i+1}-x_{i-1}}{3} y_i' + \frac{x_{i+1}-x_i}{6} y'_{i+1} = \frac{y_{i+1} - y_i}{x_{i+1} - x_i} - \frac{y_i - y_{i-1}}{x_i - x_{i-1}}.
\]  

[5-3]

which allows the calculation of the second derivatives.

The set of \( N - 2 \) linear equations for \( N \) unknowns \( y_i \); \([i=1,\ldots,N]\); requires the specification of boundary conditions at \( x_1 \) and \( x_N \). Generally, three options are available, the so-called natural, clamped and fitted boundary conditions [Bowen, 1994]. In the present work, the ‘natural’ condition, which sets the second derivatives to zero in the end points, was used at both boundaries. The system of equations [5-3] and the two end conditions are tridiagonal; therefore it can be readily solved using well-known methods such as the Thomas algorithm [Bowen, 1994] (see subroutine TDMA called by SPLINE in Appendix H).

The general strategy in using the cubic spline interpolation is comprised of two steps. First, based on a set of given data \((x_i, y_i; i=1\ldots N)\), the second derivatives are calculated via the procedure outlined above. Second, to find the corresponding \( y \) value for a desired argument \( x \), an interval search method (e.g., bisection) is applied to locate \( x \) in the interval \([x_i, x_{i+1}]\). Then Eqs. [5-1] and [5-2] are used to determine \( y \). Note, the first step has to be executed only once as long as the set of data points remains the same.

b) Non-linear Equations: Brent’s Method

There are several techniques available to solve non-linear equations [Press et al., 1992]. Among them, algorithms based on the first derivative of the equation, such as the Newton-Raphson method, are extremely popular, due especially to their very fast (i.e. quadratic) convergence in the vicinity of the root. However, the Newton-Raphson method has several disadvantages as well. First, it requires the evaluation of the first derivative, which, furthermore,
must be continuous and non-zero in the neighbourhood of the root. In other words, the function cannot have a local extremum near the root. Second, far from the root, where the higher-order derivatives are important, the Newton-Raphson method may fail to converge to the correct result. Thus, the ‘global’ convergence of the method is poor, unless an excellent initial guess is provided.

In the initial development of the present program, the Newton-Raphson algorithm coupled with root bracketing was implemented to solve for the non-linear membrane potentials at each time step of the ODE solution. Unfortunately, due to the above-mentioned problems, the failure rate of the method was unacceptably high for the diverse conditions that were simulated. Therefore, the need for a more robust, but also fast, algorithm arose.

One of the best algorithms for solving one-dimensional non-linear equations quickly and with robust convergence properties*, is the so-called Brent’s method [Press et al., 1992]. This technique makes use of root bracketing, bisection and inverse quadratic interpolation to combine the safety of bisection with the speed of a higher-order procedure.

Assuming a general non-linear equation y = f(x), inverse quadratic interpolation means that the method uses three prior root estimates to obtain x as a quadratic function of y. The new root estimate is then determined by setting y = 0 in this quadratic relationship. Without entering into a detailed mathematical treatment, the next root estimate is given by the formula [Press et al., 1992]:

\[ x = b + \frac{P}{Q}, \]

where

\[ P = S[T(R - T)(c - b) - (1 - R)(b - a)] \]
\[ Q = (T - 1)(R - 1)(S - 1), \]

with

\[ S = \frac{f(b)}{f(a)}, T = \frac{f(a)}{f(c)} \text{ and } R = \frac{f(b)}{f(c)}. \]

In the above equations, x is the next root estimate, b is the current estimate, while a and c are two prior estimates.

* In fact it is guaranteed to converge as long as the function can be evaluated in the initial interval known to contain a single root [Press et al., 1992].
Brent’s algorithm was implemented in the program as the ZBRENT external function in the form given by Press et al. [1992] (see subroutine MEMBRP). This routine proved to be extremely reliable and fast for all the simulations tested in the present study.


The general theory behind the Runge-Kutta method of ODE integration is well known [Bowen, 1994]. In order to reduce the computational effort, various Runge-Kutta methods with adaptive step-size control have been developed [Press et al., 1992]. Among them, the stepsize adjustment algorithm originally developed by Fehlberg (known otherwise as the embedded Runge-Kutta formula) gained popularity due to its efficiency and robustness.

In essence, this method relies on the observation by Fehlberg, that a certain combination of the six function evaluations required by a fifth-order method* reduces the order to four (i.e. the fourth-order formula is embedded in the fifth-order method). Furthermore, the difference between the general form fifth-order formula and the embedded fourth-order formula provides an error estimate and, hence, a basis for step-size control [Bowen, 1994; Press et al., 1992].

The general fifth-order Runge-Kutta formula is:

\[ y_{n+1} = y_n + c_1 k_1 + c_2 k_2 + c_3 k_3 + c_4 k_4 + c_5 k_5 + c_6 k_6 + O(h^6) \]  

[5-7]

The embedded fourth-order (Fehlberg) formula is expressed as:

\[ y^*_n = y_n + \frac{c_1 k_1 + c_2 k_2 + c_3 k_3 + c_4 k_4 + c_5 k_5 + c_6 k_6 + O(h^5)}{5} \]  

[5-8]

where

\[ k_1 = hf(x_n, y_n) \]
\[ k_2 = hf(x_n + a_2 h, y_n + b_2 k_1) \]
\[ \ldots \]
\[ k_6 = hf(x_n + a_6 h, y_n + b_6 k_1 + \ldots + b_{65} k_5). \]

In Eqs. [5-7] to [5-9], h is the stepsize, and \( a_2, \ldots, c_6 \) are constants. Table 5-1, shows the Cash-Karp values for the constants \( a_2, \ldots, c_6 \).

---

* A method is called \( n \)-th order if the leading error term is of \( O(h^{n+1}) \). Note also that the number of function evaluations required by Runge-Kutta formulas with an order \( M \) higher than four, are typically \( M+1 \) or sometimes \( M+2 \).
Table 5-1: Cash-Karp coefficients for the Runge-Kutta-Fehlberg method [Press et al., 1992].

<table>
<thead>
<tr>
<th>i</th>
<th>a&lt;sub&gt;i&lt;/sub&gt;</th>
<th>b&lt;sub&gt;ij&lt;/sub&gt;</th>
<th>c&lt;sub&gt;i&lt;/sub&gt;</th>
<th>c&lt;sub&gt;i&lt;/sub&gt;&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>37</td>
<td>378</td>
<td>2825</td>
</tr>
<tr>
<td>2</td>
<td>1/5</td>
<td>1/5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3/10</td>
<td>3/40</td>
<td>250</td>
<td>18575</td>
</tr>
<tr>
<td>4</td>
<td>3/5</td>
<td>3/10</td>
<td>125</td>
<td>13525</td>
</tr>
<tr>
<td>5</td>
<td>11/54</td>
<td>5/27</td>
<td>594</td>
<td>55296</td>
</tr>
<tr>
<td>6</td>
<td>7/8</td>
<td>1631/55296</td>
<td>512</td>
<td>1/4</td>
</tr>
</tbody>
</table>

The error of the Runge-Kutta-Fehlberg method is estimated as the difference between Eqs. [5-7] and [5-8]; i.e.,

\[ \Delta = y_{n+1} - y_{n+1}^* = \sum_{i=1}^{6} (c_i - c_i^*)k_i. \]  

[5-10]

According to Eq. [5-8], the error \( \Delta \) of the Runge-Kutta-Fehlberg method, scales with step-size as \( h^5 \). Therefore, if a certain step-size \( h_1 \) produced an error \( \Delta_1 \), then the required step-size \( h_0 \) to obtain the desired accuracy (or error) \( \Delta_0 \), can be estimated as:

\[ h_0 = h_1 \left( \frac{\Delta_0}{\Delta_1} \right)^{1/5}. \]  

[5-11]

Equation [5-11] controls the step-size \( h \) of the Runge-Kutta-Fehlberg algorithm as a function of the relative error (so-called adaptive step-size control).

The subroutine ODEINT uses the Runge-Kutta-Fehlberg algorithm with Cash-Karp coefficients to solve the set of twenty-two differential equations (see subroutines RKQS and RKCK). This technique was found to be reliable and fast for all the simulation scenarios considered in the present study.
Chapter 6: MODEL VALIDATION BASED ON ANIMAL STUDIES

6.1 Introduction

Confidence in the human model presented in Chapter 4 can only be gained if the predictions of this model compare well with available experimental data. In the present chapter, an initial validation of the model will be carried out based on measured data obtained from several animal experiments.

The chapter is organized according to the following topics, which all play an important role in the validation exercise:

- **Evaluation of infusion fluids**
  The fluids that have been employed in the various animal infusion experiments of relevance here include: isotonic solutions, either normal saline (NS) or Ringer's type solutions (RS); hyperosmotic saline solutions (HS); and hypertonic colloidal solutions (HSD).

- **Estimation of the permeability-surface area product and reflection coefficient for small ions**
  As was suggested in the previous chapter, the model parameters which have the highest degree of uncertainty are those related to the transcapillary transport of small ions. Hence, prior to model validation, it becomes necessary to use part of the available literature data to estimate these two essential transport parameters.

- **Validation of the model using measured data obtained following the infusion of animals with normal saline (NS), Ringer's solution (RS), and/or hyperosmotic solutions (HS)**
  A first validation of the model is based on information obtained from NS, RS and HS infusions in nephrectomized animals. The data were selected only for animals that were not subjected to any previous trauma (i.e., infusions were administered to otherwise healthy animals).

- **Discussion of the model prediction following infusions with NS, RS and HS**

6.2 Solutions for Infusion

One of the purposes of fluid therapy is to replace fluid and/or solute deficits in the body that have resulted from a traumatic condition (e.g., hemorrhage, burns, etc.). The goal in external fluid administration is to re-establish an adequate circulatory volume and, implicitly, an adequate cardiac output, blood pressure, as well as oxygen delivery to the tissues.
Over the past couple of decades, there has been a transition in fluid therapy from blood products toward plasma substitutes. This departure from the use of blood products is due to economic factors and the availability of blood, as well as concerns about the possibility of these products carrying pathogenic organisms and producing unwanted immunizations. Based on their composition, the various alternative fluids that can be used for infusions can be categorized as crystalloid or colloid solutions. A further classification of both of these types of solutions is based on their osmolarity relative to plasma. Thus, the solutions used in clinical practice are hypo-osmolar (lower osmolarity than plasma), iso-osmolar (approximately the same osmolarity as plasma), or hyperosmolar (higher osmolarity than plasma). The last two categories of infusion fluids are extensively used in the validation studies presented in this chapter and, therefore, will be discussed in more detail in the following two sections.

6.2.1 Iso-osmolar saline (Ringer's) solutions

The use of isotonic solutions is based on studies done by Ringer more than a century ago [see Weisberg, 1962]. Isotonic solutions have about the same osmolarity as the internal body fluids, i.e., intra- and extracellular fluids. It is expected, therefore, that their infusion does not alter the osmolarity of the extracellular fluid and hence causes no significant osmotic shifts of water across the capillary membrane or into or out of the cells. Infusion of large volumes of these solutions results in an initial increase in plasma volume followed by a secondary transport of fluid from plasma into interstitium because of differences in hydrostatic pressure. As a consequence, the retention of the isotonic infusate in plasma is poor; although the net result of infusion is an expansion of the total extracellular volume, this expansion occurs at the expense of an increased interstitial volume and potential edema formation.

A great many of the solutions used for infusion are described in the medical literature as belonging to the class of iso-osmolar solutions. The typical compositions of two of these solutions are presented in Table 6-1 and these are discussed briefly below.

Isoosmotic 0.9% sodium chloride solution, also known as normal saline (NS), isotonic saline or physiological sodium chloride, contains approximately 154 mEq/l of NaCl. Despite its name, however, this solution is not exactly in 'physiological' balance with the internal body fluids. As shown in a previous chapter (see Table 2-1), the sodium and chloride concentrations in the extracellular plasma and interstitial fluids (e.g., about 140 mEq/l and 100 mEq/l, respectively) are lower than those of NS. It is expected, therefore, that when NS is infused, it will
induce some minor fluid and small-ion shifts between the different fluid compartments (mainly RBCs). In addition to its limited intravascular retention that is typical of iso-osmolar solutions, the iso-osmolar NS solution has been reported to cause hemodilution, renal potassium loss, and aggravation of metabolic acidosis [Rocha E Silva et al., 1987].

To overcome the excess of sodium chloride in isotonic saline, several Ringer's type solutions (RS) have been developed over the years. As shown for the lactated Ringer's example given in Table 6-1, these contain a more balanced combination of different small ions. Other Ringer's type solutions include acetated-RS, Hartman's solution, Fox's solution, etc. Owing to their great variety, the exact composition of the infusion fluids that are used in this work for model validation and/or prediction will be indicated when discussing specific experimental protocols.

6.2.2 Hyperosmolar/hypertonic solutions

The osmolarity of these solutions is higher than that of plasma. Infusion of hyperosmolar solutions increases plasma osmolarity and therefore causes an osmotic water shift into the plasma compartment from the interstitium and also from the cells (both RBCs and tissue cells). The overall effect is a much higher initial increase in the osmolarity and volume of the extracellular fluid than can be expected from iso-osmolar solutions.

As shown in Table 6-1, the hypertonic solutions can consist of only concentrated NaCl (hypertonic saline) or a combination of a concentrated saline solution with a colloidal compound (hypertonic colloidal solution).

a) Hypertonic NaCl solutions (HS)

Numerous experiments carried out during the past several years have proven the ability of HS infusions to rapidly increase the plasma volume by two to three times the infused volume [Maningas et al., 1989; Onarheim et al., 1989; Wade et al., 1990; Onarheim, 1995; Carlson et al., 1996]. However, although HS solutions have proven to be effective in acute traumatic situations involving experimental animals, they can cause hypernatremia, hyperosmolarity, and hypokalemia, conditions that may impose limitations on their use as resuscitants [Shires et al., 1995]. Similar to RS infusions, the plasma retention of HS resuscitants is short-lived.
Table 6-1: Examples of compositions for isotonic and hypertonic fluids used most commonly for experimental infusion studies.

b) Hypertonic/hyperoncotic solutions

The marked hemodynamic improvement obtained by the addition of colloids such as dextrans in a hypertonic saline solution has been demonstrated in numerous experimental studies. The results are very promising for a variety of traumatic conditions such as hemorrhage [e.g., Sondeen et al., 1990(a)], trauma [e.g., Vassar and Holcroft, 1992] as well as burns [e.g., Kramer, 1986]. An extended literature review of more than 800 studies on this topic can be found at [http://www.hypertonic.utmb.edu/](http://www.hypertonic.utmb.edu/).

The hypertonic component (i.e., the high concentration of NaCl) of these solutions has the effect previously described for HS solutions, i.e., of rapid water shifts into the vascular compartment. The hyperoncotic component (i.e., the colloidal substance) that has a relatively good retention in the vascular space, maintains an elevated vascular volume for a longer period of time. As a result of these effects, much lower volumes of hypertonic/hyperoncotic solutions
are required to attain hemodynamic stability, compared with their RS and HS counterparts [e.g., Smith, 1985; Kramer, 1986].

Despite the numerous studies cited above, the use of hypertonic solutions or their crystalloid counterparts is still controversial [Shires et al., 1995; Tølløfsrud, 1997]. As pointed out in a recent study [Tølløfsrud, 1997], the main difficulty in establishing the optimum fluid for resuscitation and/or the optimal resuscitation protocol lies in a lack of agreement as to what constitutes an ideal physiological outcome from fluid therapy. There are a multitude of interdependent factors that influence the exchange of fluid and solutes between the vascular and interstitial compartments, as well as between the extracellular and cellular spaces. Among these factors, the physicochemical properties of the participating fluid compartments, the properties of the membranes that separate them, and the chemical or electro-chemical gradients governing transport are just a few. The full interaction between these factors is often difficult to assess from experimental studies, which are usually able to investigate only a limited number of measurable variables at a time. However, due to their ability to generate large quantities of measurable information, validated mathematical models can serve as useful tools for better understanding the roles played by the many factors implicated in physiological exchange processes.

6.3 Estimation of the Permeability-Surface Area Product and the Reflection Coefficient for the Transcapillary Transport of Small Ions

As indicated in Table 4-1 (see Chapter 4), the permeability-surface area product, \( P_{S_{ION}} \), and the reflection coefficient, \( \sigma_{ION} \), which control the transport of small ions across the capillary barrier are not well-defined. Although several authors have investigated and suggested acceptable ranges for these two parameters, there is little agreement about their precise values [Carlson et al., 1996; Curry, 1984; Wolf, 1982]. Because earlier MVES models proposed by this group [Chappie et al., 1993; Xie et al., 1995] were primarily concerned with fluid and protein redistribution, optimized values for \( P_{S_{ION}} \) and \( \sigma_{ION} \) were not determined. Thus, as part of the current study, it was necessary to make use of a portion of the available experimental data to obtain more reliable estimates of these two missing transport parameters.

To estimate \( P_{S_{ION}} \) and \( \sigma_{ION} \) and to help validate the present model, its predictions were compared with the experimental results from a previously published report by Wolf [1982]. Wolf's experimental data were selected for this purpose because 1) the data were collected from animals not subject to any type of previous trauma, 2) they include hyperosmotic infusions
where significant intercompartmental ion transfers occur, and 3) the study reports numerous data that are time-dependent and hence provide a more rigorous test of the model. To minimize the number of parameters to be determined, it is assumed that the same values of $P_{\text{Sion}}$ and $\sigma_{\text{ion}}$ apply to all of the ionic species ($\text{Na}^+$, $\text{K}^+$, $\text{Ca}^{2+}$, $\text{Cl}^-$ or $\lambda^-$) that are exchanged between the plasma and interstitial compartments. Thus, the parameters obtained can be considered as average values for all five ions.

### 6.3.1 Experimental information

In Wolf's [1982] study, normovolemic, nephrectomized dogs were infused for 6 minutes with an iso-osmotic saline solution (NS). Following a stabilization period of 45 minutes, the animals were then infused for 3 minutes with a hypertonic saline solution (HS). The resuscitation protocol reported by Wolf and subsequently used as an input to the model, is given in Table 6-2. Plasma volume and plasma osmolarity were measured continuously over a period of 3 hours. According to Wolf, all the infused volumes and times are comparable to commonly used clinical resuscitation protocols, i.e., short infusion times and relatively low infused volumes.

<table>
<thead>
<tr>
<th>Type of Infusion</th>
<th>Osmolarity (mOsm/l)</th>
<th>Volume Infused (ml/kg)</th>
<th>Duration of Infusion (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline Solution (NS)</td>
<td>303.2</td>
<td>23.15</td>
<td>6</td>
</tr>
<tr>
<td>Hyperosmolar NaCl (HS)</td>
<td>2030</td>
<td>9.25</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 6-2. Resuscitation protocols used by Wolf [1982].

### 6.3.2 Initial conditions

In Chapter 4, where the formulation of the model was first introduced, the initial conditions were given for a 70 kg 'Reference man'. For parameter estimation and for a first validation of the model, the current study relies on data from studies involving different animal species. Throughout this work, in order to make use of as much of the available experimental information as possible, there will be a continuous transition from human to animal
compartmental values. Detailed calculations exemplifying the scaling procedure used for Wolf’s dogs are given in Appendix C. The general scaling procedure will also be briefly mentioned just once in this section with reference to Wolf’s study. Thereafter, only tabulated values for the initial compartmental values will be given where appropriate.

The initial values for the fluid compartments at the start of each infusion experiment in dogs are given in Table 6-3. The initial whole body plasma and interstitial volumes were specified in Wolf’s [1982] report. As was pointed out in the previous chapter, based on studies by Bert and Pinder [1982], proteins were assumed to be excluded from 25% of the normal interstitial volume. The normal hematocrit values were also reported in these experimental studies, while the corresponding volumes for the RBC compartment were calculated based on hematocrit according to Eq. [4-1]. The tissue cell volume was assumed to constitute about 40% of the total body weight.

Table 6-3 also summarizes the properties of the lymphatic system and of the capillary membrane which separates the plasma and interstitial compartments. The majority of the parameters characterizing these two exchange pathways were obtained by scaling the values obtained by Xie et al. [1995] for humans to the appropriate animal values. The basis for this scaling was the assumption that the capillary density is about the same for all species and, hence, the area available for exchange is proportional to the volume of the interstitium, such that:

\[
\text{Transport parameter}_{\text{ANIMAL}} = \left(\frac{V_{\text{IT,ANIMAL}}}{V_{\text{IT,HUMAN}}}\right) \text{Transport parameter}_{\text{HUMAN}} \tag{6-1}
\]

To complete the description, the permeability-surface area product, \(PS_{\text{ION}}\), used to describe the rate of diffusive ion (i.e., \(\text{Na}^+, \text{K}^+, \text{C}^{2+}, \text{Cl}^-\) or \(\text{A}^-\)) transfer across the capillary membrane, is also shown in Table 6-3. This value was estimated according to the procedures outlined in Section 6.3.3. The interstitial compliance relationships were obtained by scaling, on a weight basis, the compliance relationships developed for the human MVES model by Chappie et al. [1993] to the appropriate dog values. In accordance with our past modeling practice, the compliance relationship was separated into three regions: the ‘dehydration segment’, the ‘intermediate segment’ for moderate hydration and the ‘overhydration segment’ (see Chapter 4). The range of interstitial volume values and the compliance relationships corresponding to each of the above-mentioned segments are as follows:
For dehydration, $V_{IT} \leq 4160.0\, \text{ml}$:

$$P_{IT} = -0.7 + 3.9 \times 10^{-3} (V_{IT} - 4160.0 \, \text{ml}).$$

For moderate hydration, $4160.0 \, \text{ml} \leq V_{IT} \leq 6240.0 \, \text{ml}$:

Mathematical interpolation for $P_{IT}$ versus $V_{IT}$ experimental data.

For overhydration, $V_{IT} \geq 6240.0 \,\text{ml}$:

$$P_{IT} = 1.88 + 2.1 \times 10^{-4} (V_{IT} - 6240.0 \, \text{ml}).$$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value for Dog</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, BW</td>
<td>26.0 kg</td>
<td>[Wolf, 1982]</td>
</tr>
<tr>
<td>Plasma volume, $V_{PL}$</td>
<td>49.2 ml/kg</td>
<td>[Wolf, 1982]</td>
</tr>
<tr>
<td>Hematocrit, Hct</td>
<td>43.9 %</td>
<td>[Wolf, 1982]</td>
</tr>
<tr>
<td>Red blood cell volume, $V_{RBC}$</td>
<td>30.8 ml/kg</td>
<td>calculated based on Hct</td>
</tr>
<tr>
<td>Interstitial volume, $V_{IT}$</td>
<td>159.2 ml/kg</td>
<td>[Wolf, 1982]</td>
</tr>
<tr>
<td>Excluded interstitial volume, $V_{IT,EX}$</td>
<td>40.0 ml/kg</td>
<td>calculated based on [Bert and Pinder, 1982]</td>
</tr>
<tr>
<td>Tissue cell volume, $V_{TC}$</td>
<td>400.0 ml/kg</td>
<td>calculated based on 40% BW</td>
</tr>
<tr>
<td>Fluid filtration coefficient, $k_F$</td>
<td>2.3 ml/kg-mmHg-h</td>
<td>calculated based on [Xie et al., 1995]</td>
</tr>
<tr>
<td>Permeability-surface area product for protein, $PS$</td>
<td>1.4 ml/kg-h</td>
<td>calculated based on [Xie et al., 1995]</td>
</tr>
<tr>
<td>Permeability-surface area product for small ions, $PS_{ION}$</td>
<td>4200 ml/kg-h</td>
<td>see Section 6.3.3</td>
</tr>
<tr>
<td>Normal lymph flow rate, $J_{L, NL}$</td>
<td>1.4 ml/kg-h</td>
<td>calculated based on [Xie et al., 1995]</td>
</tr>
<tr>
<td>Lymph flow sensitivity, $LS$</td>
<td>0.8 ml/kg-mmHg-h</td>
<td>calculated based on [Xie et al., 1995]</td>
</tr>
</tbody>
</table>

Table 6-3: Initial steady-state compartmental values for Wolf's [1982] dogs.
All the compartmental variables presented in Table 6-3, are extensive properties (i.e., they depend on the mass of the system). The initial values of various intensive properties are presented in Table 6-4. These are maintained constant for the simulation of all resuscitation protocols (animal or human). The initial hydrostatic and oncotic pressures as well as the protein concentrations shown are those previously presented in Table 4-1. These normal steady-state conditions correspond to a generic tissue compartment and the general circulation. The average reflection coefficients $\sigma$ (for proteins) and $\sigma_{\text{ION}}$ (for small ions) are, respectively, the value reported by Xie et al. [1993] and that estimated by curve-fitting in Section 6.3.3.

A simplified initial partition of sodium, potassium and chloride ions between the interstitial and plasma compartments is also shown in Table 6-4. The considerations, including the Donnan equilibrium, taken into account in determining these concentration values, are the same as those described in Section 4.5.4.4. Similarly, the cellular membrane transport parameters, i.e., $p_{\text{Na}}$, $p_{\text{K}}$ and $p_{\text{Cl}}$, and the contribution of the $\text{Na}^+-\text{K}^+$-pump to the active transport term, $R_P$, are unchanged from the values previously given.

All the values presented in Tables 6-3 and 6-4 are the steady-state values obtained by running the computer program associated with the model in the absence of any perturbation (i.e., fluid inputs and/or outputs), for a sufficient length of time until none of these values changed beyond a small pre-established error criterion. Hence, the modeled system was at steady state prior to simulating any disturbance due to external fluid and solute infusion. The infusion protocols reported for Wolf's experiments constitute inputs to the model as already summarized in Table 6-2. The right-hand sides of Eqs. [4-6] and [4-7], i.e., the mass balance equations for dextran in plasma and interstitium, were set to zero since they are not applicable to simulating either the work of Wolf [1982] or the other animal studies used in this chapter. As well, the urinary outputs of fluid and small ions were set to zero, and, because of the short-infusion periods studied here, the output terms for the perspiration/respiration rates of fluid and small ions are assumed negligible.
### Table 6-4. Initial steady-state compartmental values common to humans and all experimental animals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma Compartment</th>
<th>Interstitial Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>Source</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>70 g/l</td>
<td>[Kleinman and Lorenz, 1984]</td>
</tr>
<tr>
<td>Protein concentration in the available volume</td>
<td>- - -</td>
<td></td>
</tr>
<tr>
<td>Hydrostatic pressure</td>
<td>11 mmHg</td>
<td>[Xie et al., 1995]</td>
</tr>
<tr>
<td>Colloid osmotic pressure</td>
<td>25.9 mmHg</td>
<td>[Xie et al., 1995]</td>
</tr>
<tr>
<td>Albumin reflection coefficient</td>
<td>0.988</td>
<td>[Xie et al., 1995]</td>
</tr>
<tr>
<td>Small ions reflection coefficient</td>
<td>0.15</td>
<td>estimated in Section 6.3.3</td>
</tr>
<tr>
<td>Sodium concentration</td>
<td>139.7 mEq/l</td>
<td>[Onarheim, 1995]</td>
</tr>
<tr>
<td>Potassium concentration</td>
<td>4.7 mEq/l</td>
<td>[Onarheim, 1995]</td>
</tr>
<tr>
<td>Chloride concentration</td>
<td>105.7 mEq/l</td>
<td>[Onarheim, 1995]</td>
</tr>
</tbody>
</table>

6.3.3 Estimation of parameters

In the parameter estimation procedure, pairs of discrete values in the ranges of $1000 \leq PS_{\text{ION}}/PS \leq 5000$ (where $PS$ is the protein permeability-surface area product) and $0.05 \leq \sigma_{\text{ION}} \leq 0.5$ were selected as inputs to the simulation program. With all transport parameters now defined, the ordinary differential equations governing the model were integrated from $t = 0$ to $t = 4$ h, corresponding to the time course of Wolf's replicated normal saline (NS) and hyperosmotic solution (HS) infusion experiments.

In the simulations, the solution volumes and compositions, given in Table 6-2, were injected into the plasma compartment at a constant rate over the reported infusion periods. Based on the generated results, separate sums-of-squares-of-differences between the predicted and experimental plasma volumes and osmolarities were calculated. To obtain an overall sum-of-squares value for each pair of parameters, the volume and osmolarity sums were first normalized.
with respect to their minimum values over the ranges investigated, weighted by their respective number of data points, and then added together. These combined sum-of-squares values are plotted as a function of $PS_{ION}/PS$ and $\sigma_{ION}$ in Fig. 6-1.

Figure 6-1 shows that the sum-of-squares surface is minimal and relatively insensitive to the values of the two parameters over the more constrained ranges of $2000 \leq PS_{ION}/PS \leq 4000$ and $0.1 \leq \sigma_{ION} \leq 0.2$. Furthermore, there is a clear global minimum at the point $PS_{ION}/PS = 3000$ and $\sigma_{ION} = 0.15$. The model predictions, obtained with $PS_{ION}/PS = 3000$ and $\sigma_{ION} = 0.15$, and the experimental data for plasma volume and plasma osmolarity reported by Wolf are shown in Figs. 6-2 and 6-3, respectively.

Figure 6-2 shows the experimental changes in blood volume measured by Wolf [1982] for an NS infusion followed, about 40 minutes later, by an HS infusion. As can be seen in this figure, plasma volume, $V_{PL}$, increases in both experiments by about 54% above control immediately after the administration of NS at 303 mOsm/l, followed by a further experimental increase of about 65 or 105% after HS administration at 2030 mOsm/l. If the total infusate volumes had simply remained in the plasma compartment in each case, the expected increases would have been 58% and 23%, respectively.

Thus, clearly, the HS resuscitant is causing the recruitment of significant amounts of fluid from other reservoirs such as the interstitium and the plasma and tissue cells. However, the volume expansion is short-lived in both cases; as soon as the infusion ends, fluid begins leaking out of the plasma compartment such that, at least in the case of the HS infusion, a relatively stable plasma volume is reached at about 1 h after fluid administration was terminated.

Wolf's plasma osmolarity results are compared to the model predictions in Figure 6-3. Once again the agreement between the model results and the experimental measurements is very good. This is not surprising since both the plasma volume and osmolarity data were used in the estimation of $PS_{ION}$ and $\sigma_{ION}$. It should be noted that the plasma osmolarity remains near its baseline value during the normal saline infusion, but increases rapidly during the hyperosmotic infusion. The slight decrease in plasma osmolarity that occurs immediately post-NS infusion is due to the difference in composition between the NS infusion and the plasma composition, as discussed earlier in Section 6.2. At the end of the HS infusion, the osmolarity decreases sharply, as indicated by both the predicted and experimental data. Additionally, both experimental data and simulations reach an apparent steady state of about 10% above the control value within 0.5 to 1.0 hour after the infusion stops.
Figure 6-1. Combined sum-of-squares-of-differences between the model predictions and Wolf's [1982] plasma volume and plasma osmolarity measurements as a function of $PS_{ION}/PS$ and $\sigma_{ION}$, where $PS$ is the permeability-surface area for proteins. The optimal solution is obtained for $PS_{ION}/PS = 3000$ and $\sigma_{ION} = 0.15$. 
Figure 6-2. Comparison of the model predictions for plasma volume changes vs. time with experimental data from Wolf [1982]. The solid line represents the model predictions for NS followed by HS infusion while the symbols represent two sets of experimental data points for the same infusion protocol.
Figure 6-3. Comparison of the model predictions for changes in plasma osmolarity vs. time with experimental data from Wolf [1982]. The dashed line represents the model predictions for NS followed by HS infusion while the symbols represent two sets of experimental data points for the same infusion protocol.
6.4 Model Validation Based on Additional Animal Studies

One important aspect of model validation is that the model should provide a good representation of the experimental data used in the estimation of model parameters. This aspect was shown to be satisfied in the previous section. However, a more rigorous validation requires that, with the same set of estimated parameters, the model should be capable of predicting the results of other, independent, experimental studies. Based on the information available in the two additional studies, the data generated in the infusion experiments of Onarheim [1995] on normal rats and Manning and Guyton [1980] on normal dogs were selected for this purpose. These authors report the changes to such variables as plasma volumes, hematocrits, osmolarities, and ion and protein concentrations, as well as interstitial, extracellular and cellular volumes following infusions of either Ringer’s (RS) or hyperosmolar (HS) solutions.

As part of the validation procedure, the agreement, with respect to both transient and steady-state behavior, between the model predictions and these sets of available experimental data was analyzed. Whenever extensive properties (i.e., those that depend on the amount of a particular variable) are compared within a simulation or between simulations, they are done so on a relative (or percentage) basis.

6.4.1 Experimental information

In Onarheim’s experiments, normovolemic rats were infused continuously for a period of about 20 minutes with either acetated Ringer’s solution (270 mOsm/l) or with hypertonic saline solution (2400 mOsm/l). The infused volumes were selected such that the same amount of sodium was administered in both types of infusion protocols. To allow better assessment of the alterations in interstitial and intracellular fluid volumes following infusion, Onarheim chose to administer larger amounts of fluid over longer infusion times than are commonly used in clinical studies. Onarheim reports initial and final values for plasma volume, total extracellular volume, plasma sodium, potassium and chloride concentrations, hematocrit and plasma osmolarity. These were measured prior to fluid administration and 1 h after the start of the infusion, at which time an apparent steady state had been reached. Initial and post-infusion intracellular volumes for different tissues were calculated as the difference between the measured total tissue fluid volumes (determined by drying) and tissue extracellular fluid volumes (determined using tracers).
Manning and Guyton performed experiments in which dogs were infused with lactated Ringer's solution (270 mOsm/l) in amounts equivalent to 5%, 10% and 20% of the animals' body weight. The infusion times ranged from 30 to 60 minutes. In this study, the time-changes in blood volume, total extracellular volume, and plasma protein concentration were followed.

As was the case in Wolf's experiments, the animals used in these two studies were nephrectomized. Additionally, the fluid and/or solute redistribution between plasma and interstitium after RS or HS infusions is followed for cases where there was no previous trauma.

The initial compartmental values as well as the interstitial compliance relationships determined for Onarheim's rat and Manning and Guyton's dog are given in Tables 6-5 and 6-6, respectively. The resuscitation protocols reported in these two studies and used as inputs to the model are presented in Table 6-7.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, BW (kg)</td>
<td>0.255</td>
<td>20.0</td>
</tr>
<tr>
<td>Plasma volume, Vpl (ml/kg)</td>
<td>25.8</td>
<td>45.0</td>
</tr>
<tr>
<td>Hematocrit, Hct (%)</td>
<td>47.3</td>
<td>32.9</td>
</tr>
<tr>
<td>Red blood cell volume, Vpbc (ml/kg)</td>
<td>23.1</td>
<td>22.1</td>
</tr>
<tr>
<td>Interstitial volume, Vit (ml/kg)</td>
<td>174.2</td>
<td>296.3</td>
</tr>
<tr>
<td>Excluded interstitial volume, VITEX (ml/kg)</td>
<td>43.5</td>
<td>74.0</td>
</tr>
<tr>
<td>Tissue cell volume, VTC (ml/kg)</td>
<td>400.0</td>
<td>396.0</td>
</tr>
<tr>
<td>Fluid filtration coefficient, kF (ml/kg-mmHg-h)</td>
<td>2.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Permeability-surface area product for protein, PS (ml/kg-h)</td>
<td>1.52</td>
<td>2.57</td>
</tr>
<tr>
<td>Permeability-surface area product for small ions, PSION (ml/kg-h)</td>
<td>4560.0</td>
<td>7710.0</td>
</tr>
<tr>
<td>Normal lymph flow rate, JLN,NE (ml/kg-h)</td>
<td>1.57</td>
<td>2.66</td>
</tr>
<tr>
<td>Lymph flow sensitivity, LS (ml/kg-mmHg-h)</td>
<td>0.896</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Table 6-5. Initial steady-state compartmental values used in animal validation studies.
### Table 6-6: Interstitial compliance relationships for 'scaled rat' [Onarheim, 1995] and 'scaled dog' [Guyton and Manning, 1980].

<table>
<thead>
<tr>
<th>Region of Curve</th>
<th>Range of $V_{IT}$ (ml) and corresponding relationship for $P_{IT}$ (mmHg)</th>
<th>Rat</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>$V_{IT} \leq 44.42$ ml</td>
<td>$P_{IT} = - 0.7 + 0.37 (V_{IT} - 44.42)$</td>
<td>$V_{IT} \leq 5926$ ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$P_{IT} = - 0.7 + 2.78 \times 10^{-3} (V_{IT} - 5926)$</td>
</tr>
<tr>
<td>Moderate</td>
<td>$44.42$ ml $\leq V_{IT} \leq 66.63$ ml</td>
<td>Mathematical interpolation of $P_{IT}$ versus $V_{IT}$ experimental data</td>
<td>Mathematical interpolation of $P_{IT}$ versus $V_{IT}$ experimental data</td>
</tr>
<tr>
<td>Hydration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overhydration</td>
<td>$V_{IT} \geq 66.63$ ml</td>
<td>$P_{IT} = 1.88 + 1.98 \times 10^{-2} (V_{IT} - 66.63)$</td>
<td>$V_{IT} \geq 8888$ ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$P_{IT} = 1.88 + 1.48 \times 10^{-4} (V_{IT} - 8888)$</td>
</tr>
</tbody>
</table>

Table 6-7: Resuscitation protocols used as inputs to the model in the validation studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of Infusion</th>
<th>Osmolarity (mOsm/l)</th>
<th>Volume Infused (ml/kg)</th>
<th>Duration of Infusion (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Hyperosmolar NaCl (HS)</td>
<td>2400</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Acetated Ringer’s Solution (RS)</td>
<td>270</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>Dog</td>
<td>Lactated Ringer’s Solution (RS)</td>
<td>270</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Lactated Ringer’s Solution (RS)</td>
<td>270</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Lactated Ringer’s Solution (RS)</td>
<td>270</td>
<td>200</td>
<td>40</td>
</tr>
</tbody>
</table>
### 6.4.2 Comparisons with Onarheim's data

Onarheim [1995] measured several system variables before and approximately 1 h after the start of 18-minute infusions with either HS (2400 mOsm/l) or RS (270 mOsm/l). A comparison of the model predicted values of these variables with the experimentally measured data is presented in Table 6-8. In the simulations, the infused volume was added to the plasma compartment at a constant rate over the infusion period, in accordance with Onarheim's experimental protocol.

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Control</th>
<th>Acetated Ringer's Solution 270 mOsm/l</th>
<th>Hypertonic Solution 2400 mOsm/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Experiment</td>
<td>Model Prediction</td>
</tr>
<tr>
<td>Plasma Volume (% Change)</td>
<td>0.0 ±3.1</td>
<td>37.9 ±4.5</td>
<td>35.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47.4 ± 0.3</td>
<td>38.4 ±0.5</td>
<td>40.2</td>
</tr>
<tr>
<td>Plasma Osmolarity (mOsm/l)</td>
<td>303.3 ±2.0</td>
<td>294.1 ±0.5</td>
<td>296.7</td>
</tr>
<tr>
<td>[Na]_{PL} (mmol/l)</td>
<td>139.7 ± 0.8</td>
<td>139.5 ±0.4</td>
<td>140.3</td>
</tr>
<tr>
<td>[K]_{PL} (mmol/l)</td>
<td>4.7 ±0.1</td>
<td>4.5 ±0.1</td>
<td>4.6</td>
</tr>
<tr>
<td>[Cl]_{PL} (mmol/l)</td>
<td>105.7 ±0.8</td>
<td>108.0 ±0.4</td>
<td>108.7</td>
</tr>
<tr>
<td>Extracellular Volume (% Change)</td>
<td>0.0 ±0.3</td>
<td>61.5 ±3.0</td>
<td>46.9</td>
</tr>
<tr>
<td>Tissue Cell Volume (% Change)</td>
<td>0.0 ±0.9</td>
<td>-0.8 ± 2.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 6-8. Comparison between model predictions and Onarheim's [1995] experimental data at 60 minutes after fluid infusion.
One hour after beginning the fluid administration, when an apparent steady state was reported, the model predicts that the HS infusion produces a 27% increase in plasma volume above the control value, while the almost order-of-magnitude larger RS infusion caused only a 35% increase. These results are in good agreement with the experimental measurements, which indicate 28 ± 4% and 38 ± 5% plasma volume expansions, respectively. The simulations also show that, for both types of solutions infused, the plasma volume reaches its maximum expansion at the end of the infusion period. At this intermediate time, the plasma volume almost doubled with the simulated infusion of 10 ml/kg HS and increased only to about three times the initial volume for the 100 ml/kg RS infusion. If all of the infused fluid had simply been retained by the plasma compartment, the expected expansions would have been about 40% and 390%, respectively. Thus, as was the case in Wolf’s [1982] experiments, Onarheim’s HS infusion engenders additional fluid recruitment far beyond the volume administered and, following the infusion period for both types of resuscitants, fluid rapidly leaks from the plasma compartment such that an apparent steady state is reached in less than 1 h.

According to Table 6-8, blood hematocrit falls from 47% to a new steady-state value of about 40% for both the RS and HS infusions. For the RS case, Hct is predicted to drop continuously up to the end of the infusion period because of an elevated plasma volume and a relatively constant RBC volume. However, as the plasma volume returns toward its baseline value (see above), Hct is partially restored to a final predicted value of about 40%. This end-point, obtained by simulation, agrees closely with the measured value of 38% reported by Onarheim. Good agreement between the measured Hct value and the model prediction was also obtained for the HS infusion. In this case, the final plasma volume is lower, but the RBC volume is also reduced, leading to a similar steady-state Hct.

Table 6-8 indicates that, at 1 h post-infusion, the plasma osmolarity increases for the HS infusion but decreases slightly for the RS infusion. For the HS case, there is a model predicted peak increase in plasma osmolarity corresponding to about 40 mOsm/l occurring at the end of the infusion period. This is largely due to the osmotic contribution of the ionic species infused. After the HS infusion was terminated, the plasma osmolarity decreases rapidly toward a new steady state. At steady state, the model predicted a value of 338 mOsm/l which compares well with the 333 mOsm/l reported by Onarheim. For the RS infusion protocol, a maximum decrease of approximately 6 mOsm/l in plasma osmolarity is predicted during the infusion period. According to the simulations, the minimum in plasma osmolarity corresponds to the peak in
plasma volume and occurs at the end of the infusion. This decrease is due partly to dilution of plasma by the large volume of the slightly hypotonic RS infused (270 mOsm/l in RS vs. 303 mOsm/l in plasma). After the infusion stops, as the fluid and small ions are re-equilibrated between plasma and interstitium, a slight increase in overall plasma osmolarity toward the baseline value takes place. At about half an hour after the infusion has ceased, the model predicts a steady-state osmolarity of 297 mOsm/l which is in good agreement with the experimental value of 294 mOsm/l. The plasma osmolarity trends predicted for Onarheim's essentially isosmotic and hyperosmotic infusions are very similar to those measured by Wolf [1982] (see Fig. 6-3).

For the RS infusion, the predicted plasma electrolyte concentrations for sodium, potassium and chloride ions at 1 hour post-infusion are in good agreement with the values reported in Onarheim's experimental study. In fact, for this case, neither the experiments nor the model were expected to give concentration values that are significantly changed from normal for any of the ionic species. For the HS infusion on the other hand, the model predicts that the plasma sodium increases from a baseline concentration of 140 mmol/l up to about 160 mmol/l immediately post infusion. This increase is a direct result of the high sodium content of the infused HS. Once the concentration difference between the plasma and interstitium begins to dissipate, the concentration of plasma sodium decreases gradually. When steady state is achieved, the model indicates a plasma sodium concentration of 159 mmol/l which compares well to the value of 155 mmol/l reported experimentally. A similar predicted trend is observed for the chloride ion, whose concentration increases from an initial steady-state value of 106 mmol/l up to 135 mmol/l immediately post-infusion, followed by a return to 131 mmol/l when the system reaches its new steady state. This value is in good agreement with the measured value of 127 mmol/l. For K\(^+\), the model predicts that, following HS infusion, the concentration falls from the baseline value of 4.7 mmol/l to 4.4 mmol/l immediately after the infusion stops, and returns to a value of 4.5 mmol/l at the new steady state. As can be seen in Table 4, there is a disagreement between the computed and Onarheim's reported potassium ion concentration for reasons we cannot fully explain. Other series of experimental studies by the same the author, with HS/Dextran infusions following hemorrhage or burn, show that serum potassium often tends to decrease [Onarheim et al., 1989; Onarheim et al., 1990].

Onarheim [1995] also measured the changes in total extracellular fluid volume following RS and HS infusions. The simulation of the HS case, in close agreement with the experimental data, predicts a 25% increase in ECV 1 h post-infusion. However, for the RS infusion, the model
predictions underestimate the experimental values by about 15% (i.e., the model-predicted change is 47% compared to the experimental value of 62%). If, as one would anticipate for an essentially iso-osmotic infusion, little fluid exchange with the cells occurs, then the increase in extracellular volume would have been 50%, which is about 3% greater than the change predicted by the model. Onarheim [1995] also found that, for both the RS and HS infusions, ECV values measured at the end of the infusion period were very close to the final steady-state values obtained at 1 h. The model predicted a similar extracellular volume behavior; a linear increase during the infusion period followed by an almost instantaneous attainment of steady state immediately after the infusion was completed. In contrast, the predicted plasma and interstitial volumes continued to change significantly following the infusion, only approaching their new steady states about 1 h after the infusion began. These results were expected for the isotonic RS infusion where little transport to or from the cells takes place, but they also offer experimental justification for the assumption of a very rapid shift of fluid between the intra- and extracellular spaces in the case of the HS infusion.

Finally, Onarheim [1995] also provided information about the change in the cellular fluid volume of skeletal muscle following RS and HS infusions. For the RS case, the model predicts a small increase in the volume of the tissue cell compartment because the administered fluid is slightly hypotonic; the measurements corroborate this prediction, indicating virtually no change in the tissue cell volume. According to the simulations for the HS case, the tissue cells shrink gradually throughout the HS infusion, reaching an apparent steady state immediately after the infusion stops. At the final steady state, the model predicts about a 10% decrease in the volume of these cells, a value which is in good agreement with the measured 8.4% decrease reported by Onarheim.

6.4.3 Comparisons with Manning and Guyton's data

Figure 6-4 shows a comparison between the simulated and experimental blood volume changes following RS infusions, according to the experimental protocol reported by Manning and Guyton [1980]. The three panels (A, B, and C) in the figure correspond to Lactated Ringer's solution infusions equivalent to 5% body weight (BW), 10% BW and 20% BW, respectively. The trends and the magnitudes of the predicted fluid volumes are in good agreement with the experimental data, except near the end of the infusion period for the 20% BW case.
Figure 6-4. Comparison of the model predictions for blood volume change vs. time with experimental data from Manning and Guyton [1980]. The lines represent the predictions of the model while the symbols indicate the experimental results for A: 5% of body weight (BW); B: 10% of BW and C: 20% of BW RS infusions.
Note, however, that the maximum measured blood expansion is essentially the same as for the 10% BW case, even though the 20% BW infusion involved the addition of twice as much fluid over a 33% shorter period. The following values were reported by the authors at 5 hours after the infusion terminated: about a 14% increase for the 5% BW infusion, a 23% increase for the 10% BW infusion and a 25% for the 20% BW infusion. The corresponding simulated percentage increases at 5 hours post-infusion are 19%, 24% and 27% for the 5%, 10% and 20% BW infusions, respectively. The simulations and the measurements both indicate an increase in blood volume during RS infusion followed by stabilization at an elevated steady-state volume within approximately 1 hour post-infusion.

Manning and Guyton [1980] provided information about plasma protein concentration changes after RS infusions. Figure 6-5 shows their experimental results along with our model predictions. For all three levels of infusion, there is always a decrease in plasma protein concentration as a result of plasma expansion. According to the simulations, the maximum decrease is achieved immediately at the end of the infusion period. After the infusion is completed, the plasma protein concentrations increase slowly toward the control value.

However, for the 5 hours post-infusion period studied, the protein concentrations remain well below the initial value. At 5 hours post-infusion, the experimental results indicate 14%, 20% and 28% decreases from the control level for the 5%, 10% and 20% BW infusions, respectively. In good agreement with the experiments, the model-predicted values at this time are 16%, 21% and 27% below control, respectively, for the corresponding infusions.

Manning and Guyton [1980] also measured total extracellular volume changes as part of their infusion experiments with dogs. According to Fig. 6-6, the experiments and simulations both show that, during RS infusions, the total extracellular volume increases proportionally with the fluid infused, for the duration of the infusion period.

However, once the infusion is terminated, a new elevated steady-state ECV level is almost immediately reached. A similar behavior was observed by Onarheim in his RS and HS infusion experiments. The post-infusion predictions are in reasonable agreement with the measured ECV values for all three of Manning and Guyton’s experiments.
Figure 6-5. Comparison of the model predictions for plasma protein concentration change vs. time with experimental data from Manning and Guyton [1980]. The lines represent the predictions of the model while the symbols indicate the experimental results for the A: 5% of BW; B: 10% of BW and C: 20% of BW RS infusions.
Figure 6-6. Comparison of the model predictions for extracellular volume change vs. time with experimental data from Manning and Guyton [1980]. The lines represent the predictions of the model while the symbols indicate the experimental results for the A: 5% of BW; B: 10% of BW and C: 20% of BW RS infusions.
6.4.4 Summary of validation study

The results presented above suggest that the model's predictions are well supported by the experimental results for hyperosmotic as well as iso-osmotic or essentially iso-osmotic solutions. The comparisons for fluid volumes and solute concentrations proved to be satisfactory for the first 1-5 hours post-infusion, and no additional volume compensatory mechanisms needed to be accounted for in the mathematical formulation. The experimental data provided by Wolf [1982] for NS and HS infusions, as well as by Manning and Guyton [1980] for RS infusions, provided information about the transient phase of plasma expansion in dogs. As shown in Figs. 6-2 and 6-4, the time-course of the predicted plasma volume changes was in good agreement with the experimental data. As well, the model provided excellent predictions of the steady-state plasma volume changes in rats measured by Onarheim for RS and HS infusions. These results provide confidence that this improved four-compartment model has the ability to provide reliable predictions of plasma expansion, even for cases where ionic resuscitants cause significant cellular volume changes.

6.5 Implications of the Model

One of the main advantages of the validated model is its ability to predict simultaneously a large number of both experimentally accessible and difficult to measure (or experimentally inaccessible) variables. This wealth of information can help contribute to a better understanding of the phenomena occurring at both the microvascular and cellular levels following resuscitation. Based on the predictions of the model (some of which were used in the previous section for model validation), the compartmental fluid and solute changes which occur after infusion of either HS or RS/NS will be discussed in more detail in this section.

6.5.1 Iso-osmotic solution (NS or RS) infusions

Infusion with iso-osmotic solutions represents the less complex of the two cases studied using the model. Since no osmotic disturbance is present at the boundary between the extra- and intracellular compartments, the cells play an essentially passive role and the vascular and interstitial compartments are the only ones which undergo significant changes in volumes and protein concentrations.
Chapter 6: Model validation based on animal studies

**Infusion period:** Corresponding to Wolf's experiments, the computed plasma volumes increase during NS infusion to about 50% above the control value, a peak which is slightly less than that predicted if all the infused fluid is retained in plasma. Based on his transient experimental data, Wolf reported a 91% vascular retention for NS when plasma expansion is at its peak. According to the simulations, which indicate a similar value of about 88%, the incomplete retention of infused fluid is due to a continuous fluid shift from the vascular to the interstitial compartment caused by an increased hydrostatic pressure and decreased colloid osmotic pressure in the vasculature. As illustrated in Fig. 6-7 for this experiment, the model predicts an increase of about 16 mmHg in the plasma hydrostatic pressure (Fig. 6-7, panel A) and a decrease of about 8 mmHg in the vascular colloid osmotic pressure (Fig. 6-7, panel B). The model also predicts (although it is not shown here) that, during NS infusion, the amount of protein in plasma remains near the control value; however, the protein concentration decreases due to plasma dilution.

Manning and Guyton [1980] similarly reported that, while the plasma protein content remained essentially constant, a decrease in plasma protein concentration occurred for each of the RS infusions they studied (see Fig. 6-5). Simulations of their experiments also demonstrate that increased hydrostatic pressures and decreased colloid osmotic pressures in plasma favor fluid filtration across the capillary barrier toward the interstitium. As illustrated in Fig. 6-8, which shows model simulations for the relative transcapillary fluid flow corresponding to Manning and Guyton's experimental conditions, fluid is shifted from the vascular to the interstitial compartment at an increasing rate throughout the infusion period. The model predicts that the fluid continues to be filtered into the interstitium more rapidly than it can be removed by the lymphatics. As shown in Fig. 6-8, the relative transcapillary flow increases much more dramatically than does the lymphatic flow. The net result is the accumulation of fluid in the interstitium. The compliance-engendered increase in the interstitial hydrostatic pressure causes slightly higher fluid and protein return rates through the lymphatics.

**Post-infusion period:** In agreement with the experimental results, the simulations for all the cases considered show a near equilibration of fluid, proteins and small solutes between the plasma and interstitium within about 0.5 to 1 hour post-infusion. When final steady states are achieved, the hydrostatic pressures are increased and the colloid osmotic pressures are decreased in both the vascular and the interstitial compartments. No experimental information was available regarding the interstitial hydrostatic and colloid osmotic pressure changes.
Figure 6-7. Model predictions performed according to the experimental protocol described by Wolf [1982] for the NS infusion period: A. hydrostatic pressure vs. time and B. colloid osmotic pressure vs. time, for the vascular compartment; and C. hydrostatic pressure vs. time and D. colloid osmotic pressure vs. time, for the interstitial compartment.
Figure 6-8. Model predictions for the relative transcapillary flow (i.e., $J_{IT}/J_{IT,NL}$) vs. time, for 5% of BW (solid line), 10% of BW (dotted line) and 20% of BW (dashed line), RS infusions. The simulations were performed corresponding to the experimental protocol described by Manning and Guyton [1980]. The upper r. h. s. corner of the graph also gives the relative lymph flow (i.e., $J_{L}/J_{L,NL}$) vs. time, corresponding to the three RS volumes infused.
The simulations for the NS infusion case, based on the experiments by Wolf [1982] and shown in Fig. 6-7 (C and D), suggest, however, an increase of about 1.5 mmHg in interstitial hydrostatic pressure and an approximately 2 mmHg decrease in the interstitial colloid osmotic pressure for the post-infusion steady-state period. The increased interstitial hydrostatic and decreased colloid osmotic pressures are, as mentioned before, a direct consequence of fluid being shifted from the vasculature.

From Fig. 6-5, which showed comparisons between computed and experimental plasma protein concentrations for Manning and Guyton's [1980] experiments, it can be observed that, following a decrease during infusion, the protein concentration returns toward its baseline value. However, both the simulations and experiments demonstrate that the protein concentrations remain 15-30% below control for the entire post-infusion period, depending on the particular experimental protocol. In all cases, the net effect of RS infusion is to produce hemodilution.

In accordance with experimental values reported by Manning and Guyton [1980], the model output shows that, at steady state, only about 10-20% of the Ringer's solution infused is retained in the vascular compartment. Similarly, based on data from Onarheim [1995], a 10% isosmotic fluid retention within the vasculature was calculated from his measurements and also predicted by the model.

6.5.2 Hyperosmotic solution (HS) infusions

For the HS infusions, according to the model predictions for both infusion conditions described; namely, short-term [Wolf, 1982], and long-term with large infusion volume [Onarheim, 1995], the plasma volume increases up to a maximum, which coincides with the end of the infusion period and accounts for far more than the volume of fluid infused. This condition is followed by a decrease in plasma volume with a significantly reduced retention of infusate at steady state. In accordance with the transient experimental data reported by Wolf [1982] and presented in Fig. 6-2, the model predicts an increase in plasma volume of about 70% at its peak, the equivalent of about three times the infused volume. Furthermore, for Onarheim's [1995] experiments involving larger volumes and longer infusion times, the simulations show a plasma volume elevation of more than two times the infused volume at the end of the infusion period. At this time, plasma osmolarity is increased by approximately 40 mOsm/l as compared to controls.
**Infusion period:** During HS infusion, at least for the two experimental conditions simulated in this work, the model suggests the following events that account for fluid and solute shifts between compartments. The highly hyperosmotic state created in plasma by the HS infusion has an initial impact on the red blood cells as well as on the transcapillary transport of small ions and fluid. The red blood cells reduce their fluid volume in order to achieve an osmotic balance with plasma. As shown in Fig. 6-9 (solid line), the simulations corresponding to Onarheim’s [1995] experimental protocol indicate that the volume of these cells is rapidly reduced by about 15% from control during the infusion. Additionally, the infused 7.5% NaCl solution creates large ionic concentration differences across the capillary wall. Measurements of plasma Na$^+$ and Cl$^-$ concentrations during the transient infusion period were not reported in any of the experimental studies. For these experiments, however, the simulation predicts increases of about 20 mmol/l and about 30 mmol/l in the plasma sodium and chloride concentrations, respectively. The hemodynamic effects of HS infusion are short-lived since the small ions leak rapidly through the highly permeable capillary wall. The model predicts that these large differences in ion concentration begin to dissipate quickly (within seconds after the beginning of infusion) by both an enhanced transcapillary transport of small ions toward the interstitium by diffusion and a fluid absorption from the interstitium due to the increased plasma osmolarity.

![Figure 6-9](image-url)

Figure 6-9. Model predictions for changes in cell volume vs. time following HS infusion. The simulations shown are for RBC (solid line) and tissue cells (dashed line), and are performed according to the experimental protocol described by Onarheim [1995].
As a consequence of both of these effects, the interstitial osmotic pressure increases and in turn has an osmotic impact on the tissue cells. As shown by the model predictions in Fig. 6-9 (dashed line), fluid is also fairly rapidly mobilized from the tissue cells into the interstitial reservoir. The simulations demonstrate that the shift of fluid from the tissue cells takes place throughout the infusion period, independent of whether the infusion is short-term [Wolf, 1982] or long-term [Onarheim, 1995]. The fluid mobilized from these cells causes an increase in the interstitial volume throughout this period. However, the interstitial fluid participates also in elevating the plasma volume, mainly by absorption to plasma (driven by increased transcapillary osmotic pressure differences) and, to a lesser extent, through an enhanced lymphatic transport (due to elevation of the interstitial hydrostatic pressure). Simulations of the transcapillary fluid flow, for both Onarheim's [1995] HS experiment, presented in Fig. 6-10(A), and Wolf's [1980] infusions, Fig. 6-10(B), show a continuous absorption of fluid into plasma from the interstitium during HS infusion. According to the simulations, the fluid absorption to plasma lasts as long as the HS solution is infused, regardless of the duration of the infusion. The progressive increase in the interstitial volume (not shown) paralleled by a continuous fluid absorption into the vasculature suggest that the two- to three-fold plasma expansion relative to the infused volume, noted at the end of the infusion period, is due to fluid recruited from the cellular compartments and mainly from the tissue cells.

Post-infusion period: Both the experimental results and the model predictions demonstrate that the redistribution of fluid and small ions between plasma, interstitium and cells is essentially complete within 30 minutes post-infusion. When the post-infusion steady state is achieved, the plasma volume remains elevated compared to its pre-infusion control value.

The experimental results for the two HS studies of Wolf [1982] and Onarheim [1995], as corroborated by the simulations, show an approximate 30% elevation in plasma volume (see Fig. 6-2 and Table 6-8) at steady state. The data and the simulations corresponding to Onarheim's [1995] experiments indicate that, when steady state is reached, the final plasma expansion is about 70% of the infused fluid volume. Clearly, for this case, the HS resuscitant results in a greater expansion of the vasculature than does an essentially isosmotic infusion which, as was mentioned earlier for Onarheim's RS experiment, results in only 10% of the infused fluid being retained in the plasma compartment.
Figure 6-10. Model predictions for the relative transcapillary fluid flow vs. time after HS infusion. The simulations are performed according to the experimental protocols described by Onarheim [1995] (panel A) and Wolf [1982] (panel B).
As shown in Fig. 6-9 for Onarheim’s experimental protocol, immediately after the HS infusion was terminated, the RBC volume increases slightly up to about 10% below control where it remains for the entire post-infusion period. These changes reflect directly the osmotic conditions in the plasma. At the final steady state, the model also predicts a 10% volume decrease for the tissue cells. Based on his experimental measurements (see Table 6-8), Onarheim reported an approximately 10% decrease in muscle cell volume. The experimental results as well as the interpretation of the model predictions presented above indicate that, following an HS infusion, the increase in the steady-state plasma volume is caused by the recruitment of cellular fluid as well as by the infused fluid.

6.6 Conclusions from Validation Studies

In a first validation, for all the cases explored, the model predictions compared well with experimental results for compartmental fluid volumes as well as small ion and protein contents. The experimental data for validation were chosen so that comparisons with several variables were possible.

An important aspect of the overall exchange process, which the model helps to clarify, is whether the increase in plasma volume that results from an HS infusion is due to fluid transfer from the cellular compartments alone, or if changes in interstitial volume are involved as well. The model simulations suggest that, during infusion with hyperosmolar NaCl solutions, all of the fluid that contributes to an increase in plasma volume is recruited from the infusate and from the cellular compartments, mainly from the tissue cells. The simulations also predict a continuous absorption of fluid into plasma throughout the HS infusion period, independent of its duration and the volume of the infusate. In agreement with other studies [Cervera and Moss, 1974; Mazonni et al., 1988; Onarheim et al., 1990; Wolf, 1982], this model predicts that once the infusion period is over, the elevated plasma volume rapidly declines such that, at the new steady state, plasma retention of the infusate is poor. The model simulations indicate that the transitory nature of plasma elevation is strictly dependent on the duration of the infusion; i.e., a longer infusion period results in a longer duration of fluid absorption into the vasculature. Among other factors, this might be one of the reasons why Onarheim et al. [1989] reported hemodynamic improvements when lower resuscitation rates and increased durations of HS infusion were used. However, more experimental data and analyses are required in order to confirm these results.
Chapter 7: MODEL VALIDATION BASED ON HUMAN STUDIES

7.1 Introduction

A further logical step in the validation of a model which yields the dynamics of mass exchange in the body would be to compare the model predictions with the experimental results obtained from studies of fluid infusion in humans. To this author's knowledge, an attempt to predict the changes associated with fluid balances in humans following different types of infusions by using a compartmental model such as the one described in Chapter 4 has never been carried out before. Particularly for humans, where the experimental and clinical studies involve a limited number of invasive measurements, a validated human model would be a welcome addition to help understand the complex interactions that exist between the various fluid compartments of the body.

The proposed purpose of the present chapter is, therefore, to provide a validation of the human model. The experiments selected for this validation exercise involved administration of two types of infusions: 1) isotonic normal saline (NS) and 2) hypertonic saline with Dextran 70 (HSD). As discussed in the previous chapter, despite the large amount of information already published about HSD infusions, they are still under intensive clinical investigation in order to meet regulatory approval for use in humans. The HSD simulations represent the greater challenge of the two since the hypertonic solution causes a significant alteration of the cellular compartments, while the added dextrans contribute, along with proteins, to the plasma and interstitial oncotic pressures. Additionally, the implications of administering HSD infusions were examined for both normal and 10% hemorrhaged subjects. As was the case in the previous chapter, data from infusions on otherwise healthy subjects (including those with a 10% hemorrhage) were chosen for validation. As an added feature, compared with the validation described in Chapter 6, the model must also be updated to account for the effects of external losses of fluid and solutes through urine.

The rest of the chapter is organized as follows. First, the minor changes required by the model in order to describe losses of blood are discussed. Then, a comparison is presented between the simulated results and measured data for two sets of human infusion experiments. The experimental data include the changes with time of plasma and interstitial fluid volumes, plasma and interstitial colloid osmotic pressures, hematocrit, plasma solute concentrations (both
Chapter 7: Model validation based on human studies

sodium and total osmolarity) and transcapillary flow rates. This validation is then followed by a brief discussion concerning the implications and applicability of the model. Specifically, the model is used to investigate mechanisms associated with the redistribution and transport of fluid and solutes administered following a mild blood removal, and to speculate on the relationship between the timing and amount of fluid resuscitation and subsequent blood volume expansion in an attempt to optimize fluid resuscitation protocols.

7.2 Model Validation for Human Studies

7.2.1 Model description

The only modifications required for the human model described in Chapter 4 are those related to the removal of whole blood from the vascular compartment. Whole blood loss implies the loss of red blood cells with their cellular contents of small ions, as well as the loss of plasma volume together with its proteins and small ion constituents. This scenario is represented schematically in Figure 7-1, where the symbol HEM signifies the rates of all the lost materials described above.

The model relies on the time-dependent values taken by the hematocrit (calculated from Eq. [4-1]) to establish, based on the known rate of whole blood loss, both the rates of plasma volume loss and red blood cell loss. All the terms that describe these rates were accounted for in the mass balance equations for the plasma and red blood cell compartments (see Eqs. [4-2], [4-4],[4-5], [4-8] and [4-9] for the plasma compartment and Eqs. [4-10], [4-11] and [4-12] for the RBC compartment).

7.2.2 Available experimental data

The validation of the model for human subjects is carried out by comparing the model predictions with measured data provided by two independent experimental studies. The first study, by Watenpaugh et al. [1992], reports measured and calculated isotonic plasma volume expansion data for normal humans, following short infusions of normal saline (NS). The data were collected as function of time up to three hours post-infusion. The large number of measured physiological variables such as plasma volume, plasma oncotic pressure, renal output, together with calculated values for the net whole-body transcapillary fluid transport and interstitial fluid volume, make this study an ideal choice for model validation.
Figure 7-1. A schematic diagram depicting the compartments and flows which comprise the model. The four compartments include the plasma and red blood cells as well as the interstitial fluid and its associated cells. J represents the flow of fluid, R represents the flow of Dextran, M represents the transport of small solutes and HEM refers to whole blood loss via hemorrhage. The subscripts are as follows: IT represents the interstitial fluid, TC represents the tissue cells, RBC denotes the red blood cells, L corresponds to the lymph, UR represents urine, ION represents all the small solutes individually and RES corresponds to resuscitation fluids. PER and ISL corresponds to perspiration and solute free insensible losses, respectively.
The second study, by Tølløfsrud et al. [1997], is more complex and involves fluid administration of hypertonic saline containing 6% Dextran 70 (HSD) to normal human subjects. This latter study reports the effects of small bolus HSD infusions on fluid and solute distribution among the different body compartments (i.e., plasma, interstitium and cells). A total of five women (averaging 62 kg) and four men (averaging 74 kg) in resting position, all healthy individuals with no history of renal dysfunction, were subjected to fluid administration. Furthermore, two distinct cases were investigated by Tølløfsrud et al. [1997]: one in which the HSD infusion was administered to normovolemic humans, and another where the same volume of HSD solution was administered to 10% (by volume) hemorrhaged volunteers.

The resuscitation protocol performed by Watenpaugh et al. [1992] is presented in Table 7-1. Stated briefly, this protocol consists of an intravenous infusion of about 30 ml/kg of NS solution; specifically a rate of 100 ml/min was administered for 24 minutes. The average inputs to and outputs from the subjects are shown in Table 7-1; i.e., inputs in the form of infused fluid and solutes, and outputs such as urine as well as other losses such as sensible (perspiration) and insensible (e.g., respiration) losses. All of these quantities were measured as part of the experimental data except for the sensible and insensible fluid losses that were estimated by Watenpaugh et al. to be around 46 ml/h*. Of this, for lack of better data, the value of about 0.03 ml/(kg-h) reported in the literature [e.g., Altman and Dittmer, 1971; Krieger and Sherrard, 1991] for a normal 70 kg human, was considered to be a reasonable approximation for the perspiration rate. The normal concentrations of small ions eliminated via perspiration are also given in Table 7-1. The distribution of solutes between the compartments under study was recalculated to account for compartmental small ion values that are more descriptive of humans [Tølløfsrud et al., 1997] and is presented in Appendix D.

In order to accommodate the different body weights of the volunteers involved in these two experimental studies, the coefficients and properties of the model fluid compartments were scaled to the average weight and height of the individuals in each test. The weight and height of the volunteers in Watenpaugh’s study averaged 76.9 kg and 177 cm, respectively.

* Note that the sensible/insensible losses estimated by these authors are the same on a per kg basis as those of a normal human, i.e., ~ 0.6 ml/(kg-h).
| INPUTS | OUTPUTS |
|-----------------|-----------------|-----------------|-----------------|
| Infusion Fluid: NS | Urine | Perspiration | Insensible Losses |
| **Volume Infused at t = 0.6 h** | **Duration of Infusion** | **Composition** | **Duration of Collection** | **Average Rate** | **Average Composition** | **Rate and Composition** | **Rate** |
| 30 ml/kg | 0.4 h | Na⁺: 150 mEq/l | 3.4 h | 3.5 ml/(kg-h) | Na_U⁺: 114 mEq/l | 0.03 ml/(kg-h) | Na_PER⁺: 45 mEq/l | 0.57 ml/(kg-h) |
| | | Cl⁻: 150 mEq/l | | | K_U⁺: 50 mEq/l | | K_PER⁺: 4 mEq/l | |
| | | | | | Cl_U⁺: 110 mEq/l | | Cl_PER⁺: 49 mEq/l | |
| | | | | | A_U⁺: 70 mEq/l | | | |
| | | | | | C_U⁺²: 16 mEq/l | | | |

Table 7-1. Inputs and outputs used for the model infusions of NS solution in human subjects. The data correspond to the seven healthy subjects used in the experiment of Watenpaugh et al. [1992]. Time, t = 0.6 h, indicates the start of infusion which lasts up to 1 h. All outputs from the model are considered from the start of infusion and up to the end of experiment, i.e., from t = 0.6 h to t = 4 h.
Experimental procedure of Tøløfsrud et al.

The experimental protocols for the two separate but related studies carried out by Tøløfsrud et al. [1997] are presented in Tables 7-2 and 7-3. According to the experimental procedure of the first study reported by these authors, 4 ml/kg of HSD solution (75 mg/ml) containing 60 mg/ml of Dextran 70 was infused into the human subjects for 10 minutes. Data were taken for these individuals for up to 3 hours post-infusion and then averaged. This first study will be referred to as the "normovolemic case", NV.

A week later, the same individuals served as their own controls for a second study, in which a mild hemorrhage of 10% of their calculated blood volume was removed over a 15 minute period. After a 90 minute stabilization period, an identical HSD infusion protocol as for the NV case was applied. This latter study will be referred to as the "hypovolemic case", HV.

The infused fluids, with the volumes and compositions indicated in Tables 7-2 and 7-3, were treated as inputs to the model. The urinary outputs shown in the table are reported by Tøløfsrud et al. [1997] in their original study, while the urinary concentrations of small ions were supplied by one of the original researchers [Tøløfsrud, personal communication]. Due to a lack of better information, the rates for fluid and ion losses via perspiration/respiration are considered to be unchanged from those typically found in 70 kg normal healthy individuals [Altman and Dittmer, 1971; Krieger and Sherrard, 1991]. That is, in agreement with an observation made by one of the authors of the study [Tøløfsrud, personal communication], for the short duration of these experiments, the loss of fluid by this route was not significantly different from that of normal humans. Hence, the perspiration/respiration loss is unaffected by either the HSD infusion or hemorrhage.

7.3 Comparison of Model Predictions with Experimental Data

In the work of Watenpaugh et al. [1992], experimental data were obtained for the dynamic behavior of the plasma volume, $V_{PL}$, hematocrit, Hct, plasma colloid osmotic pressure, $\pi_{p,PL}$, net transcapillary fluid transport, $J_{IT}$, and interstitial fluid volume, $V_{IT}$. With the exception of the hematocrit, the model-predicted and measured variables were compared based on their relative changes from the normal steady-state conditions at $t = 0$, as shown in the five panels of Figure 7-2. The data and their errors (where available) are shown individually along with a solid line which represents the continuous predictions of the model. For the Hct and net transcapillary fluid transport the standard errors of the mean ($\pm$ SEM) are taken from the original study; while
### NORMOVOLUMIC CASE:

<table>
<thead>
<tr>
<th>INPUTS</th>
<th>OUTPUTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion Fluid: HSD</td>
<td>Urine</td>
</tr>
<tr>
<td>Volume Infused at t = 0 h</td>
<td>Duration of Infusion</td>
</tr>
<tr>
<td>4 ml/kg</td>
<td>0.2 h</td>
</tr>
<tr>
<td>Cl⁻: 1200 mEq/l</td>
<td></td>
</tr>
<tr>
<td>Dex⁷₀: 60 g/l</td>
<td></td>
</tr>
<tr>
<td>(1-3) h</td>
<td>3.3 ml/(kg-h)</td>
</tr>
</tbody>
</table>

Table 7-2. Inputs and outputs used for the model infusions of HSD solution in human subjects. The data correspond to the normovolemic case, NV, reported by Tølløfsrud et al. [1997]. Time, t = 0 h, indicates the start of the infusion.
### HYPOVOLEMIC CASE:

<table>
<thead>
<tr>
<th>Hemorrhage at 1.5 h prior to infusion</th>
<th>INPUTS</th>
<th>OUTPUTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion Fluid: HSD</td>
<td>Volume Infused at t = 0 h</td>
<td>Duration of Infusion</td>
</tr>
<tr>
<td>Duration: 0.25 h</td>
<td>4 ml/kg</td>
<td>0.2 h</td>
</tr>
<tr>
<td>Degree: 10% BV₉₀</td>
<td>(1-3) h</td>
<td>2 ml/(kg-h)</td>
</tr>
</tbody>
</table>

Table 7-3. Inputs and outputs used for the model infusions of HSD solution in human subjects. The data correspond to the hypovolemic case, HV, reported by Tølløfsrud et al. [1997]. Time, t = 0 h, indicates the start of the infusion.
Figure 7-2. The five panels in this figure compare experimental data from Watenpaugh et al. [1992] with predictions of the model for changes in plasma volume ($V_{pl}$), (panel A), colloid osmotic pressure of plasma ($\pi_{pl}$), (panel B), and interstitial fluid volume ($V_I$), (panel E). In addition, comparisons of the hematocrit (Hct) (panel D), and net transcapillary fluid flow (panel C) are also shown. All experimental data are shown as individual points (with the standard errors included, where appropriate). The simulation predictions for the variables are given as continuous solid lines. Control values are those between $t = 0$ to 0.6 h and the infusion of normal saline, NS occurs during the times between the arrows shown on the time axis. The total duration of the experiment is about 4 hours.
for $V_{PL}$ and $\pi_{P,PL}$, a conservative estimate of the standard error of the difference of the means was evaluated (i.e., calculated assuming statistical independence between consecutive average means and by treating the average measurement and its error at each time during the course of the experiment as representative of a distinct population) [Mandel, 1984; Altman, 1991].

For changes in $V_{PL}$, $\pi_{P,PL}$, and $V_{IT}$ as well as for Hct and $J_{IT}$, the simulated values follow the experimental results closely. In the majority of cases, the simulations fall within (or are very close to) the standard errors of the mean or the errors in the difference of means as shown. An exception occurs for the net capillary fluid transport data point at $t = 1$ h. At this time, however, one can expect dramatic changes to occur in this quantity. Furthermore, this is not a measured variable; rather, it was calculated by Watenpaugh et al. [1992] based on other measured parameters. Nonetheless, the relative changes in this variable are well characterized by the model-simulated results at all subsequent times.

The trends predicted by the model corroborate what is expected clinically following an NS fluid infusion. The saline solution increases the plasma volume throughout the duration of the infusion (panel A). This volume increase is paralleled by hemodilution as reflected by both the changes in plasma colloid osmotic pressure (panel B) and the hematocrit (panel D). Immediately after the infusate is administered, there is increase in the transcapillary fluid flow with a direct consequence of interstitial fluid accumulation (panels C and D). As soon as the infusion terminates, plasma fluid quickly begins to dissipate into the neighboring interstitium.

The study by Tøllofsrud et al. [1997] provided an additional source of data for validation of the model predictions. They measured changes in various vascular and interstitial compartment variables following HSD infusion, for a post-infusion period of up to 3 hours. A comparison between their results and the model predictions for the NV case is given in panels A to D of Fig. 7-3. This figure shows the time-dependent changes (compared with control) in blood volume (BV), plasma and interstitial colloid osmotic pressures ($\Pi_{PL}$ and $\Pi_{IT}$, respectively), and the Na$^+$ concentration in plasma ($Na_{PL}$). The capital letter, $\Pi$, (see panels B and C of Fig. 7-3) indicates the total colloid osmotic pressure due to the contributions of proteins and dextran (see Eq. [4-41]). The data in these figures are shown as mean values of differences, along with the estimates of the standard errors of differences of the means.

As can be seen from Fig. 7-3, the agreement between the data and the model predictions is good for all the variables considered. The model has predicted especially well the changes in plasma sodium concentration (panel D) and, as shown further in Table 7-4, in plasma osmolarity.
Figure 7-3. The four panels, A through D, presented in this figure compare model predictions with data from Tøllofsrud et al. [1997]. In the text, this data set is referred to as the NV case. The data are shown as individual points (with standard error of measurement), while the model predictions are given as continuous solid lines. Control values are those at time = 0 h and the infusion of hypertonic saline with Dextran (HSD) occurs during the times between the arrows shown on the time axis.
The predictions for the behavior of the Na\(^+\) concentration in plasma show that this important variable related to transcapillary exchange reached a reasonably stable value within 15 minutes following the infusion.

Since the original estimation of the parameters associated with transcapillary transport of small ions was based on animal studies (see Section 6.3), it was of interest to see whether potential interspecies differences might affect the model predictions with respect to the transport of small ions. The good predictions for the changes in human sodium and osmolarity values offer some reassurance that these parameters are essentially the same for all species, and encourage further application of the model to other human studies.

<table>
<thead>
<tr>
<th>Case of study</th>
<th>Change in Plasma Osmolarity (mOsm/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Normovolemic</td>
<td>Experiment</td>
</tr>
<tr>
<td></td>
<td>Model</td>
</tr>
<tr>
<td>Hypovolemic</td>
<td>Experiment</td>
</tr>
<tr>
<td></td>
<td>Model</td>
</tr>
</tbody>
</table>

Table 7-4: Comparison between the model predictions and experimental results for the changes in plasma osmolarity at the start of the protocol (i.e., 2 h before infusion), immediately prior to fluid administration (\( t = 0 \) h) and at 1 h post-infusion. The experimental results are from Tøløfsrud et al. [1997].

Figure 7-4 shows the results obtained for the case of the hypovolemic study by Tøløfsrud et al. [1997]. The same variables as in Fig. 7-3 are chosen to exemplify the comparison between the data and predictions for the HV case. The fit of the model to the data is generally good for this case, as well.

As for the NV case, it can be observed that the measured blood volume changes during most of the post-infusion period are somewhat 'flatter' than the model predictions. However, the absolute values of the differences between the data and model predictions are extremely small. Similarly, the model-predicted changes in plasma colloid osmotic pressure are somewhat lower.
Figure 7-4. This figure, which contains data or simulations of the work by Tøløfsrud et al. [1997], is similar to Fig. 7-3 except that during the time -1.50 to -1.25 hours, 10% of the blood volume of the subjects is withdrawn. This is shown to occur in the figure between the arrows on the time axis labeled 10% HEM. This data set is referred to in the text as the "hypovolemic" or HV condition.
than the measured data, but the differences are generally of the order of 2 mmHg and the model matches the trend reasonably well. The plasma Na\(^+\) concentration is once again well predicted by the model.

The good comparison between model predictions and experimental data, as shown for different conditions and for different variables in Figs. 7-2 to 7-4, presents a good case for validation of the human model.

### 7.4 Implications of the Model

A more detailed interpretation of the mechanisms associated with fluid infusion will be given in this section, based either on the results already presented in the validation section or on additional predictions of the model. The discussion is largely concerned with the study of Tølløfsrud et al. [1997], since this work is the more complex and interesting of the two human studies considered in this chapter.

Figures 7-3 and 7-4 show that, in both the NV and HV cases, there is a rapid expansion of blood volume throughout the HSD administration, leading to a maximal value at the end of the infusion period. Recall that the same volume of HSD, 280 ml, was infused at the same rate for both the NV and HV cases. It is therefore not unexpected that, as can be seen from panels A in Figs. 7-3 and 7-4, the maximal expansion of blood volume in both cases is similar, i.e., approximately 900 ml from the start of the infusion. Thereafter, again for both cases, the blood volume decreases to a relatively constant value by 2 hours post-infusion. At this time, however, the HSD infusion has a more significant absolute effect on plasma volume expansion in the hypovolemic than in the normovolemic subjects. Based on the model predictions at 3 hours after the start of the infusion (i.e., end of the experiment), the blood volume expansion is approximately 350 ml in the hypovolemic subjects, as opposed to about 200 ml in the normovolemic ones. The result predicted by the model is consistent with the conclusion drawn in the original paper by Tølløfsrud et al. [1997]. Therefore, the dynamic fluid and solute exchanges that occur after blood removal prior to HSD infusion, as well as those following it, deserve further discussion.

Panel A of Fig. 7-4, which shows the model blood volume changes for the hypovolemic study, demonstrates that, immediately after a 10% hemorrhage, the blood volume is slowly restituted for the entire 90 minutes that precede the actual infusion. That is, after about 530 ml of the blood has been removed, there is an expansion (autotransfusion) in the vasculature of about
130 ml of fluid derived from the neighboring compartments. According to the model predictions shown in Fig. 7-5, the source of this fluid that compensates the hypovolemic insult is the interstitium.

As can be seen in Fig. 7-5, which depicts the volume changes in the interstitial and cellular compartments, the interstitial volume starts to decrease shortly after the start of blood removal. This decrease continues for about 45 minutes post-hemorrhage and then reaches a stabilized plateau. Thus, the simulations corroborate Tølløfsrud's [1997] correct assessment that a 90-minute stabilization period is required for human subjects prior to administration of the hypertonic infusate. Figure 7-5 also shows that, between the end of the hemorrhage and the start of the HSD infusion, the interstitial fluid volume decreased by about 130 ml, corresponding to the net amount of fluid transferred into the circulation during the same period. The model predictions also indicate that, except for the loss of RBCs during the hemorrhage, the interstitial and red blood cell volumes do not change during the entire pre-infusion period.

![Figure 7-5](image-url)  
Figure 7-5. Model predictions for the hypovolemic condition described in Tølløfsrud et al. [1997] for interstitial fluid volume ($V_{IT}$), red blood cell volume ($V_{RBC}$) and tissue cell volume ($V_{TC}$). The periods of hemorrhage and infusion are the same as in Fig. 7-4.
After the HSD infusion, in agreement with the discussion offered by Tøløfsrud et al. [1997], the model shows that the volumetric expansion of either the blood (Fig. 7-4) or interstitial (Fig. 7-5) volume exceeds the volume infused. This condition requires that some of the extracellular fluid must also be derived from intracellular sources, which include both the red blood cell and tissue cell volumes. The intracellular water mobilization is due to the high concentration of salt in the infusate (2400 mOsm/l HS) and is similar to the effect observed in Onarheim's experiments (Fig. 6-9), where only an HS infusion was simulated. As shown in Fig. 7-5, at the end of the experiment, the total interstitial tissue cell volume ($V_{TC}$) has decreased by about 1200 ml, while the interstitial fluid volume ($V_{IT}$) has increased by about 400 ml. The relative changes in red blood cell volume are consistent with those predicted for tissue cells. The RBC volume decreases over the entire infusion period and reaches its local minimum at the end of the infusion. The red blood cells and tissue cells have similar time-dependent behaviors; however, since the vascular compartment is the direct recipient of the infusion, the RBCs will be the first to undergo changes. After the HSD infusion, while $V_{TC}$ continues to decrease for a few minutes, $V_{RBC}$ increases for about the same period of time. This period corresponds approximately to the time required for small ions to equilibrate across the capillary and cell membranes. This behavior can be very closely correlated with the dynamic adjustment of the Na\textsuperscript{+} concentration in plasma (panel D in Fig. 7-4).

After about 0.5 hours post-infusion, the red blood cell volume remains essentially constant over the remainder of the 3 hour experimentation interval. Overall, there is a predicted small decrease in $V_{RBC}$ of less than 100 ml (i.e. <5%) between the pre-infusion value and that at 1 hour after the start of infusion. This is consistent with the increased osmolarity of plasma between the pre-infusion and post-infusion periods. The dynamic behavior of this decrease is also consistent with the dynamic behavior of plasma sodium (and implicitly, plasma osmolarity). Contrary to the model predictions, Tøløfsrud et al. [1997] did not find a statistically different MCV (mean corpuscular volume) for these times. The difference in results might be due to their measurement errors (which are also of the order of 5% for mean corpuscular volumes).

Based on interstitial compliance measurements, Tøløfsrud et al. [1997] reported that, by 1.5 hours post-infusion, the total extracellular volume (i.e., plasma together with interstitium) was expanded. This is in agreement with the simulations shown in Fig. 7-5, which predict that the interstitial fluid volume remains expanded for the entire post-infusion period. When
compared to its pre-infusion value (see Fig. 7-4), the blood volume also remains expanded during this period.

The model also predicts that during the entire experiment (i.e., up to three hours post infusion), essentially all of the dextran infused remains within the circulation. Therefore, the conjecture by Tølløfsrud et al. [1997] that plasma volume expansion can be increased through the use of more dextran, at least for the times investigated and likely also for longer times, seems reasonable.

The ability of the model to evaluate the transcapillary fluid flow ($J_{IT}$) yields further useful insights about the fluid dynamics of the system. This important variable, that reflects the balance of Starling forces across the capillary wall, can only be, at best, roughly estimated from other experimental measurements. A negative value of $J_{IT}$ implies vascular absorption, while a positive value corresponds to filtration. Before blood removal, the system is at steady state and, hence, the filtration rate is exactly balanced by lymph flow. As shown in Fig. 7-6, following the onset of hemorrhage, the model predicts that $J_{IT}$ decreases and changes from filtration to absorption as blood is withdrawn. The absorption then continues for the entire duration of the blood removal. Following termination of blood withdrawal, as forces within the system readjust, $J_{IT}$ reaches its new steady-state value. In agreement with the previous discussion regarding the changes in interstitial volume and the time required by this compartment to reach a new steady state, absorption changes back to filtration at about 45 minutes post-hemorrhage (i.e., at $t \sim -0.75$ h in Fig. 7-6).

The onset of HSD infusion has the highest impact on transcapillary fluid absorption from the interstitium. This effect is the result of an increased osmolarity in plasma due to the infusion. After the infusion period is over, the rate of absorption decreases and, once again, concomitant with the equalization of Na$^+$ concentration in plasma and interstitium, filtration from plasma to the interstitium is established within a few minutes. This filtration flow is driven primarily by a hydrostatic pressure increase inside the capillaries (as a result of increased blood volume). Filtration then continues at an increasingly lower rate until the end of the simulated period. About 2 hours after the start of the infusion, the filtration flow rate ($J_{IT}$) approaches very low values. The predictions for lymph flow ($J_L$) are also shown in Fig. 7-6.
Figure 7-6. Model predictions for the hypovolemic condition described in Tølløfsrud et al. [1997] for transcapillary fluid exchange ($J_{IT}$) and lymph flow ($J_{IL}$). The periods of hemorrhage and infusion are the same as in Fig. 7-4. Based on mass balances in plasma, $J_{IT} - J_{IL}$ is equivalent to the net intravascular fluid shift as discussed by Tølløfsrud et al.

According to the plasma fluid mass balance, capillary filtration minus lymph flow for a specified time interval is equal to the volume infused in that time interval ($V_{RES}$) minus the urinary output ($\Delta Ur$) and the change in plasma volume ($\Delta V_{PL}$); that is, the net vascular fluid shift can be defined as follows:

$$ (J_{IT} - J_{IL})_{at} = \frac{V_{RES} - \Delta Ur - \Delta V_{PL}}{\Delta t} $$

[7-1]

According to the model, the term ($J_{IT} - J_{IL}$), that becomes negative during hemorrhage and even more so during the infusion, will return to positive values within minutes after the termination of the infusion. Eventually, this quantity declines to negative values at about 1 h post-infusion. This
prediction is in contrast with the calculations of transcapillary fluid shifts reported by Tøløfsrud et al. [1997]. In their work, the net vascular fluid shift is calculated from Eq. [7-1] with the exception that the term ΔV_{PL} is replaced by BV; i.e., the study assumes that there are no changes in the volume of red blood cells. They also reported continuously negative values for (J_{IT} - J_{L}) over the entire period following the infusion. It is not clear if these negative values are statistically different from zero or even positive values. Nonetheless, Tøløfsrud et al. [1997], when discussing net vascular fluid shifts, state that they did not find the rapid leakage of fluid back into the extravascular space as reported by Velasco et al. [1989] and as predicted by the model. A direct comparison between the two studies is not possible due to differences in experimental protocol, but it would appear that more experimentation would be desirable to eliminate this controversy. All the trends described by our model for plasma volume expansion are consistent with the findings of Velasco et al. [1989], in that it predicts a rapid expansion after infusion followed by a significant decline in this volume over the next 3 hours. It might very well be that the calculated values for the net intravascular fluid shifts in Tøløfsrud's work contain significant errors associated with experimental determination of blood volume changes. At least two possible explanations can be given for these errors: firstly, the changes in blood volume were calculated based on the variations in hemoglobin concentrations and, secondly, these authors chose a relatively large time interval for averaging blood volume changes. Based on their published report, this interval can be estimated to be somewhere between 10-20 minutes. As a result, the possibility of positive values of net transcapillary fluid shifts for some periods following the infusion may be obscured.

From the analysis of the model predictions for the particular hypovolemic case studied by Tøløfsrud et al. [1997], one can gain insight into the mechanisms associated with the distribution and transport of fluid, solutes and macromolecules following infusion of HSD. In general, the model corroborated many but not all of the potential mechanisms associated with the behavior of the system as discussed in the original work.

### 7.5 Application of the Model to Fluid Resuscitation

The validated model can be used to analyze the dynamic behavior of the system under investigation for different resuscitation protocols. For example, it would be useful to determine the effect of changing the rate of HSD infusion on blood volume dynamics. Clinically, these
experiments would require not only a considerable amount of time but also a large number of volunteers willing to subject themselves to lengthy procedures.

The predicted changes in blood volume for the infusion of 280 ml HSD over different time periods for a 70 kg reference man are shown Fig. 7-7. Following a 10% hemorrhage for 15 minutes and a 1.5-hour stabilization period, HSD infusions were carried out for either 12 minutes (Tøllofsrud et al.'s [1997] original protocol), 1 hour or 2 hours. For all cases, the urinary output was taken to be the same as that for the original hypovolemic case presented in Table 7-2.

For the cases where resuscitation was carried out over a 1 or 2 hour period, there is no relatively large overshoot of the normal blood volume as in the case of the 12-minute infusion. As expected, longer resuscitation times, utilizing the same fluid volume, result in smaller fluctuations in blood volume. In all cases, about the same steady value of blood volume is achieved by about 3 hours post-infusion.

Based on this hypothetical scenario, the predictions of the model suggest a reasonable protocol to return and maintain blood volume near its normal value following hemorrhage, when an HSD infusion is the therapy of choice. In designing such a protocol, one has to keep in mind the important objectives to be achieved by therapy when a patient is in a hypovolemic state.

The immediate short-term goal is to restore the blood deficit as soon as possible to near normal values and, thus, to improve promptly all the hemodynamic variables without overloading the heart. The long-term goal is to maintain the blood at a normal volume. Based on this description, instead of administering a single HSD bolus, several successive infusions at different rates can be employed as follows. A short, low-volume HSD infusion would restore the blood volume rapidly. This infusion could be followed by one or more slower infusions over longer periods in order to maintain the blood volume at or near its normal level. Although, as shown in Fig. 7-7, after all of the HSD is dispensed, the blood volume eventually returns to about the same level, there will be an overall improvement in hemodynamic maintenance.

It should also be pointed out that the blood volumes shown in Fig. 7-7 may very well be underestimated for the two longer infusion rates. According to the literature, it is reasonable to expect that blood volume is one of the determinants of urine production [Linko and Makelainen, 1988; Aukland, 1989]. The model predictions presented in Fig. 7-7 assumed the high urinary output rates obtained by Tøllofsrud et al. [1997] for a 12-minute infusion. It would be expected that a more moderate fluid and salt overload on the cardiovascular system would result in lower urinary output.
The above is just a single example demonstrating how a validated model might be used to optimize fluid administration protocols to achieve a specific physiological endpoint. An alternative approach might be to manipulate both the amount and the rate of the HSD infusion in order to maintain the blood volume at its normal value for a maximum period of time without the rapid excursion caused by the initial administration of fluid. In this manner, model-based information can be used to develop testable hypotheses and/or to assess, based on physiological endpoints, which fluid infusion protocol is likely to have the optimal outcome.
7.6 Conclusions from Validation Studies

Even though a variety of human data were compared with model predictions (i.e., volumes, solute concentrations, colloid osmotic pressures, and transcapillary transport rates), the model was able to provide a good description of almost all of this experimental and/or calculated information. This fact is encouraging, since some of the descriptive parameters needed to characterize the model were obtained from independent studies on species other than humans. Confidence in the model is further bolstered by its ability to make very reasonable predictions when new and different types of fluid inputs (such as HSD) and outputs (such as urine with varied composition of small ions) are considered.

However, it would be inappropriate to claim that the model, as it stands, would be suitable for describing more severe pathophysiological cases such as, for example, those occurring when significant amounts of blood are lost. The physiological mechanisms and succession of events encountered in severe hemorrhage are fairly complex and some of them have not yet been elucidated. A possible application of this model to the study of hemorrhage will be presented in Chapter 9. However, it is expected that the model will need to undergo significant modification in order for it to describe this traumatic condition. The use of Tølløfsrud et al.'s [1997] HV study as part of the validation of the present human model was only possible because 1) only a small fraction of the blood volume was removed from the subjects (such a mild hemorrhage is known not to produce major disturbances) and 2) the subjects were young, healthy, hydrated individuals, who had not suffered any real previous traumas.
Chapter 8: MODEL OF THE RENAL FUNCTION

8.1 Introduction

The cells in the human body are responsible for a variety of physiological functions that depend on the constancy of both the intracellular and extracellular environments. The urinary system, which is involved in highly complex excretory functions, is one of the systems responsible for assuring the constancy (homeostasis) of the extracellular fluid.

Specifically, the homeostatic roles of the kidney are as follows [see e.g., Brenner and Rector, 1996]:

- maintenance of fluid balance and plasma volume (an important determinant of blood pressure)
- regulation of blood osmolarity
- maintenance of electrolyte balance within the extracellular fluid
- regulation of blood pH
- excretion of wastes, such as creatinine, urea, etc., as well as of excesses of fluid and all small ions
- production of hormones (e.g., erythropoietin, renin, etc.).

To take a more simplistic view, however, the basic function of the kidney is to excrete excess amounts of solutes and water from the extracellular environment. The kidney model developed in this chapter will take this primary function into account. Furthermore, it will be considered that, as a regulatory system, the kidney responds via a negative feedback to any changes in the plasma environment such that this environment will be brought back to normal. That is, an increase (or decrease) in either plasma volume and/or plasma osmolarity from a normal 'set-point' will cause a proportional increase (or decrease) of the urinary output and/or solute excretion from their normal values.

8.2 Some Aspects of Kidney Anatomy and Physiology

The major renal components of the kidney are the parenchyma and the collecting system [e.g., Krieger and Sherrard, 1991; Guyton, 1991]. The parenchyma filters plasma and modifies the filtrate. This renal structure is composed of an outer portion, the cortex, and an inner portion,
the medulla. The collecting system has the main function of transporting urine and is comprised of the calix, the pelvis and the ureter.

The present work proposes to establish relationships between the urinary fluid and solute excretion rates and the properties of the plasma and its dissolved small solutes. Therefore, it is mainly the physiology associated with filtration and the modification of filtrate, i.e., the processes that occur in the parenchyma, which will be outlined further.

8.2.1 The nephron

The filtration and modification of plasma filtrate by reabsorption or secretion occurs in the nephrons. Each kidney contains about 1.5 to 2 million nephrons and each of them has the ability to form urine independently [Guyton, 1991; Greger, 1996]. Two main types of nephrons can be identified in humans, i.e., the cortical and the juxtamedullary nephrons. Their characteristics are dependent on their location within the kidney mass. The anatomical organization of the two different types of nephrons is illustrated in Fig. 8-1. The cortical nephrons are situated close to the outer surface of the kidney, in the cortical area, and produce dilute urine [Brenner and Rector, 1996; Guyton, 1991]. About one third of the nephrons, called juxtamedullary nephrons, are situated deep in the renal mass and produce mainly concentrated urine [Brenner and Rector, 1996; Guyton, 1991].

A schematic of a functional nephron and its components is depicted in Fig. 8-2. As can be seen from this figure, the nephron is composed of four main sections [e.g., Krieger and Sherrard, 1991]: 1. the renal corpuscle, 2. the proximal convoluted tubule, 3. the loop of Henle and 4. the distal convoluted tubule.

The essentially protein-free filtrate is collected in the Bowman's capsule and then flows into the proximal tubule. Both the renal corpuscle and the proximal tubule are situated in the renal cortex. From the proximal tubule, the filtrate descends toward the medulla into the loop of Henle. The loop of Henle is composed of two limbs; one carrying the filtered plasma from the cortex to the medullary portion of the loop of Henle, i.e., the descending limb, and one bringing back the filtrate into the cortex, i.e., the ascending limb. Each limb is divided in turn into two segments. Toward the outer part of the medulla, the walls of the limbs are thick; usually they are simply called thick descending and thick ascending limbs, respectively. In the inner medullary zone of the kidney, the walls of the tubules are very thin, and thus are called thin descending and ascending limbs, respectively. After exiting the loop of Henle, the filtrate passes through the
Figure 8-1: Differences between a cortical nephron situated in the upper portion of the kidney and a juxtamedullary nephron nestled deep in the renal mass, close to the outer medullary area. From Guyton [1991].

Figure 8-2: Schematic of a functional nephron indicating the renal corpuscle composed of the glomerulus and Bowman's capsule and a series of tubules (starting on the right hand side of the renal capsule) that transport and modify the filtrate. Starting in the proximal tubule (thick segment), the filtrate descends into the loop of Henle (thin segment) and then ascends once again into the distal tubule (thick segment). The left side of this figure illustrates the renal peritubular capillary system with the vasa recta (for more discussion see text, Section 8.2.2) From Guyton [1991]
distal tubule into the collecting tubule. Both of these tubules are situated in the cortex.

The initial filtrate resulting from the passage of blood through the glomerulus has a composition similar to that of plasma with the main exception being that the plasma proteins are highly restricted in their passage through the glomerular filter. However, throughout the tubules, at each individual segmental level, the filtrate undergoes continuous modification, i.e., the solutes and fluid are continuously either reabsorbed or secreted* by both active and passive processes occurring across the cellular walls of the tubules. Thus, at the exit from the final collecting tubule, the urine formed has a totally different composition from that of the initial filtrate.

From the collecting tubule, the urine passes once again from the cortex downward into the medullar collecting ducts. Several collecting ducts merge to form increasingly larger ducts that empty the final urine into the renal pelvis. Each of the estimated 250 of these large collecting ducts carries the urine produced by about 4000 nephrons [Guyton, 1991].

About 99% of the fluid in the glomerular filtrate is reabsorbed in plasma during its passage through the tubules. It is the remaining approximately 1% of the filtered fluid that undergoes changes to form urine. A quantitative analysis of the compositions of the filtrate and excreted urine that is relevant to this work will be provided in the next section.

8.2.2 Nephron capillary system

The capillary bed associated with the nephron has specific characteristics, different from those of the muscle and skin tissues described in previous chapters (see Chapter 3). Therefore, a brief description of this particular capillary network will be presented in this section.

There are two capillary beds associated with the nephron [e.g., Guyton, 1991; Brenner and Rector, 1996]: the glomerulus, described previously as part of the anatomical structure of the nephron, and the peritubular capillaries (see also Fig. 8-2).

As previously mentioned, the blood from an afferent arteriole is filtered across the glomerulus and exits through an efferent arteriole. The efferent arterioles constitute a capillary segment which offers a high resistance to flow; therefore, this capillary bed is also called the high pressure bed of the nephron [Guyton, 1991]. This is the site in the nephron where

* In renal physiology the term reabsorption refers to the transport of fluid and solutes from the tubules into the plasma; the term secretion refers to the secretion of solutes into the tubules from the plasma (or the surrounding interstitium).
continuous transcapillary filtration takes place. In many ways this bed resembles the arterial end of other tissue capillaries within the body (e.g., such as those of skeletal muscles). In normal humans, the total surface area of the glomerular capillary bed is estimated to be about 1.6 m$^2$ [Krieger and Sherrard, 1991; Greger, 1996]. A fraction of about 2 to 3% of this area is available for glomerular filtration [Guyton, 1991].

After exiting the glomerulus through the efferent arteriole, the blood flows into the peritubular capillaries. The peritubular capillary bed, also called the low pressure bed, of the nephron functions somewhat similarly to the venous end of other tissue capillaries, i.e., it allows for a continuous absorption of fluid back into the plasma. This capillary network surrounds the entire tubular system of the nephron, and follows the same winding route as the tubules of the nephron through the interstitium, i.e., from cortex down to medulla and back in the cortex [Guyton, 1991] (see also Fig. 8-2). The branching loops of the capillaries, which are associated with (and parallel to) the loop of Henle, are called the vasa recta. Although only about 1 to 2% of the renal blood flow, RBF, flows through the vasa recta, this segment is important in the formation of concentrated urine [Krieger and Sherrard, 1991]. The blood that flows from the peritubular capillaries then empties into what is known as the cortical veins.

It is important to mention at this point that, unlike the previous description of the microvasculature of the skeletal muscle tissue of the body, the segmental arteries, which supply the blood to the afferent arteriole to the kidney, are end arteries. This implies that blockage (or long blood stoppage) of a segmental artery will lead to ischemia and death of the particular vascular segment that follows it [Guyton, 1991]. Such a scenario arises in the present work in relation to the vasoconstriction produced by an external loss of blood.

### 8.3 Quantitative Analysis of the Kidney Function

The important objectives of this chapter are to develop and then validate a model of the kidney which will provide a quantitative, clinically acceptable description of the renal outputs of fluid and solutes. This section therefore summarizes the following aspects of the kidney function:

a) the mechanisms of urine formation,

b) the equations used to assess the renal output in clinical settings, and

c) the normal physiological excretion values for fluid and some of the main solutes considered in this work, that correspond to a well-hydrated, 70 kg "Reference man" whose kidney function is normal.
8.3.1 Urine formation

To summarize briefly the previous discussion, urine is produced in the kidney by three major successive mechanisms: 1. glomerular filtration, 2. tubular reabsorption and, 3. tubular secretion. Based on the physiological background presented in the preceding sections, a simplified schematic diagram that depicts these three mechanisms is shown in Fig. 8-3.

![Diagram of urine formation](image)

Figure 8-3. The three main mechanisms of urine formation consist of (see thick arrows and their corresponding numbers): (1) filtration across the glomerulus, (2) reabsorption from tubules, and (3) secretion within the renal tubules. Modified from Krieger and Sherrard [1991].

(1) Glomerular Filtration

The glomerulus of a normal, well-hydrated 70 kg human filters an average of 180 l of plasma per day, or the equivalent normal average *glomerular filtration rate* (GFR) of 125 ml/min [e.g., Brenner and Rector, 1996; Krieger and Sherrard, 1991; Guyton, 1991]. In humans, this filtration is brought about by a *renal blood flow* (RBF) of about 1200 ml/min (i.e., about 20% of the total cardiac output). Correspondingly, for a normal average systemic Hct of about 42%, a
normal renal plasma flow (RPF) would be about 700 ml/min. Any significant decrease in RBF results in a decrease in GFR.

(2) Tubular reabsorption

Tubular reabsorption is the process that allows the filtered fluid and solutes to be partially (e.g., fluid and sodium) or totally (e.g., glucose and amino acids) reabsorbed from the tubular lumen to the vascular compartment. From the total of 180 l of plasma filtered per 24 hours, a normal human will excrete a urinary volume in the range of only 0.6 to 2.5 l. These values indicate that approximately 99% of the water filtered through the glomerulus undergoes reabsorption within the tubules. Thus, the normal excretion of water in humans could be anywhere between 0.5 to 3 ml/min, with an average of around 1 ml/min [Guyton, 1991].

(3) Tubular Secretion

In addition to glomerular filtration, tubular secretion represents an additional route for solute transport from the vascular compartment into the tubular lumen. Small solutes such as potassium ions and hydrogen ions, as well as a multitude of organic acids, are eliminated from plasma into the final urine by means of this latter mechanism [e.g., Brenner and Rector, 1996]. As will be shown shortly, the renal model proposed in this work accounts for the renal excretion of water and small ions. These materials are subjected, independently and at different levels in the tubules, to transport via one or all of the three mechanisms described above. The model proposed below does not explicitly take the tubular transport into account, but relies instead on generalized mass balances of fluid and solutes across the tubules. In its most general form, the mass balance equation for the net tubular transport of material can be expressed as follows:

$$EXCRETION = FILTRATION - REABSORPTION + SECRETION \quad [8-1]$$

Each of the terms on the right hand side of Eq. [8-1] is directly dependent on the changes in plasma fluid volume and solute concentrations from their normal values. Therefore, a direct link between renal excretion and the plasma fluid and solute status can be established. The model is based on clinical concepts used to assess renal function (such as renal clearances) that are defined in the next section.
8.3.2 Renal clearances

The clearance of a solute is defined as the volume of plasma from which the solute is removed (cleared) by the nephrons per unit time in order to supply the amount of this substance that is measured in the urine over the same period of time [e.g., Koushanpour, 1976]. Thus, the renal clearance of the solute $S$, $C_{ls}$, is given by:

$$C_{ls} = \frac{[S]_u \cdot J_u}{[S]_{pl}}$$

where $J_u$ is the urine output rate expressed in ml/min, while $[S]_{pl}$ and $[S]_u$ are the solute concentrations in plasma and urine, respectively. The units for clearance are ml/min while the concentrations are usually expressed in mEq/l or mM. Every solute is characterized by its own normal clearance value. Some clearance values representative of a well-hydrated 70 kg human, which are important to this work, are presented in Table 8-1.

The renal clearance of a substance represents the combined effect of filtration, absorption and secretion within the nephron. For instance, if a solute (e.g., inulin) is totally cleared from plasma only by filtration, its clearance will reflect the value of the glomerular filtration rate as follows:

$$\text{GFR} = C_{l_{\text{inulin}}} = \frac{[\text{inulin}]_u \cdot J_u}{[\text{inulin}]_{pl}} = 125 \text{ (ml/min)}$$

where the symbols were previously defined.

The osmolar clearance can be calculated in a similar fashion as the individual solute renal clearance by replacing the plasma and urine solute concentrations in Eq. [8-2] by the plasma osmolarity, $[\text{Osm}]_{pl}$ and the urinary osmolarity $[\text{Osm}]_u$, respectively. Thus, the osmolar clearance, $C_{l_{\text{Osm}}}$ is obtained from

$$C_{l_{\text{Osm}}} = \frac{[\text{Osm}]_u \cdot J_u}{[\text{Osm}]_{pl}}$$

Depending upon whether the osmolar ratio, $[\text{Osm}]_u/[\text{Osm}]_{pl}$, is greater or less than unity, the kidney will either retain or eliminate an excess of water.

Clinicians often prefer to report the free water clearance rather than the osmolar clearance. The free water clearance, $C_{H_2O}$, represents the theoretical volume of water that must be
removed from urine in order to make it isosmolar to plasma, and is given by the following expression [Koushanpour, 1976]:

\[ C_{H_2O} = J_U - C_{I_{Osm}} \]  

[8-4]

It can be observed from Eqs. [8-3] and [8-4] that \( C_{H_2O} \) is zero when the osmolar ratio, \([Osm]_U/[Osm]_PL = 1\). This case represents the excretion of isosmolar urine; that is, the water is not reabsorbed during its passage through the distal tubules.

If \([Osm]_U/[Osm]_PL > 1\), the excretion of hyperosmolar (concentrated) urine with respect to plasma takes place. The free water clearance, \( C_{H_2O} \), in this case is negative. This scenario is indicative of an active reabsorption of water in the terminal tubules (usually in the presence of antidiuretic hormone, ADH). Reabsorption of water occurs when the kidney attempts to conserve the water in the body. Such circumstances are encountered, for example, during hemorrhage (i.e., the loss of plasma volume causes a decrease in fluid excretion), or during isovolemic increases in plasma osmolarity (i.e., water must be conserved in order to decrease the osmolarity of the extracellular fluids within the body) [Dunn et al., 1973].

If \([Osm]_U/[Osm]_PL < 1\), then the free water clearance is greater than unity (i.e., \( C_{H_2O} > 1 \)), indicating water diuresis (i.e., excessive water elimination through the kidney). Diuresis takes place, for example, if there is an isosmolar (or hypo-osmolar) increase in plasma volume. Maximal free water clearances under these circumstances have been measured at around 10 to 15 ml/min in humans. This high value reflects the ability of a normal kidney to excrete, in extreme conditions, as much as to 20 l of water in 24 hours.

### 8.3.3 Average values for urine and solute excretions in humans

Table 8-1 shows the average human rates of glomerular filtration and urinary excretion for fluid as well as for some of the ions of interest to this work. These are representative of a normal, well-hydrated 70 kg human. Also given in the table are the concentrations of small solutes in the fluid and excreted in urine. The difference between the amount of a solute filtered (column 2) and the amount of solute excreted (column 4) represents the net transport through the tubules, i.e., the combined effect of the reabsorption and secretion mechanisms according to Eq. [8-1]. The last column of Table 8-1 presents the average normal clearances of the sodium, potassium, and chloride ions.
For comparison, Table 8-1 also provides data for glucose, a solute that is normally found in plasma at a concentration 100 mg/100 ml. When present at normal plasma concentrations, glucose is first filtered across the glomerulus and then completely reabsorbed from the tubules such that, under normal conditions, its excretion rate and clearance are zero. A hemorrhage-induced increase in glucose concentration in the extracellular fluid is taken into account in Chapter 9. Even in this case, however, it is assumed that there is no renal excretion of glucose.

The initial conditions for renal excretion used in this work will be presented shortly, after the formulation of the renal model.

Table 8-1: Average concentrations of some solutes of interest to this work, i.e., sodium, potassium, chloride and glucose in the protein-free glomerular filtrate and in the urine. The data in this table are obtained from Guyton [1991]; however they approximate fairly well information in other literature reports [e.g., Greger, 1996; Brenner and Rector, 1996].

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Glomerular Filtrate 125 ml/min</th>
<th>Urine 1 ml/min</th>
<th>Clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solute</td>
<td>Quantity/min mEq/min Concentration mEq/l</td>
<td>Quantity/min mEq/min Concentration mEq/l</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>17.7 142</td>
<td>0.128 128</td>
<td>0.9</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.63   5</td>
<td>0.06 60</td>
<td>12</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>12.9 103</td>
<td>0.134 134</td>
<td>1.3</td>
</tr>
<tr>
<td>glucose</td>
<td>125 mg/min 100 mg/100ml</td>
<td>0 mg 0 mg/100 ml</td>
<td>0</td>
</tr>
</tbody>
</table>

8.4 Formulation of Model for Renal Excretion of Fluid and Solutes

8.4.1 Modeling background

The objective in the present work is to formulate an empirical kidney model, based on well-accepted clinical knowledge, which will predict the renal response to the specific conditions studied in this thesis. The conditions for which the kidney model is initially formulated and then tested are:

- **Infusions of solutions with different solute compositions in otherwise non-traumatized humans.**
Both iso-osmolar infusates (e.g., Ringer's (RS) or normal saline (NS) solutions) as well as hypertonic infusates (e.g., hypertonic saline solution (HS) or saline solution with a colloidal component such as dextran (HSD)) will be considered.

- **Traumatic conditions such as hemorrhage**

The integrated mechanisms of urine formation are fairly complex. Therefore, the task of formulating a model that completely describes the kidney and its multiple hormonal and transport mechanisms is a fairly challenging one. Nonetheless, many authors have attempted to develop such models. Kidney models commonly found in the literature can be generally divided into the following two categories. One group of models provides very detailed descriptions of certain aspects of kidney function such as specific transport mechanisms within the renal tubules or the effect of hormones on excretion rates [e.g., Dehaven and Shapiro, 1970; Blaine et al., 1972; Packer and Packer, 1973]. The second group are much more empirical in nature and are intended to provide an integrated representation of the renal/body fluid system [e.g., Guyton et al., 1972; Bigelow et al., 1972; Cameron, 1977; Uttamsingh et al., 1985; Arturson et al., 1989; Carlson et al., 1996; Coleman, 2000]. The first type of model is very useful in understanding and testing hypotheses regarding specific segments of the renal system. However, they lack general applicability when a description of fluid and solute transport in the whole body is desired. Thus, models in the second category are more appropriate for use in this work. However, these complex models cannot be easily incorporated in systems with perturbations other than the ones for which they were initially designed.

Failed attempts to adapt and incorporate any of the existing empirical kidney models into the model proposed in Chapter 4, justified the formulation of a new kidney model as part of this study. This new model, which is also empirical in nature, can be used in conjunction with the microvascular and cellular exchange model formulated earlier to provide information related to the short-term dynamic exchange of material within the body under normal as well as traumatic conditions. It is envisaged that this model will be applied only for conditions similar to those for which it is initially formulated and tested; it may not prove to be reliable for other, alternative scenarios outside this range.
8.4.2 Renal response to perturbed extra-renal conditions

Prior to describing the model assumptions and the formulation of the pertinent equations, it is necessary to briefly review the renal response to the perturbed conditions imposed in this work, (i.e., the elevation or depletion of plasma volume as a result of infusion or blood loss, respectively). The left-hand panels of Table 8-2 show the typical changes in plasma volume, sodium content and concentration that result following various perturbations to the vascular compartment. The right-hand panels indicate the renal responses to these changes. It is assumed in these figures that the changes depicted take place over the entire period of perturbation. No post-perturbation changes are shown; however, they will be briefly discussed later in this section.

8.4.2.1 Renal response to isosmolar Ringer’s (RS) or normal saline (NS) infusions

As shown in Table 8-2, Fig. A, an infusion of NS or RS solution will increase the plasma volume for the entire duration of infusion. Both NS and RS solutions contain sodium ions; therefore, when infused, they will produce an increase in the amount of sodium in plasma. However, since the infusate has a similar sodium concentration as plasma, \([\text{Na}]_{PL}\) remains fairly constant after infusion. The increase in plasma volume, however, will cause an increase in blood pressure and, consequently, in the glomerular filtration rate (GFR).

Figure B of Table 8-2 shows that, during NS and RS infusions, urine excretion is also increased (a direct result of the increase GFR). This type of renal response to iso-osmolar infusions has been widely reported in the literature for different animal species [e.g., Shackford et al., 1988] as well as for humans [e.g., Shackford, 1987; Watenpaugh et al., 1992]. The amount of sodium eliminated in urine increases from normal while the concentration of this ion is either constant or, at high plasma volumes and urinary rates, can even be slightly decreased [Shackford et al., 1987; 1988]. When these types of infusions are administered, the total urine osmolarity is generally decreased, i.e., there is an increased water diuresis (see also the discussion of negative osmolar clearance in Section 8.3.2).

After the infusion stops (results not shown), as either the NS or RS infusate dissipates into the interstitium, plasma volume start to decline and, therefore, the urine excretion returns toward its normal value.
<table>
<thead>
<tr>
<th>Type of Perturbation</th>
<th>Changes in Vascular Compartment</th>
<th>Changes in Excretion Values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS or NS</td>
<td><img src="image" alt="Graph A" /></td>
<td><img src="image" alt="Graph B" /></td>
<td>e.g.,</td>
</tr>
<tr>
<td></td>
<td>$V_{PL}(ml)$</td>
<td>$J_U \ (ml/h)$</td>
<td>Linko et al., 1988</td>
</tr>
<tr>
<td></td>
<td>$Na_{PL}$ (mEq)</td>
<td>$M_{Na,U}$ (mEq/h)</td>
<td>Shackford et al., 1987</td>
</tr>
<tr>
<td></td>
<td>$[Na]_{PL}$ (mEq/l)</td>
<td>$[Na]_U$ (mEq/l)</td>
<td>Shackford et al., 1988</td>
</tr>
<tr>
<td></td>
<td>Infusion</td>
<td>Infusion</td>
<td>Sondeen et al., 1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Watenpaugh et al., 1992</td>
</tr>
<tr>
<td>HS or HSD</td>
<td><img src="image" alt="Graph C" /></td>
<td><img src="image" alt="Graph D" /></td>
<td>e.g.,</td>
</tr>
<tr>
<td></td>
<td>$V_{PL}$</td>
<td>$J_U \ (ml/h)$</td>
<td>Shackford et al., 1988</td>
</tr>
<tr>
<td></td>
<td>$Na_{PL}$ (mEq)</td>
<td>$M_{Na,U}$ (mEq/h)</td>
<td>Sondeen et al., 1990</td>
</tr>
<tr>
<td></td>
<td>$[Na]_{PL}$ (mEq/l)</td>
<td>$[Na]_U$ (mEq/l)</td>
<td>Tellefsrud et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Infusion</td>
<td>Infusion</td>
<td></td>
</tr>
</tbody>
</table>

Table 8-2: The renal and vascular response to fluid infusion: changes in plasma, volume $V_{PL}$, sodium concentration $[Na]_{PL}$ and sodium amount $Na_{PL}$, and the corresponding changes in urine rate $J_U$, sodium excretion rate $M_{Na,U}$ and sodium concentration in urine $[Na]_U$. 
<table>
<thead>
<tr>
<th>Type of Perturbation</th>
<th>Changes in Vascular Compartment</th>
<th>Changes in Excretion Values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEM</td>
<td><img src="image" alt="EFFECT OF HEMORRHAGE" /></td>
<td><img src="image" alt="EFFECT OF HEMORRHAGE" /></td>
<td>e.g., Lucas and Ledgerwood, 1984</td>
</tr>
<tr>
<td></td>
<td>$V_{PL}$</td>
<td>$J_U$  (ml/h)</td>
<td>Shackford et al., 1987</td>
</tr>
<tr>
<td></td>
<td>$Na_{PL}$ (mEq)</td>
<td>$M_{Na,U}$ (mEq/h)</td>
<td>Sondeen et al., 1990</td>
</tr>
<tr>
<td></td>
<td>$[Na]_{PL}$ (mEq/l)</td>
<td>$[Na]_U$ (mEq/l)</td>
<td>Stone and Stahl, 1970</td>
</tr>
<tr>
<td></td>
<td>Hemorrhage</td>
<td>Hemorrhage</td>
<td></td>
</tr>
</tbody>
</table>

-Cont- Table 8-2: The renal and vascular response to fluid hemorrhage: changes in plasma, volume $V_{PL}$, sodium concentration $[Na]_{PL}$ and sodium amount $Na_{PL}$ and the corresponding changes in urinary rate $J_U$, sodium excretion rate $M_{Na,U}$ and sodium concentration in urine $[Na]_U$. 

165
8.4.2.2 Renal response to hyperosmolar (HS) or hyperosmolar/hyperoncotic (HSD) infusions

During either HS or HSD administration, there is an increase in plasma volume and plasma sodium amount and concentration as well as in total plasma osmolarity (Table 8-2, Fig. C). This hyperosmolar plasma expansion is the result of the high hyperosmolarity of the infusate together with fluid mobilization into the plasma compartment from intracellular sources [e.g., Kramer et al., 1986; Holcroft et al., 1987; Shackford et al., 1987]. It has been reported that the increase in plasma volume following HS and HSD infusions is accompanied by an increase in arterial pressure, RPF (renal plasma flow) and GFR in humans [e.g., Cannon et al., 1970] and other animal species [e.g., Ericson et al., 1976; Sondeen et al., 1990(a)]. At the same time, as a result of HS or HSD administration, there is also a decrease in the renal vascular resistance [e.g., Cannon et al., 1970; Ericson et al., 1976; Sondeen et al., 1990(a)]. As qualitatively shown in Table 8-2, Fig. D, in response to these alterations, the urinary excretion rates of water and sodium are increased [e.g., Cannon et al., 1970; Sondeen et al., 1990(a); Tølløfsrud et al., 1997]. Such increases in diuresis and natriuresis were reported in even earlier studies on dogs [e.g., Selkurt and Post, 1950; Howards et al., 1968]; the combination of increased plasma volume and sodium concentration along with the increased glomerular filtration rate exceeds the reabsorptive ability of the proximal tubules and, as a result, there is an increased excretion of both water and sodium through the kidney tubules. Even when only a small volume of hyperosmolar fluid is infused, such that the arterial pressure does not change significantly from its control value, the GFR is still increased and the renal vascular resistance decreased, such that the pattern in urinary output presented in Fig. D can be observed [e.g., Cannon et al., 1970; Selkurt and Post, 1950]. These reports suggest a direct correlation between the urinary output and changes in plasma volume, rather than changes in blood pressure. The effect observed is most probably due to the release of ANP in response to plasma volume expansion.

It was shown by Cannon et al. [1970] that, for hyperosmolar infusions, the increased excretion rate of sodium in humans is closely accompanied by similar changes to other small ions such as Ca\(^{2+}\), K\(^+\) and Cl\(^-\). Similarly, Shackford et al. reported that, in humans [1987] or pigs [1988], there is a linear correlation between the sodium and potassium excretion rates. This fact suggests that administration of an hyperosmolar infusate results in an overall increase in the urine osmolarity.
normal [Shackford et al., 1988]. The beneficial effect of plasma volume elevation due to HS infusions is short-lived compared with the same effect caused by HSD infusions. Experimental studies have shown that, accordingly, the renal sodium and water excretion rates are much higher and remain elevated for a longer duration following HSD compared with HS infusions [e.g., Sondeen et al., 1990(a)].

The trends depicted in Table 8-2, Figs. C and D, are valid only for the HS and HSD infusion cases discussed here. An earlier study by Selkurt and Post [1950] showed that, for an isovolemic increase in plasma sodium concentration, sodium excretion can remain normal. It is possible that in these instances the ADH will cause a marked tubular water reabsorption such that the sodium concentration in plasma is regulated at normal levels through this route. Such scenarios will not, however, be simulated in this study.

### 8.4.2.3 Renal response to hypovolemia caused by external hemorrhage

In general, the response of the kidney to hypovolemia is designed to compensate for the effect of the hypovolemic insult on the cardiovascular system. During plasma volume depletion, the cardiac output is reduced. This effect, in addition to the renal vasoconstriction, causes a decrease in RPF and GFR [e.g., Stone and Stahl, 1970; Shackford et al., 1988; Sondeen et al., 1990(b)]. As shown in Table 8-2, Fig. E, while both plasma volume and amount of sodium decrease during hemorrhage, the plasma sodium concentration (at least for a mild hemorrhage) is approximately constant. In a severe hemorrhage, however, due to plasma volume restitution (i.e., fluid mobilization into the plasma from the interstitium), [Na]_Pl is often slightly decreased. The qualitative renal response to hypovolemia is shown in Table 8-2, Fig. F. As illustrated in this figure, the kidney responds to the hypovolemic insult by reducing both its urinary output and sodium excretion rate. The sodium concentration in urine during hemorrhage has been reported to remain fairly close to normal (mild hemorrhage) or to be significantly reduced (moderate hemorrhage) [Stone and Stahl, 1970; Sondeen et al., 1990(b)].

Table 8-3 presents a compilation of data, obtained from experiments on humans, that further exemplify the previous discussion. The table shows that there is a direct correlation between the volume of blood lost and the renal blood flow, the glomerular filtration rate, the urine flow rate, and the sodium clearance. Although data are not presented, the osmolar clearance also parallels closely the renal excretion of sodium [Lucas and Ledgerwood, 1984]. As the plasma volume deficit increases, renal antidiuresis as well as reduced C_iNa and C_iOsm follow
shortly. Corresponding to a severe plasma depletion of 35 to 45%, the urinary and sodium excretions fall to zero. Several other experimental studies in humans [Stone and Stahl, 1970] and animals [Shackford et al., 1988] corroborate the data presented in Table 8-3.

It is interesting to note that, for mild hemorrhages of about 15%, plasma volume, RPF and U decrease while GFR is maintained essentially constant by renal vasoconstriction (autoregulation of the kidney). In this case, the lower urine and sodium excretions are due to continuous fluid and water reabsorption in the renal tubules. For a mild to moderate hemorrhage, however, there is a gradual decline in GFR along with the decreased plasma volume.

A great deal experimental effort has been expended over the years to demonstrate that the renal response to hypovolemia is brought about by a synergistic action of neural and hormonal mechanisms (e.g., secretion of renin, aldosterone, and antidiuretic hormone) to produce vasoconstriction and to alter the tubular processes. There is no argument that, because of the actions of these hormones, the renal blood flow can be reduced dramatically (e.g., for a 35% degree of hemorrhage in humans, the RBF can be reduced from 1200 ml/min to less than 100 ml/h within minutes). Similarly, many studies have demonstrated the hormonal effects triggered by ANP and ADH when there is an increase in the extracellular volume and sodium concentration, respectively.

<table>
<thead>
<tr>
<th>Severity of Hemorrhage</th>
<th>Blood Volume (ml)</th>
<th>Blood Pressure (mmHg)</th>
<th>Plasma Volume (ml) and PV Lost (%)</th>
<th>RPF Relative to Normal (%)</th>
<th>GFR Relative to Normal (%)</th>
<th>Urine Flow (ml/min)</th>
<th>Clearance of Na (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5500</td>
<td>120-80</td>
<td>3200</td>
<td>100</td>
<td>100</td>
<td>1.5-1</td>
<td>2.0-0.9</td>
</tr>
<tr>
<td>Mild</td>
<td>4700</td>
<td>115-75</td>
<td>2750 ~15%</td>
<td>70</td>
<td>100</td>
<td>0.7-0.5</td>
<td>1.5-0.7</td>
</tr>
<tr>
<td>Moderate</td>
<td>4200</td>
<td>110-70</td>
<td>2450 ~25%</td>
<td>30</td>
<td>50</td>
<td>0.3-0.2</td>
<td>0.7-0.3</td>
</tr>
<tr>
<td>Severe</td>
<td>3700</td>
<td>90 –60</td>
<td>2100 ~35%</td>
<td>8</td>
<td>10</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Irreversible Shock</td>
<td>3200</td>
<td>&lt; 50</td>
<td>1850 ~45%</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 8-3: Vascular and renal responses to different degrees of hemorrhage. Adapted from Lucas and Ledgerwood [1984].
However, in the final analysis (for the hyper- and hypovolemic cases described above and, Furthermore, in the absence of any chronic disease related to the body fluid balance and an otherwise normally functioning kidney), all of these hormonal actions are caused by a departure from normal plasma volume and/or normal plasma osmolarity (as represented by the sodium concentration).

The model, that follows, attempts to establish a direct empirical correlation between plasma and its sodium concentration with the renal excretion of water and sodium. Furthermore, to keep the renal model as simple as possible, an attempt is also made to correlate the renal excretion of the other small solutes of interest with the renal excretion of sodium.

8.5 Model Description
8.5.1 General assumptions

This model is intended to reasonably describe the behaviour of the normally functioning kidney of a well-hydrated 70 kg 'Reference man'. This statement has the following two implications:

First, it is quite possible that, even after scaling, the model will not be able to accurately predict the renal output of other animal species. It is well established that, with a few exceptions (e.g., swine), there are anatomical and physiological differences between humans and other animals with respect to both the nephron anatomy and the tubular secretion and absorption processes [Weisberg, 1962; Mahmood, 1992].

Second, it is considered that the excretory ability of the kidney is unperturbed by the traumatic conditions studied. Where the model will be applied to describe a traumatic condition, such as hemorrhage, it will be assumed that the extra-renal traumatic insult was either mild or of a relatively short duration such that there were no pathophysiological changes that alter the normal operation of the kidney.

The assumptions upon which the model is formulated are listed as follows:

- The renal urinary output reflects changes that occur in the vascular compartment only.

The majority of textbooks stipulate that the role of the kidney is to maintain an adequate volume and composition of the total extracellular volume, ECV, as well as the total body fluid content [e.g., Brenner and Rector, 1996; Guyton, 1991; Koushanpour, 1976]. However, as stated in a review by Aukland [1989] or as can be observed from studies of fluid infusion [e.g., Linko and Makelainen, 1988], in those conditions where interstitial edema might be present after
infusion (e.g., RS fluid overload), the urinary output decreases despite a generalized increase in the total ECV. It appears, therefore, that it is reasonable to correlate the renal response with the fluid status of the plasma, rather than with the total ECV. That is, the urinary output increases as long as the plasma volume is elevated; however, once the plasma volume starts to decrease, the urinary output decreases as well.

When applied to the urinary excretion of small solutes, this assumption is justified by the fact that such solutes, having small transcapillary reflection coefficients, will equilibrate relatively rapidly (i.e., within approximately 5 to 10 min) between the plasma and interstitial compartments. Hence, the concentrations of small solutes in plasma are approximately the same as those in the extracellular volume.

- The model takes into account the renal excretion rates of the following materials: fluid or urine ($J_U$), sodium ($M_{Na,U}$), potassium ($M_{K,U}$), chloride ($M_{Cl,U}$) and all the remaining cations ($M_{C,U}$) and anions ($M_{A,U}$).

- The renal outputs of fluid and sodium are direct consequences of changes in the plasma volume and plasma sodium concentration, respectively, from their normal value. In addition, except in the case of HSD infusions, sodium excretion is coupled with fluid excretion. The rationale behind this assumption is discussed later when the pertinent model equations are presented.

- The renal excretion rates of all small ions other than sodium are proportional to the excretion rate of sodium, $M_{Na,U}$.

This assumption is based on experimental studies involving hyperosmolar infusions in humans that report a direct link between the excretion rate of potassium [Cannon et al., 1970; Shackford et al., 1988], calcium, chloride and possibly other ions (e.g., urate) [Cannon et al. [1970] and the renal sodium excretion rate. A similar correlation between sodium excretion and the overall osmolites present in urine was mentioned by Lucas and Ledgerwood [1984] from their studies of hemorrhage in humans.

- There is no renal excretion of plasma proteins [Guyton, 1991; Brenner and Rector, 1996].

Although the glomerular filter is impermeable to plasma proteins, occasionally a small amount of these species do escape into urine. When the kidney functions normally, the concentration of protein in urine is estimated to be very low, i.e. < 0.01 g/l [Guyton, 1991]. Therefore the renal output rate for plasma proteins is set to zero.
Other, more specific assumptions of the kidney model are specified in the following section when the appropriate model equations are presented. A schematic diagram showing the kidney module incorporated into the global microvascular and cellular fluid and solute exchange model is presented in Fig. 8-4.

### 8.5.2 Model equations

#### 1. Fluid excretion

The urine excretion equation assumes a first-order negative-feedback response of the kidney to any change in plasma volume from its normal set-point, $V_{PL,NL}$. Thus, the linear proportionality between renal excretion and the changes in plasma volume can be expressed as:

$$J_U = k_U \cdot \frac{(V_{PL} - V_{PL,NL})}{V_{PL,NL}} + J_{U,NL}$$

[8-5]

where $J_U$ is the urinary output rate expressed in (ml/h), $V_{PL}$ is the plasma volume expressed in (ml) with a normal value $V_{PL, NL}$ of 3200 ml, while $J_{U,NL}$ is the normal urinary output rate. The value of $J_{U,NL}$ for a standard 70 kg human is taken as 60 ml/h [Guyton, 1991] (see also Table 8-1).

The proportionality constant $k_U$ in Eq. [8-5] has units of ml/h and needs to be estimated from experimental data. Based on experimental evidence concerning the effect of plasma volume depletion [Lucas and Ledgerwood, 1984] or plasma elevation [Linko and Makelainen, 1988; Tøllofsrud et al., 1997; Watenpaugh et al., 1992] on renal fluid excretion, it is clear that $k_U$ must have two distinct values, corresponding to a dehydrated and overhydrated plasma volume. Thus, it is assumed that Eq. [8-5] has the following two segments:

1. a renal 'volume-depleted response' segment, that corresponds to a plasma volume lower than normal, i.e.,

$$J_U = k_U^D \cdot \frac{(V_{PL} - V_{PL,NL})}{V_{PL,NL}} + J_{U,NL} ; \quad V_{PL} < V_{PL, NL}$$

[8-6]

2. a renal 'volume-elevated response' segment that corresponds to a plasma volume greater than or equal to normal, i.e.,

$$J_U = k_U^E \cdot \frac{(V_{PL} - V_{PL,NL})}{V_{PL,NL}} + J_{U,NL} ; \quad V_{PL} \geq V_{PL, NL}$$

[8-7]
Figure 8-4. Schematic representation of the kidney module and its relationship to the overall model of whole-body fluid and solute exchange. Fluid and solutes are excreted from the plasma compartment. $J_U$ represents the urinary rate while $M_{Na,U}$, $M_{K,U}$, $M_{Cl,U}$, $M_{C,U}$ and $M_{A,U}$ represent the excretion rates of sodium, potassium, chloride and the remaining positive and negative charges, respectively. The concentrations of small ions in urine are shown underneath each corresponding excretion rate. The dashed arrows within the kidney module indicate that, in most cases (except for increased sodium plasma sodium concentration), the urinary output determines the magnitude of the sodium excretion rate, which in turn, as shown by the solid arrow, directly determines the excretion rates of potassium, chloride and the remaining cations. See text for more discussion.
The two constants $k^D_U$ and $k^E_U$ are determined by statistically fitting experimental data from humans that involve either hemorrhage (i.e., depleted plasma volume) or infusion (i.e., elevated plasma volume). The estimation of $k^D_U$ and $k^E_U$, together with their tabulated values is presented in Section 8.5.4.

2. Sodium excretion

In order to avoid the complex tubular processes involved in sodium reabsorption and secretion, the equation that describes the effective sodium excretion was based on the clinical concept of renal clearance. Thus, if it is assumed that the renal clearance of sodium is proportional to the change in sodium plasma concentration at a given time, then:

\[
C_{INa} = \left( k_{Na} \cdot \frac{([Na]_{PL} - [Na]_{PL,NL})}{[Na]_{PL,NL}} + C_{INa,NL} \right) \cdot F(U)
\]  

[8-8]

where $C_{INa}$ is the renal clearance of sodium expressed in ml/h, $C_{INa,NL}$ is the normal value of $C_{INa}$, $[Na]_{PL}$, expressed in mEq/l, is the concentration of sodium in plasma which has a normal value of $[Na]_{PL,NL}$, and $k_{Na}$ is a proportionality constant which relates the sodium clearance with the change in the plasma sodium concentration from its normal set-point and has units of ml/h. The estimation of $k_{Na}$ is also presented in Section 8.5.4. $F(U)$ is a variable that couples the renal excretion of sodium with the urinary flow rate. Its meaning is explained below.

By considering the definition of the sodium clearance expressed as $C_{INa} = \frac{[Na]_U \cdot J_U}{[Na]_{PL}}$, and re-arranging Eq. [8-8], the rate of sodium excretion, $M_{Na,U}$, can be written as:

\[
M_{Na,U} = [Na]_U \cdot J_U = \left( k_{Na} \cdot \frac{[Na]_{PL}}{[Na]_{PL,NL}} ([Na]_{PL} - [Na]_{PL,NL}) + C_{INa,NL} \cdot [Na]_{PL} \right) \cdot F(U)
\]

[8-9]

where $M_{Na,U}$ has units of mEq/h.

The variable $F(U)$ which is a function of urinary output and establishes an interdependence between the sodium excretion rate and the urine flow rate, is defined as follows:
Chapter 8: Model of the renal function

\[
F(U) = \frac{J_U}{J_{U,NL}} \quad \text{when } [\text{Na}]_{\text{PL}} < [\text{Na}]_{\text{PL}}^T \quad \text{[8-10]}
\]

\[
F(U) = 1 \quad \text{when } [\text{Na}]_{\text{PL}} \geq [\text{Na}]_{\text{PL}}^T, \quad \text{[8-11]}
\]

where \([\text{Na}]_{\text{PL}}^T\) is defined as a threshold sodium concentration in plasma. Its value was arbitrarily assumed to be equal to the maximal value of the normal physiological range that can be taken by the plasma sodium concentration, i.e., around 146 mEq/l. By imposing the above conditions for \(F(U)\), Eqs. [8-8] implies that, as long as the plasma sodium concentration is near or less than its normal value, the sodium clearance will depend on the urinary flow rate.

Thus, in conditions characterized by a depleted plasma volume such as hemorrhage, where \([\text{Na}]_{\text{PL}}\) has near normal values and where an antidiuretic renal response occurs, \(F(U)\) is less than unity. According to the model, \(F(U) < 1\) translates into a reduced sodium clearance and implicitly a reduced sodium excretion rate; a response that is in agreement with experimental information (see Table 8-3). Physiologically, this condition corresponds to an enhanced reabsorption of sodium from the tubules that results in a decreased excretion rate.

For iso-osmolar plasma volume expansions such as those that occur after the infusion of RS or NS solutions (where \([\text{Na}]_{\text{PL}}\) is less than \([\text{Na}]_{\text{PL}}^T\)), \(F(U)\) is greater than unity. This condition assures a slightly increased sodium excretion rate. Note however that, although \(M_{\text{Na,U}}\) is increased in this case, for the high urinary rates that are usually recorded after NS or RS infusions, the concentration of sodium in urine is actually less than normal. Physiologically, this case would correspond to a reduced reabsorption of sodium from the tubules to plasma and an enhanced renal diuretic response which is typically observed after NS or RS administration [Krieger and Sherrard, 1990].

In conditions of plasma hyperosmolarity, when the plasma sodium concentration increases above its threshold value of \([\text{Na}]_{\text{PL}}^T\), \(F(U) = 1\). For this case the sodium excretion rate becomes independent of that of fluid, although both the urine and sodium excretion rates are increased. This scenario corresponds to the enhanced diuretic and natriuretic renal responses that are well described in studies involving HSD infusions [see e.g., Shackford et al., 1988].

From Eq. [8-9], it is clear that the concentration of sodium in urine can be expressed in terms of the sodium excretion rate as,
Chapter 8: Model of the renal function

3. Renal excretion of other small ions

Studies of hypertonic saline infusion in humans by, e.g., Cannon et al. [1970] and Shackford et al. [1988], showed that there is a linear correlation between the fractional excretion of sodium and the excretion rates of chloride, calcium, magnesium and potassium ions. This observation is the basis for formulating the excretion rate equations for all the other ions considered in the kidney model.

It is assumed, therefore, that the rate of potassium excretion, $M_{K,U}$, expressed in mEq/h, is given by

$$M_{K,U} = [K]_U \cdot J_U = [K]_{U,NL} \cdot J_{U,NL} + k_K \cdot ([Na]_U \cdot J_U - [Na]_{U,NL} \cdot J_{U,NL})$$

or, more concisely, as

$$M_{K,U} = M_{K,U,NL} + k_K \cdot (M_{Na,U} - M_{Na,U,NL})$$

where $[K]_U$ is the potassium concentration in urine with its normal value of $[K]_{U,NL}$, while $k_K$ is the proportionality constant between the potassium and sodium excretion rates. The product $[K]_{U,NL} U_{NL}$ is equal to the normal urinary excretion rate, $M_{K,U,NL}$ expressed in mEq/h. The rest of the symbols were previously defined.

Similar equations can be written for the excretion rates of chloride ions, $M_{Cl,U}$, and $C^{2+}$ cations, $M_{C,U}$, i.e.,

$$M_{Cl,U} = [Cl]_U \cdot J_U = [Cl]_{U,NL} \cdot J_{U,NL} + k_{Cl} \cdot ([Na]_U \cdot J_{U,NL} - [Na]_{U,NL} \cdot J_{U,NL})$$

$$= M_{Cl,U,NL} + k_{Cl} \cdot (M_{Na,U} - M_{Na,U,NL})$$

and
\[ M_{C,U} = [C]_U \cdot J_U = [C]_{U, NL} \cdot J_{U, NL} + k_c \cdot ([Na]_U \cdot J_U - [Na]_{U, NL} \cdot J_{U, NL}) \]

\[ = M_{C,U, NL} + k_c \cdot (M_{Na,U} - M_{Na,U, NL}) \]  

where \([Cl]_U\) and \([C]_U\) are the urinary concentrations of Cl\(^-\) and C\(^{2+}\), respectively, expressed in mEq/l; \(k_{Cl}\) and \(k_c\) are the proportionality constants for the Cl\(^-\) and C\(^{2+}\) excretion rates, respectively; \(M_{Cl,U} = [Cl]_U J_U\) and \(M_{C,U} = [C]_U J_U\) are the excretion rates of Cl\(^-\) and C\(^{2+}\), expressed in mEq/h; and \(M_{Cl,U, NL}\) and \(M_{C,U, NL}\) are their normal steady-state values.

It was assumed that the remaining negative ions, i.e., the A\(^-\) species, are excreted such that the electroneutrality of urine is respected. Thus the urinary excretion of A\(^-\) ions, \(M_{A,U}\), expressed in mEq/h, is given by:

\[ M_{A,U} = M_{Na,U} + M_{K,U} + M_{C,U} - M_{Cl,U} \]  

Note that Eq. [8-16] assumes an ionic charge of charge of +1 for Na\(^+\) and K\(^+\), -1 for Cl\(^-\) and A\(^-\), and +2 for C\(^{2+}\).

The urinary concentrations for K\(^+\), \([K]_U\), Cl\(^-\), \([Cl]_U\), C\(^{2+}\), \([C]_U\), and A\(^-\), \([A]_U\), ions can be determined from the following equations:

\[ [K]_U = \frac{M_{K,U}}{J_U} \]  

\[ [Cl]_U = \frac{M_{Cl,U}}{J_U} \]  

\[ [C]_U = \frac{M_{C,U}}{J_U} \]  

\[ [A]_U = \frac{M_{A,U}}{J_U} \]
8.5.3 Initial conditions for renal excretion in humans

The normal renal excretion values corresponding to a 70 kg, well-hydrated human are provided in Table 8-4. This table was put together in the following manner. The average normal urinary rate of 60 ml/h was obtained from standard literature sources [e.g., Guyton, 1991] as were the normal renal clearance values for sodium, potassium and chloride ions. The clearance of $C^{2+}$ cations was assumed to be similar to that of calcium ions and therefore a value of 72 ml/h was assigned to $C_{C^{2+},NL}$ [Guyton, 1991]. For a given small ion, based on its normal renal clearance value and known plasma concentration value, its concentration in urine together with its excretion rate can be calculated from equations similar to Eqs. [8-2] and [8-9], respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Rate, $J_{U,NL}$</td>
<td>60 ml/h</td>
</tr>
<tr>
<td>Renal Sodium Clearance, $C_{I_{Na},NL}$</td>
<td>54 ml/h</td>
</tr>
<tr>
<td>Sodium Excretion Rate, $M_{Na,U,NL}$</td>
<td>7.6 mEq/h</td>
</tr>
<tr>
<td>Sodium Concentration in Urine, $[Na]_{U,NL}$</td>
<td>127.7 mEq/l</td>
</tr>
<tr>
<td>Renal Potassium Clearance, $C_{I_{K},NL}$</td>
<td>720.0 ml/h</td>
</tr>
<tr>
<td>Potassium Excretion Rate, $M_{K,U,NL}$</td>
<td>3.0 mEq/h</td>
</tr>
<tr>
<td>Potassium Concentration in Urine, $[K]_{U,NL}$</td>
<td>50.0 mEq/l</td>
</tr>
<tr>
<td>Renal Chloride Clearance, $C_{I_{Cl},NL}$</td>
<td>78.0 ml/h</td>
</tr>
<tr>
<td>Chloride Excretion Rate, $M_{Cl,U,NL}$</td>
<td>8.2 mEq/h</td>
</tr>
<tr>
<td>Chloride Concentration in Urine, $[Cl]_{U,NL}$</td>
<td>136.9 mEq/l</td>
</tr>
<tr>
<td>Renal $C^{2+}$ Clearance, $C_{I_{C^{2+}},NL}$</td>
<td>72.0 ml/h</td>
</tr>
<tr>
<td>$C^{2+}$ Excretion Rate, $M_{C,U,NL}$</td>
<td>1.6 mEq/h</td>
</tr>
<tr>
<td>$C^{2+}$ Concentration in Urine, $[Cl]_{U,NL}$</td>
<td>26.8 mEq/l</td>
</tr>
<tr>
<td>$A^+$ Excretion Rate, $M_{A,U,NL}$</td>
<td>3.2 mEq/h</td>
</tr>
<tr>
<td>$A^+$ Concentration in Urine, $[A]_{U,NL}$</td>
<td>67.6 mEq/l</td>
</tr>
</tbody>
</table>

Table 8-4: Initial values for renal excretion in a 70 kg normal human. The standard literature source for the values presented in this table is Guyton [1991]; however, all data are in agreement with other sources [e.g., Greger, 1996; Brenner and Rector, 1996].
8.5.4 Parameter estimation

Before the renal excretion model can be used, the following six parameters: \( k^D_u \), \( k^E_u \), \( k_{Na} \), \( k_K \), \( k_{Cl} \) and \( k_C \) need to be determined. These parameters were estimated by using four sets of human experimental data as follows:

1) \( k^E_u \), the proportionality constant that characterizes the urinary output during plasma overhydration, was estimated from data reported by Watenpaugh et al. [1992] for iso-osmotic infusions.

2) \( k^D_u \), the proportionality constant for urinary output corresponding to a depleted plasma volume, was obtained using data reported by Lucas and Ledgerwood [1984] for humans undergoing hemorrhage.

3) \( k_{Na} \), \( k_K \), and \( k_{Cl} \), the proportionality constants required to determine the urinary outputs for small ions, were estimated using data obtained by Tølløfsrud et al. [1997] for HSD infusions in normovolemic humans.

4) \( k_C \), the proportionality constant needed to calculate the urinary rate for \( C^{2+} \) was estimated from data reported by Cannon et al. [1970] for HS infusions in normovolemic humans.

All of these parameters were estimated by least-squares fitting using the commercial software package MicroMath Scientist®. The technique used by this package for minimizing the sum of the squared residuals is based on Powell's method [Press et al., 1992].

8.5.4.1 Estimation of \( k^E_u \) using data from Watenpaugh et al. [1992]

The experimental protocol employed by Watenpaugh et al. [1992] was given in Section 7.2.2. Stated briefly, this study involved infusing normal, well-hydrated human volunteers with 30 ml/kg of an isotonic saline solution for a period of 24 min. Among other variables, plasma volume and urinary output were monitored during and up to 3 hours after the infusion.

For the estimation of \( k^E_u \), each experimental urinary rate measured by Watenpaugh et al. [1992] was averaged over the measurement time interval. The model-predicted changes in plasma volume for this study (see Section 7.3) were also averaged over the same time intervals used for averaging the urinary values. Following this, the average urinary output rates predicted by Eq. [8-7] using the average plasma volume from the model, were fitted to the averaged urinary values reported in the experiment. The estimated \( k^E_u \) value obtained by this
procedure is given in Table 8-5. Figure 8-5 shows a comparison between the average experimental urine excretion rates measured by Watenpaugh et al. [1992] and the average model-predicted urinary output values calculated using the best estimate of $k_U^E = 1250 \text{ ml/h}$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (ml/h)</th>
<th>Corresponding equation</th>
<th>R-squared</th>
<th>Standard Deviation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_U^E$</td>
<td>1250</td>
<td>Urine$_{Ov} \text{ hydration}$ Eq. [8-7]</td>
<td>0.94</td>
<td>± 142</td>
<td>[Watenpaugh, 1992]</td>
</tr>
<tr>
<td>$k_U^D$</td>
<td>168</td>
<td>Urine$_{D} \text{ dehydration}$ Eq. [8-6]</td>
<td>0.97</td>
<td>± 12</td>
<td>[Lucas and Ledgerwood, 1984]</td>
</tr>
<tr>
<td>$k_{Na}$</td>
<td>3080</td>
<td>Na$_{+} \text{ Excretion}$ Eq. [8-9]</td>
<td>0.92</td>
<td>± 163</td>
<td>[Tøllofsrud et al., 1997]</td>
</tr>
<tr>
<td>$k_K$</td>
<td>0.48</td>
<td>K$_{+} \text{ Excretion}$ Eq. [8-13]</td>
<td>0.95</td>
<td>± 0.04</td>
<td>[Tøllofsrud et al., 1997]</td>
</tr>
<tr>
<td>$k_{Cl}$</td>
<td>1.28</td>
<td>Cl$_{-} \text{ Excretion}$ Eq. [8-14]</td>
<td>0.92</td>
<td>± 0.08</td>
<td>[Tøllofsrud et al., 1997]</td>
</tr>
<tr>
<td>$k_{Cu}$</td>
<td>0.23</td>
<td>C$_{2-} \text{ Excretion}$ Eq. [8-15]</td>
<td>0.9</td>
<td>± 0.03</td>
<td>[Cannon et al., 1970]</td>
</tr>
</tbody>
</table>

Table 8-5: Set of estimated parameters of the kidney model obtained by least-square fitting technique.

8.5.4.2 Estimation of $k_U^D$ using data from Lucas and Ledgerwood [1984]

The value of the proportionality constant that applies to the hypovolemic case, i.e., $k_U^D$, was obtained from data reported by Lucas and Ledgerwood [1984]. These data, shown in Table 8-3, represent a compilation of literature information on plasma volumes and urine flow rates obtained from several humans who sustained different degrees of hemorrhage. The urine output rates were collected subsequent to hemorrhages that were not followed by infusions.

In carrying out the fitting procedure, the lower limits of the ranges of urinary values presented in Table 8-3 were chosen for determining $k_U^D$. Thus, for a normal human plasma volume of 3200 ml, the urinary output was taken to be 60 ml/h. The urine production stops for plasma values lower than approximately 1850 ml (i.e., a degree of hemorrhage of about 45%). The estimated $k_U^D$, obtained by fitting Eq. [8-6] to this $J_U$ vs. $V_{PL}$ data, has a value of 163 ml/h and is presented in Table 8-5 together with the goodness-of-fit statistical data.
Figure 8-5: The upper panel shows the average experimental urinary output reported by Watenpaugh et al. [1992] (solid circles) vs. average model predicted urinary outputs (dashed lines) obtained by using the estimated parameter $k_U^E = 1250$ ml/h. The lower panel gives the instantaneous urinary output rates predicted using this value of $k_U^E$. 
Based on the two distinct segments determined for Eq. [8-5], the renal output of fluid that characterizes either an increase or decrease in plasma volume from its normal value of 3.2 l is depicted in Fig. 8-6. In agreement with general physiological knowledge, this figure illustrates the role of the kidney in preserving fluid in the body during dehydration through a reduced fluid excretion rate and, increasing fluid output more dramatically during overhydration.

Figure 8-6: Calculated urinary output rate vs. change in plasma volume obtained by using the estimated $k_D^U$ and $k_U^D$ constants. The solid circle represents the normal conditions.
8.5.4.3 Estimation of $k_{Na}$, $k_{K}$ and $k_{Cl}$ using data from Tølløfsrud et al. [1997]

The experimental protocol for the normovolemic conditions studied by Tølløfsrud et al. [1997] was discussed previously in Section 7.2.2. In this study, human volunteers were infused with a 4 ml/kg HSD solution for 10 minutes. Among other variables, the plasma volume as well as the plasma small ion concentrations were followed for 3 hours after the beginning of fluid administration.

The urinary fluid output rates, together with the excreted small ion concentration values, recorded for the same period of time, were provided to us by one of the authors [Tølløfsrud, personal communication]. These values were also presented in Table 7-2.

The work of Tølløfsrud et al. [1997] is used for two purposes. An initial preliminary validation of the urinary output equation is achieved by comparing the model predictions with the experimental data reported by these authors. Following this step, the proportionality constants required to determine the small solute renal excretion rates are estimated.

The model predicted plasma volumes for the normovolemic HSD case were presented in Chapter 7. Based on these model-generated plasma values and making use of the $k_{ij}$ value estimated previously, the urinary output is predicted using Eq. [8-7]. The two panels of Fig. 8-7 present the model-predicted urinary output rates obtained by this procedure. The upper panel of Fig. 8-7 shows a comparison between the simulated (dashed lines) and experimentally measured (solid circles) results for the average urinary rate. As can be seen from this figure, as a consequence of HSD administration, the renal excretion of fluid, for the time interval from $t = 0$ to $t = 1$ h, increases markedly from the baseline value of 60 ml/h to 260 ml/h (measured) or 270 ml/h (predicted). For the following 2 h (until the end of the experiment), the model predicts an average renal output of 180 ml/h. Although this value underestimates the experimentally reported figure of 230 ml/h, it is well within the error of the measurement. Presented in the lower panel of Fig. 8-7 is the continuous variation in urinary output predicted by the model. In agreement with the report of Tølløfsrud et al. [1997] as well as information from other authors [e.g., Sondeen et al., 1990(a)], the model predicts the enhanced fluid elimination through the kidney that results following HSD fluid administration.

The experimental information regarding the urine elimination of sodium, potassium and chloride concentrations provided by Tølløfsrud was used to calculate the average excretion rates of these solutes. As reported by this author, urine was collected for the time intervals: $t = -1.5$ to $t = 0$ h, $t = 0$ to $t = 1$ h, and $t = 1$ to $t = 3$ h. This information was used to estimate values for
Figure 8-7: The upper panel presents a comparison between the computed average urinary rates (dashed lines) and the experimentally measured values (solid circles) from the work of Tollefsrud et al. [1997]. The lower panel illustrates the continuous urinary output predicted by the model. The error bars on both figures correspond to the experimentally measured standard deviations reported in the experimental study. The duration of infusion is indicated on the time axis.
k_{Na}, k_{K}, and k_{Cl} as follows. From the model simulations (see the Section 7.3 results for the normovolemic HSD case), the predicted plasma concentrations of sodium were averaged for the same three time intervals used for urine collection. By using these values, the average sodium excretion rates, M_{Na,U}, predicted by Eq. [8-9] were fitted to the experimental average excreted sodium rates, in order to estimate k_{Na}. Then, after inserting the experimental average M_{Na,U} rates into Eq. [8-13a] and [8-14], k_{K} and k_{Cl} were obtained by forcing the predicted results from these two equations to fit the experimental average potassium and chloride excretion rates. These three estimated parameters (i.e., k_{Na}, k_{K}, and k_{Cl}) obtained from the fitting procedure, are presented in Table 8-4.

Figures 8-8 to 8-13 present comparisons between the experimental excretion results for sodium, potassium and chloride ions and the corresponding model-predicted values that were obtained with the newly estimated parameters. The upper panels of these figures illustrate the average excretion rates and concentrations while the lower panels show the continuous changes in these variables predicted by the model.

8.5.4.4 Estimation of k_{C} using data from Cannon et al. [1970]

Tøløfsrud et al. [1997] did not measure the excretion rates or urine concentrations of any ions other than sodium, potassium and chloride. Therefore, the value of k_{C} was determined from data available from an earlier study by Cannon et al. [1970], who reported the values for various small ion excretion rates after infusions of 2.5% HS solution in humans. In this study, the excretion rates of several ions such as sodium, potassium, chloride and calcium ions, were measured prior to infusion and at peak natriuresis.

The excretion rates of calcium reported by Cannon et al. [1970] were considered to approximate fairly well the C^{2+} excretion rates described in the present work. Thus, the experimentally measured changes in the excretion rate of calcium were plotted as a function of the changes in the excretion rate of sodium in Fig. 8-14. The slope of the regression line is k_{C}, and it has a value of 0.23. The statistical information associated with the determination of this constant is presented in Table 8-4.
Figure 8-8: The upper panel presents a comparison between the computed averages for sodium excretion rates (dashed lines) and the corresponding experimentally measured values (solid circles) from the work of Tøløfsrud et al. [1997]. The lower panel illustrates the model-predicted continuous changes for the sodium excretion rate. The error bars indicated on both figures represent standard deviations.
Figure 8-9: The upper panel presents a comparison between the computed averages for sodium concentration in urine (dashed lines) and the experimentally measured values (solid circles) from the work of Tølløsfjord et al. [1997]. The lower panel illustrates the model predicted continuous changes of the sodium concentration in urine. The error bars indicated on both figures represent standard deviations.
Figure 8-10: The upper panel presents a comparison between the computed averages for potassium excretion rates (dashed lines) and the corresponding experimentally measured values (solid circles) from the work of Tølløfsrud et al. [1997]. The lower panel illustrates the model-predicted continuous changes of the potassium excretion rate. The error bars indicated on both figures represent standard deviations.
Figure 8-11: The upper panel presents a comparison between the computed averages for potassium concentration in urine (dashed lines) and the experimentally measured values (solid circles) from the work of Tøllefsrud et al. [1997]. The lower panel illustrates the model predicted continuous changes of the potassium concentration in urine. The error bars indicated on both figures represent standard deviations.
Figure 8-12: The upper panel presents a comparison between the computed averages for chloride excretion rates (dashed lines) and the corresponding experimentally measured values (solid circles) from the work of Tølløfsrud et al. [1997]. The lower panel illustrates the model-predicted continuous changes of the chloride excretion rate. The error bars indicated on both figures represent standard deviations.
Figure 8-13: The upper panel presents a comparison between the computed averages for chloride concentration in urine (dashed line) and the experimentally measured values (solid circles) from the work of Tølløfsrud et al. [1997]. The lower panel illustrates the model predicted continuous chloride concentration in urine. The error bars indicated on both figures represent standard deviations.
Figure 8-14: Least-squares fitting of the experimental data for the change in \( \text{C}^{2+} \) excretion rate vs. the change in sodium excretion rate at peak natriuresis after HS fluid infusions. Data were obtained for \( \text{Ca}^{2+} \) from the study of Cannon et al. [1970] and involve normotensive (solid circles) and hypertensive (open circles) humans with no history of renal disease.

8.5.5 Model validation and discussion

Model validation was carried out by comparing the model predictions with new experimental data (i.e., data that were not used for parameter estimation) for renal output and/or other compartmental variables of interest (e.g., plasma volume, Hct, small ion concentrations, etc.). The experimental studies chosen for comparison involve two species, humans and pigs, and include the following three cases:

1. Normal saline (NS) and hypertonic/hyperoncotic saline (HSD) infusions in normal swine that suffered no previous trauma [Sondeen et al., 1990(a)].

2. HSD infusions in normal human volunteers that had undergone a mild, 10% external hemorrhage [Tøløfsrud et al., 1997].

3. Severe blood losses in swine, equivalent to 42% of the normal blood volume, not followed by resuscitation [Sondeen et al., 1990(b)].
Although the application of the model to the study of hemorrhage and the compensatory mechanisms that may take place after this type of insult is the subject of the next chapter, the renal response to a depleted plasma volume that results from hemorrhage will be discussed here. However, the kidney model will be tested only for those cases that do not require additional modification of the model. For instance, as will be shown in the next chapter, one of the additional mechanisms that has to be accounted for post-hemorrhage is the presence of hyperosmolarity due to the release of (mainly) glucose in the plasma. Therefore, in order to avoid this confounding hyperglycemic mechanism, the only studies used for kidney model validation are those that involve animals that were deprived of food for at least 18 h prior to experimentation. Under these conditions, glucose mobilization does not take place.

8.5.5.1 Validation based on NS or HSD infusions in normal swine

The experiments of Sondeen et al. [1990(a)] involved pigs having an average body weight of 21.2 kg that were previously splenectomized. Although these animals had been deprived of food, they were otherwise well hydrated. The study describes changes in the vascular compartment and the renal response following short 2 - 3 (average 2.4) min, 4 ml/kg infusions of NS solutions (i.e., normal saline at 0.9% NaCl) or HSD solutions (i.e. hypertonic saline with 7.5% NaCl and 6% Dextran 70).

The initial compartmental values as well the interstitial compliance relationships were determined as described previously for other animal species (see Section 6.3.2 or Appendix C). These values are reported in Appendix E.

Sondeen et al. [1990(a)] measured plasma compartment variables such as Hct and sodium concentration as well as the renal excretion rates of fluid and sodium. Comparisons of the model-predicted results with the experimentally measured data are shown in Figs. 8-15 to 8-17 for the NS infusion case and in Figs. 8-18 to 8-20 for the HSD case. In the simulations, the system is maintained at steady state between 0 to 0.75 h, i.e., there are no inputs or outputs from any of the fluid compartments. According to the author's report, for this period of time, the bladder of the animals is emptied and no fluid intakes are allowed. From 0.75 h until the end of the experiment at 4 h, renal eliminations of fluid and solutes are reported in the experimental study and are also accounted for by the model. At 1.94 h after the beginning of the experiment, the 0.04 h (2.4 minute) infusions of either NS or HSD are included in the model, in accordance with Sondeen et al.'s experimental protocol.
Figure 8-15: Comparison between model predictions (solid line) and experimental data (solid circles) for Hct values. The experimental data reported by Sondeen et al. [1990(a)] involve a short 0.04 h NS infusion in swine. The infusion period is indicated by the marker on the time axis.
Figure 8-16: Model-predicted continuous changes in plasma volume (upper panel) and the renal response to an NS iso-osmolar plasma fluid expansion (lower panel). Experimental urinary output data are from the study of Sondeen et al. [1990(a)] and are represented by symbols (solid circles) in the lower panel. The infusion period is indicated by the marker on the time axis.
Figure 8-17: Experimental (solid circles) and model-predicted (solid line) changes in the concentration of sodium in plasma (upper panel) and the relative change in the sodium excretion rate, i.e., $\frac{M_{Na,U}}{M_{Na,U,NL}}$ (lower panel). Experimental data for the NS case are from the study of Sondeen et al. [1990(a)]. The infusion period is indicated by the marker on the time axis.
Figure 8-18: Comparison between model predictions (solid line) and experimental data (solid circles) for Hct values. The experimental data reported by Sondeen et al. [1990(a)] involve a short 0.04 h HSD infusion in swine. The infusion period is indicated by the marker on the time axis.
Figure 8-19: Model-predicted continuous changes in plasma volume (upper panel) and the renal response to an HSD hyperosmolar plasma fluid expansion (lower panel). Experimental urinary output data are from the study of Sondeen et al. [1990(a)] and are represented by symbols (solid circles) in the lower panel. The infusion period is indicated by the marker on the time axis.
Figure 8-20: Experimental (solid circles) and model-predicted (solid line) changes in the concentration of sodium in plasma (upper panel) and the relative change in the sodium excretion rate, i.e., $M_{Na,U}/M_{Na,U,NL}$ (lower panel). Experimental data for the HSD case are from the study of Sondeen et al. [1990(a)]. The infusion period is indicated by the marker on the time axis.
Figure 8-15 illustrates the model-predicted changes in hematocrit (solid line) and the measured Hct data (solid circles) for the NS case. In agreement with the experimental measurements from 0.75 to 1.93 h, where plasma fluid is lost only through the kidney, there are insignificant changes in the Hct values. Once the NS bolus is administered, the Hct decreases as a direct result of the increase in plasma volume. As predicted by the model, a maximal decrease in plasma Hct to ~ 26.5% (compared to an initial value of 28.8%) is achieved by the end of the infusion.

The experimental study does not report any measurements at the end of infusion period; however, in agreement with the experimental data, at about 5 to 10 minutes post-infusion, the Hct reaches a value of about 27%. According to the simulations, the entire post-infusion period is characterized by a return of Hct toward its normal value, which it has essentially reached at t = 4 h. Although, with the exception of the value at t = 4 h, the model-predicted Hct values are within the standard errors of the measurement, the simulations appear to overestimate the experimental data. Note, however, that the experimental measurements post-infusion seem somewhat suspicious. Although the 4 ml/kg of NS infused produces a small transient increase in plasma volume, this volume returns very quickly toward its control value as soon as the infusion stops (see Fig. 8-16, upper panel). Such a return is normally associated with an increase in Hct toward normal. As shown in the figure, the model predicts that the NS infusion causes an immediate increase of about 7% in plasma volume, but by the end of the experiment, all of this increase is dissipated to the neighboring interstitium or lost through urine. At t = 4 h, there is no significant plasma retention of the NS infusate.

The changes in plasma volume are reflected in the fluid excretion by the kidney as illustrated in Fig. 8-16, lower panel. In good agreement with the experimental data, the simulations show that the only significant changes in the urinary rate from its baseline value take place during, and about 1 h after the infusion. At subsequent times, both the model and the experiments indicate that the urinary rate returns to near normal values. The average computed urinary output rate for the time interval between t = 1.84 and t = 2.35 h (i.e., the time interval that includes the fluid administration) is 142 ml/h; a value that compares very well with the measured urinary rate of 145 ml/h reported for the same time interval. For the last hour of the experiment the average model-predicted urinary output is 105 ml/h and is, once again, in close agreement with the value of 95 ml/h reported by Sondeen et al., 1990(a)].
Figure 8-17 presents the model-predicted changes as well as the experimental results for the plasma sodium concentration (upper panel) and the renal excretion rate of sodium (lower panel). As shown in this figure, the simulated changes in $[\text{Na}]_{PL}$ and $M_{Na,U}$ are well corroborated by the experimental results of Sondeen et al. The administration of NS results in a small, short-lived decrease in $[\text{Na}]_{PL}$. Within about 10 minutes post-infusion and up to the end of the experiment, the concentration of this ion increases by about 0.5 mEq/l compared to the infusion level, a value that has no statistical significance. Although the NS infusion does not change $[\text{Na}]_{PL}$ significantly, the total amount of sodium in the extracellular compartment is slightly increased. The renal response, presented in the lower panel of Fig. 8-17, attempts to counterbalance the increase in plasma sodium by increasing its excretion rate. According to the model predictions, the renal sodium excretion increases immediately after infusion and remains slightly elevated up to $t = 4$ h (i.e., the end of the experiment). From $t = 2$ to $t = 4$ h, both model and experiments show that the average sodium excretion rate is twice its normal value.

Figure 8-18 shows a comparison between the model predicted (solid line) and the measured (solid circles) Hct values for the HSD infusion case. Once again, as soon as the HSD bolus is administered, the Hct starts to decrease; a direct consequence of the increase in plasma volume. The model predicts that Hct decreases from an initial value of 30% to approximately 23% at the end of the infusion period. Once the infusion is over, Hct then returns toward normal, reaching a model-predicted and experimental value of about 27.5% at $t = 4$ h.

A comparison between the model-predicted Hct changes when the same volume of either NS (see Fig. 8-15) or HSD (see Fig. 8-18) is administered shows that the HSD infusion is characterized by a marked plasma hemodilution. As discussed in Chapter 7, the marked decrease in Hct is due to fluid mobilization into plasma from the cellular and interstitial reservoirs. By the end of the experiment, the Hct has decreased by about 3.5% for the HSD infusion and only by about 0.5% for the NS case. The final Hct value for the HSD infusion is a reflection of the improved plasma fluid retention in this case. As is illustrated in Fig. 8-19, upper panel, the plasma volume starts to increase as soon as the HSD infusion begins and reaches a maximum of about 30% above normal at the end of the infusion period. Although some of the plasma fluid is then transferred to the interstitium or lost through the kidneys, at 2 h post-infusion, the model predicts a plasma retention of about 0.92 ml/ml-HSD infused.

The higher urinary output rates associated with HSD administration are shown in the lower panel of Fig. 8-19. Although, with the exception of the value for the last hour of the
experiment, the predictions using the proposed kidney model are within the experimental error of
the measurements reported by Sondeen et al., the model generated results seem to overestimate
somewhat the experimental data points. However, both model and experiment show a significant
increase in urinary output immediately after the start of infusion and about 1 h thereafter.
According to the study of Sondeen et al., when the same volume of either NS or HSD is
administered, the same value for urinary output, i.e., of 95 ml/h, is achieved at 2 hour post-
infusion. For this interval however, the simulations predict an average urinary rate of about 180
ml/h. This predicted result is more in agreement with the experimental study Tølløfsrud et al.

The dynamic changes predicted by the model for the plasma sodium concentration and
the sodium excretion rate are illustrated, together with the experimental data, in the two panels of
Fig. 8-20. As predicted by the model, following HSD administration, $[\text{Na}]_{\text{PL}}$ increases, reaching
a maximum of about 19 mEq/l above its baseline value by the end of infusion. This increase is
however very short-lived and the excess plasma sodium dissipates rapidly to the interstitium over
the next 10 minutes. As shown in the lower panel, the renal excretion of sodium also increases in
parallel with $[\text{Na}]_{\text{PL}}$. According to both the simulated and experimental results, for the time
period between 1.84 h and 2.35 h, the kidney produces an average excretion rate that is 4-5 times
higher than normal; a direct response to the high sodium content of plasma resulting from the
HSD infusion. By the end of the experiment, in agreement with the experimental data, the model
predicts an increase in the plasma sodium concentration which is about 6 mEq/l above normal
(upper panel). As shown in the lower panel, the kidney responds to this increase in $[\text{Na}]_{\text{PL}}$ via an
enhanced excretion of sodium which, as indicated by both the model and experiment, remains
elevated at the levels reached immediately post-infusion.

8.5.5.2 Validation based on data from mild hemorrhage followed by HSD administration

The experimental information relating to this case was described previously in Section
7.2.2. Stated briefly, in the study of Tølløfsrud et al. [1997], 10% of the normal blood volume of
several human volunteers was removed over a 15 minute period. After 45 minutes of
stabilization, an infusion of 4 ml/kg HSD solution was administered for 10 minutes. The urinary
output rates together with the excreted small ion concentrations for this mildly hypovolemic case
were separately provided by one of the authors of this study [Tølløfsrud, personal
communication].
This HSD infusion case was simulated according to the experimental protocol using the initial conditions for renal excretion given in Table 8-4. Figure 8-21 illustrates the renal output of fluid resulting from the mild plasma volume depletion followed by a hypertonic/hyperoncotic volume expansion. The upper panel of the figure shows a comparison between the computed average urinary rates predicted by the model (dashed lines) and the average values measured by Tøløfsrud et al. (solid circles). The lower panel of this figure plots the continuous profile of urinary output predicted by the model. The three time intervals used for calculating average rates are: \( t = -1.5 \) to \( t = 0 \) h (symbolized by H) corresponding to the period of blood removal followed by a 45 minutes of stabilization; \( t = 0 \) to \( t = 1 \) h (I) corresponding to the period of HSD infusion and plasma volume expansion; and \( t = 1 \) to \( t = 3 \) h (R) corresponding to post-infusion recovery period.

As indicated in the upper panel of Fig. 8-21, the mild hemorrhage results in a slight decrease in urinary output from an initial value of 60 ml/h to an average value of about 50 ml/h. Similar to the study of Sondeen et al. [1990(a)] presented earlier, as a result of HSD infusion, the kidney responds with a marked diuresis for the entire (I) interval. In good agreement with Tøløfsrud's measurements, the model predicts an increase in urinary output of about 3.7 times its normal value. The diuresis continues for up to at least 3 hours after the administration of fluid. Corresponding to the (R) interval, the model predicts an increased urinary rate of 131 ml/h, while the experimental value was 140 ml/h (see also Table 8-6).

Table 8-6 presents a comparison between the results of the simulations and data for the excretion rates and concentrations of sodium, potassium and chloride ions provided by Tøløfsrud [personal communication]. All of the values generated by the model are well corroborated by the experimental measurements.

As shown in Table 8-6, both the (I) and (R) intervals are characterized by an enhanced natriuresis despite an initial blood loss. As predicted by the model, the sodium excretion rate decreases slightly after hemorrhage from a control value of about 7.7 to 6.9 mEq/h, but after HSD administration, this ion is eliminated at high rates from plasma. The predicted values of 31.5 and 31.3 mEq/h for the (I) and (R) intervals, respectively, are in good agreement with the measured mean values of 31.3 and 30.8 mEq/h, respectively, for the same intervals of time.

Also in good agreement with the experiments, the model predicts that HSD infusion results in an increased excretion of potassium and chloride ions from the body. According to the model predictions, the excretion rate of potassium decreases slightly from an initial control value of
Figure 8-21: The upper panel presents a comparison between the computed average urinary rates (dashed lines) and experimentally measured values (solid circles) for the hypovolemic HSD case investigated by Tøløfsrud et al. [1997]. The lower panel shows the continuous urinary output values predicted by the model. The error bars indicated on both figures correspond to the experimentally measured standard deviations reported by the experimental study. The arrows on the time axis indicate the duration of hemorrhage as well as of HSD infusion.
Variable | Time Interval | Model predicted value | Experimental value
---|---|---|---
$J_U$ (ml/h) | (H) | 50.3 | ---
 | (I) | 221.9 | 224.0 ± 80
 | (R) | 131.0 | 140.0 ± 50
$M_{Na,U}$ (mEq/h) | (H) | 6.9 | ---
 | (I) | 31.5 | 31.3 ± 8.2
 | (R) | 31.3 | 30.8 ± 11
$[Na]_U$ (mEq/l) | (H) | 137.2 | ---
 | (I) | 141.9 | 140.0 ± 35.2
 | (R) | 238.9 | 220.0 ± 47.8
$M_{K,U}$ (mEq/h) | (H) | 2.6 | ---
 | (I) | 14.5 | 17.4 ± 4.2
 | (R) | 14.3 | 14.9 ± 6.4
$[K]_U$ (mEq/l) | (H) | 51.7 | ---
 | (I) | 65.3 | 78.0 ± 20.5
 | (R) | 109.1 | 106.0 ± 28.0
$M_{Cl,U}$ (mEq/h) | (H) | 7.2 | ---
 | (I) | 39.3 | 39.8 ± 10.5
 | (R) | 38.9 | 34.9 ± 11.4
$[Cl]_U$ (mEq/l) | (H) | 144.1 | ---
 | (I) | 177.1 | 174.0 ± 46.5
 | (R) | 296.9 | 266.0 ± 65.6

Table 8-6: Renal excretion of fluid, sodium, potassium and chloride after a 10% hemorrhage followed by 4 ml/kg HSD infusion: comparison between model predictions and experimental data of Tøløfsrud et al. [1997] and [Tøløfsrud, personal communication]. Data represent averages for the following intervals: (H) t = -1.5 to t = 0 h; (I) t = 0 to t = 1 h; (R) t = 1 to t = 3 h.
3 mEq/h to about 2.6 mEq/h after the mild hemorrhage, but in agreement with the measurements, is increased by about five times for the entire post-infusion period. The model shows a decrease of 1 mEq/h in the average excretion rate of chloride ion after the blood removal. Once again, after HSD administration, both the experiments and the model indicate an enhanced elimination of this ion from the body. For the (I) and (R) intervals, the model predicts chloride excretion rates of 39.3 and 38.9 mEq/h, respectively; the experimental values are 39.8 and 34.9 mEq/h, respectively.

The good agreement for the urinary output rates and the excretion rates of sodium, potassium and chloride is reflected in the urinary concentration of these ions. As shown in Table 8-6, all the values measured are closely predicted by the model.

Fig. 8-22 shows the model-predicted values for sodium clearance that correspond to both the normovolemic HSD case and hypovolemic HSD cases studied by Tølløfsrud et al. [1997]. Illustrated in this figure is the reduced sodium clearance that occurs after the blood is removed compared to the non-hemorrhage case. As predicted by the model, during and after HSD administration, both hemorrhaged and non-hemorrhaged humans manifest increased sodium clearances, although in the former case, as expected, the values are lower.
8.5.5.3 Validation based on data from graded hemorrhages in pigs

The experiments of Sondeen et al. [1990(b)] involved normal swine having an average body weight of 21.2 kg that were splenectomized. The animals were deprived of food for 24 h prior to experimentation, but were allowed access to water. The pigs were subjected to graded hemorrhages up to a total external blood loss of 42% of the initial blood volume. To achieve this objective, blood was removed in 4 increments of 7 ml/kg over 9, 10, 12, and 13 min. The removal of blood was not followed by any type of resuscitation. The purpose of this study was to provide an insight into the hemodynamics and excretory properties of the kidney after a hemorrhage severe enough to produce shock. Several experimental studies [e.g., Zimpfer et al., 1982] had shown that anesthesia alters the renal response to hemorrhage. Thus, in order to obtain renal outputs corresponding to a real trauma the animals in this study were not anesthetized.

The initial compartmental values for hemorrhaged pigs were not provided by Sondeen et al. [1990(b)]. Therefore, the same compartmental values as were employed for simulating the results of Sondeen et al. [1990(a)], were chosen. These values, including the interstitial compliance relationships described previously for other animal species (see Section 6.3.2), are reported in Appendix E (see also [Hannon et al., 1990] for normal physiological values in swine). Appendix E also includes the average initial urinary output and sodium excretion rates reported by of Sondeen et al. [1990(b)]. These renal values were imposed as initial conditions for the simulations.

Among other variables, the study of Sondeen et al. [1990(b)] provides information about the renal excretion rates of fluid and sodium. Additionally, based on reported measurements of glomerular filtration rates together with the fractional excretions of sodium, the renal clearances of the sodium ion could be calculated and compared with the values predicted by the model.

A comparison between the model predictions and experimental measurements for urinary output is shown in Fig. 8-23. The upper panel plots the continuously falling urinary output that is predicted in response to the depleted plasma volume resulting from blood removal. The lower panel provides a direct comparison between the computed averages obtained by the model (dashed lines) and the measured data (closed circles). Both the model and experiments indicate that, up to a 21% degree of hemorrhage, the urine output, although it decreases, still falls within the normal physiological range of 50-150 ml/h (for normal range of urinary output in pigs see also Table E-3 in Appendix E). According to the simulations, for a 21% hemorrhage, the urinary rate decreases from a normal value of 92.4 ml/h to an average of about 56.6 ml/h. For degrees of
hemorrhage above 21%, the urinary rate is significantly decreased. According to Sondeen et al. [1990(b)], at the end of the 31.5% hemorrhage, the urinary rate decreases by about 70 - 74%. For this degree of hemorrhage, the model-predicted decrease in urinary rate is about 60% from normal; a value that is within the error of the measurement. As blood continues to be removed, the renal fluid output decreases even further. After 42% blood removal, the urine output decreases by about 82% or 90% from normal as predicted by the model or reported by Sondeen et al., respectively.

The renal elimination of sodium in response to a graded hemorrhage is illustrated Fig. 8-24. The figure demonstrates that, throughout the hemorrhage period, there is a reduced elimination of sodium from plasma. Both simulations and measurements show that the excretion rate for this ion decreases from a normal value of about 5.9 mEq/h at the beginning of the experiment to zero mEq/h after 42% of the blood has been removed.

The last set of results presented in Fig. 8-25, increases our confidence that the proposed kidney model, although simple in nature, is formulated upon reasonable clinical assumptions. This figure shows a comparison between the model-predicted renal clearances of sodium with the corresponding clearance values calculated from the experimental data of Sondeen et al. As mentioned in the introductory section, the renal clearance reflects the combined effect of glomerular filtration in conjunction with the intrinsic tubular transport processes. As indicated in Fig. 8-25, the model yields very good predictions of the experimental data and demonstrates that, at the end of 42% hemorrhage, the kidney responds by altering the excretion rate of sodium until the clearance value of this small ion is virtually zero. For completeness, the lower panel of Fig. 8-25 illustrates how the model predicts a gradual decrease in the urinary sodium concentration as the deficit in plasma volume increases.

8.6 Conclusions

The basis upon which the model for renal excretion was formulated assumes that a departure of plasma volume from a given 'set-point' determines a change in the renal urinary output. In addition, a change in the plasma sodium concentration from its normal 'set-point' is responsible for altering the sodium excretion rate. Additionally, with the exception of the hyperosmolar infusion cases, the term F(U) in Eq. [8-8] makes sodium excretion (and implicitly the excretions of all other small solutes) directly dependent on the changes in renal fluid flow rate.
Figure 8-23: The upper panel shows the continuous urinary output profile predicted by the model for increasing degrees of hemorrhage (solid line) while the lower panel presents a comparison between the computed average urinary rates (dashed lines) and the experimentally measured values (solid circles) based on the work of Sondeen et al. [1990(b)]. The arrows on the time axis indicate the intervals of blood removal. The % 'Hem' values represent the cumulative blood losses. Note that the percentage of blood removed during each time interval is always 10.5% of the normal blood volume.
Figure 8-24: The upper panel plots the continuous sodium excretion rate profile predicted by the model for increasing degrees of hemorrhage (solid line), while the lower panel presents a comparison between the computed average sodium excretion rates (dashed lines) and the experimentally measured values (solid circles) reported by Sondeen et al. [1990(b)]. For more details see the caption of Fig. 8-23.
Figure 8-25: The upper panel shows a comparison between the model-predicted renal clearance of sodium and the values calculated using measured data from Sondeen et al. [1990(b)]. The lower panel presents the continuous changes in the urinary sodium concentration predicted by the model. More details are provided in the caption of Fig. 8-23.
The proposed model for renal excretion provided satisfactory predictions for the following three important cases encountered in clinical settings: plasma volume deficit (i.e., hemorrhage not followed by resuscitation), isotonic plasma volume expansion (i.e., by NS or RS infusions) and hypertonic/hyperoncotic volume expansion (i.e., HSD infusions). Since a hemorrhage model has not been validated at this point, the renal response to hemorrhage followed by the administration of different types of resuscitants was not discussed in this chapter.
Chapter 9: APPLICATION OF THE MODEL TO THE STUDY OF HEMORRHAGE

9.1 Introduction

Mathematical models can also be used to describe and assess mass-transfer related mechanisms associated with the pathophysiological conditions that follow a severe trauma. In this chapter, we extend the validated model of human microvascular exchange to study the consequences of hypovolemic conditions. Hypovolemia can result from different traumatic factors such as external blood loss (e.g., external hemorrhage), internal blood or plasma loss (e.g., burns, soft tissue trauma), etc. The primary objective of this chapter is to demonstrate the applicability of the model to the study of hypovolemia and hypovolemic shock produced by external hemorrhage.

Hemorrhagic shock is one of the most commonly encountered types of shock and probably the one that has been most extensively studied. However, the pathophysiological disturbances occurring during the progressive evolution of this type of shock are not completely understood.

The term hypovolemia means a depletion of the circulatory volume. The basic function of the circulatory system is 'to service' the needs of all the tissues in the body (i.e., to transport the nutrients and hormones to the tissues and to remove the waste products of cellular metabolism from the tissues). Hence, a reduction in the circulatory volume diminishes or even impedes completely the ability of the circulatory system to maintain the optimal, constant environment required by the cells of the body. Hypovolemic shock is not a disturbance that can be described as a single event. Rather, it is a succession of disturbances, a syndrome [Shoemaker, 1979], that can be defined as a generalized failure of the circulation to meet the tissue demands. Initially only peripheral tissues are compromised, but ultimately, vital tissues are also affected [Haljamae, 1985]. Shock occurs when tissues become severely damaged due to reduced blood flow and/or nutrient and oxygen delivery [Haljamae, 1985].

Generally, a multitude of compensatory mechanisms (nervous reflexes as well as hormonal mechanisms) act synergistically to counteract an initial hypovolemic derangement and to restore the normal body homeostasis. This phase is commonly referred to as the compensatory stage. However, if the hypovolemic insult is severe enough and/or alternatively, of long duration
and is left untreated, then hypovolemic shock ensues. This second phase is known as the decompensatory stage of shock.

The first part of this chapter attempts to provide a better understanding of the hypovolemic events that follow the external loss of blood through hemorrhage. The discussion is mainly qualitative and targets the following issues:

- a description of the magnitude and the time-frame of the sequence of disturbances that occur during the evolution of a hemorrhagic episode.
- a presentation of the most commonly held views about the pathophysiology of shock-induced disturbances.

The second part of this chapter provides information about the disturbed transport of fluid and solutes that occurs during a hemorrhagic event as predicted by a modified version of the compartmental human model developed in Chapters 4 and 8. The purpose of this preliminary investigation is to demonstrate that the model can be successfully applied to assist our understanding of the pathophysiological changes that arise following a severe hemorrhage. It is envisaged that the model will benefit from a more sound formulation and validation in the near future.

9.2 Hemorrhagic Shock

9.2.1 Classification of hemorrhagic shock

There are varying sequential characteristic disturbances that correspond to different degrees of hemorrhage. These disturbances are met in turn by different compensatory mechanisms or, alternatively, a lack thereof. Based on the sequence of events that occur, hemorrhagic shock can generally be divided into three major stages [Guyton, 1991]: 1) the compensatory (non-progressive) stage, 2) the progressive stage and 3) the decompensatory (irreversible) stage. All three stages of shock appear in Fig. 9-1, which illustrates the relationship between the change in arterial pressure and the degree of the untreated hemorrhage [Guyton, 1991].

The non-progressive stage of shock, as shown in Fig. 9-1, corresponds to relatively low blood volume losses (i.e., typically up to 10-15% of normal). During this stage, compensatory mechanisms are able to provide a full or partial recovery of the hemodynamic variables even in the absence of adjuvant fluid therapy. Usually the arterial pressure and cardiac output are maintained at or near normal values.
Figure 9-1. The figure illustrates the results of several experiments in which dogs were bled rapidly up to a given degree of hypotension. All dogs for which the arterial pressure was not lowered by more than 50% recovered (cases I, II, III corresponding to a blood loss <30%). As expected the degree of compensation is inversely related to the degree of hemorrhage. In those cases where the arterial pressure was lowered more than about 50% of normal (i.e., cases IV, V and VI where the blood loss is >30%) the animals eventually died of hypovolemic shock. The plateau observed between 60 and 180 min, indicates an interplay of compensatory mechanisms to maintain a constant arterial pressure. From this illustration, one can identify a maximum blood loss below which the compensatory mechanisms successfully restore the arterial pressure (i.e., between curves III and IV). Even a few degrees of hemorrhage above this threshold will cause irreversible damage. From Guyton [1991].

The progressive stage occurs as a result of mild to severe blood losses of up to 15-30% of normal. Immediately after the onset of such a hemorrhage, the compensatory mechanisms are able to provide a partial recovery of the decreased arterial pressure and cardiac output. However, if therapy is not provided, the shock will gradually worsen eventually causing death. The progressive stage was described by Guyton [1991] as the stage where "shock breeds more shock", i.e., the same compensatory mechanisms that were activated to compensate an initial hypovolemic insult, cause a positive feedback control that produces irreversible damage.

The irreversible (decompensatory) stage of shock is characterized by the inadequacy of either internal compensatory mechanisms or external fluid therapy to save the victim’s life. This type of shock normally corresponds to a severe hemorrhage of more than about 35% of normal.

It should be noted that the percentages of blood loss mentioned above for defining the different stages of shock are only approximate. For instance, a 25% blood loss that is not
immediately treated by adjuvant therapy, will induce a severe hypotension which in turn will lead to irreversible damage. Additionally, the degree of hemorrhage, which can be sustained by different animal species, varies widely. For example, based on percentage of blood loss, cats and dogs can withstand a higher degree of hemorrhage than humans. However, as it will be shown shortly, a reasonable choice of a 'threshold' degree of hemorrhage required to produce a severe hypotension for several animal species including humans, is about 30%. Additionally, there are other factors, beside the percentage of blood lost, including the nutrition and hydration status of the individual and the acuteness of the insult, that influence the progression of hemorrhagic shock. Ultimately, it is a combination of clinical and hemodynamic variables that determines the stage of shock.

9.2.2 Progression of hemorrhagic shock

After the onset of the hemorrhage, as a direct result of a depleted intravascular volume, the cardiac output and arterial pressure are reduced. Following the hemorrhage, the body activates a series of defense mechanisms whose objectives are [Haljamae, 1993]: a) to maintain sufficient blood flow to the central organs (such as the heart and the brain) at the expense of tissues less important for immediate survival (such as the peripheral tissues - mainly skeletal muscle, skin and intestines) [Mellander, 1978] and b) to prevent any further fluid and solute losses from the body. Some of the most frequently described physiological changes associated with these compensatory actions are as follows [Haljamae, 1985; Darlington et al., 1995]:
- increased vascular constriction in peripheral tissues (such as skin and skeletal muscle),
- decreased vascular resistance in vital tissues such as brain and heart,
- increased heart rate and myocardial contractility, and
- reduced renal function in order to prevent any further fluid losses from the body (see also Chapter 8).

These defense mechanisms are triggered by increases in both neural and hormonal activation [Chien, 1967]. As pointed out by Drucker et al. [1981], the diversion of the flow away from the peripheral tissues might very well be an anatomical coincidence. The sympathetic fibers are more concentrated in the vasculature of skin, kidney, muscle and intestines, but are sparse along the vessels perfusing the brain, heart and lungs [Drucker et al., 1981; Guyton, 1991]. Thus, the sympathetic discharge associated with any type of bodily stress, including hemorrhage, will have a higher impact on constricting the peripheral vessels, with a net effect of diminishing the
blood flow to these tissues. As a result of peripheral vasomotor reflexes, the hemorrhage will produce important disturbances in terms of transport and perfusion at the microvascular level. Observation of the microvasculature in skeletal muscles by microscopic techniques [Amundson et al., 1980] revealed that, following an acute hypotension producing hemorrhage (i.e., the hemorrhage produces a decrease in arterial pressure to about 40 to 50 mmHg), the intense peripheral vasoconstriction that takes place can cause a complete cessation of flow for as long as 5-15 min. When the flow reappears, the vasomotion typical of resting skeletal muscles is replaced by an uneven, low flow. It was also estimated that the number of perfused vessels is reduced to 30-50% of the normal value [Amundson et al., 1980; Haljamae, 1985].

A direct consequence of the peripheral vasoconstriction and the reduction of blood flow to these tissues, is a decrease in the average capillary pressure in the peripheral microvascular beds. Experimental induction of hemorrhage in animals has shown that this decrease leads to a net fluid absorption into the vascular compartment [Mellander and Lewis, 1963]. Additionally, it has also been proposed that the increased output in cathecolamine immediately post-hemorrhage activates glycogenolysis and produces hyperglycemia and implicitly, plasma hyperosmolarity [e.g., Jarhult, 1973; Gann, 1982]. Plasma hyperosmolarity constitutes a second factor that contributes to a net fluid mobilization into the plasma compartment. This compensatory mechanism occurs at the expense of the extravascular fluid (interstitial and/or tissue cellular fluids), and is known as transcapillary refill [Drucker et al., 1981] or blood (plasma) volume restitution [Gann et al., 1982].

The sequence of events described up to this point are representative of the compensatory (non-progressive and progressive) stages of hemorrhage. With prolonged hypovolemic shock or, conversely, after a specific high threshold value of blood loss, there is a gradual decline in the effectiveness of the compensatory mechanisms that assure adequate perfusion of the vital tissues. This gradual breakdown is possibly a reflection of the generalized depletion of energy within the body. Based on literature reports, the possible events of compensatory deterioration that lead to shock and/or are characteristic of shock can be summarized as follows:

1) The fluid absorption into plasma ceases and the transcapillary refill fails to be effective [Gann et al., 1981].

2) The peripheral vascular wall no longer responds to the vasoconstrictor sympathetic activity [Mellander, 1978; Mellander and Lewis, 1963].
3) The liver is depleted of its glycogen reserve and therefore glucose mobilization into plasma ceases [Jarhult, 1973].

4) The peripheral tissues become highly hypoxic [Amundson et al., 1980; Guyton, 1991].

5) The interstitium accumulates toxic metabolites [Jennische et al., 1978(b)].

6) The interstitial fluid along with the plasma volume is reduced [Shires et al., 1972].

7) The skeletal muscle cells and possibly the RBCs start to manifest a defect in the transport of small ions and water which is reflected by the depolarization of the cell membrane potential [Haljamae, 1970; Shires et al., 1972; Trunkey et al., 1973; Nakayama et al., 1984].

8) As a result of the defective transcellular transport, the cells swell and accumulate sodium.

   It is not clear which of the above-mentioned disturbances are of primary importance in determining the final stage of decompensated shock. It is possible that each of the enumerated events, or the combined effect of some of them, can trigger the irreversibility of shock.

In what follows, a more quantitative description of the mechanisms that take place after blood removal and their relevance to the changes in the four compartments of interest to this work is presented. The discussion assumes the accepted hypothesis that the hemorrhagic shock takes place in two stages: a hemodilution phase (i.e., where compensatory fluid absorption occurs) and a hemoconcentration phase (characterized by decompensation).

9.2.3 Hemodilution phase

For more than a century, it has been suggested that the initial stage of hemorrhage is characterized by hemodilution. As mentioned above, this distinct period of time that follows a hemorrhagic event is also referred to as the transcapillary refill, blood volume restitution, or compensatory hemodilution phase. Evidence for such a phase is provided by observations of decreased Hct and plasma colloid osmotic pressure values in the period immediately following the hemorrhage. Several studies [e.g., Chien, 1958; Haddy et al., 1965; Jarhult, 1973; Gann et al., 1982] have confirmed the above conclusions in experiments involving both animal and human subjects. The hemodilution is due to a continuous absorption of fluid into plasma from interstitium and/or the intracellular fluid compartments. This transcapillary absorption, that largely compensates the fluid lost from plasma, occurs mainly in skin and skeletal muscles and has a maximal rate within one hour post-hemorrhage, irrespective of the degree of hemorrhage [Mellander and Lewis, 1963; Jarhult, 1975; Gann et al., 1982]. Plasma volume restoration via
fluid absorption is due to the combined effect of a decrease in the capillary pressure and an increase in plasma osmolarity. Each of these factors is discussed separately below.

a) Plasma hemodilution due to a decrease in capillary pressure

The reduction in plasma volume due to hemorrhage and the consequent peripheral vasoconstriction causes a decrease in the capillary pressure, $P_c$. According to the Starling equation (Eq. [4-15]), depending on the magnitude of the decrease in $P_c$, there will be a reduction of net fluid filtration or, alternatively, a reabsorption of fluid into the vasculature from the neighboring interstitium. Zweifach [1974] reported that, even for a large hemorrhage, the reduction in capillary pressure is relatively small (i.e., within 10-20% below normal), however, $P_c$ still becomes sufficiently low to cause fluid absorption into the vasculature. Due to difficulty in measuring $P_c$, the values presented by Zweifach [1974] might be underestimated. Nonetheless, it can be calculated that, in the absence of changes in any of the other Starling forces, a 15% reduction of the capillary pressure from its normal value of 11 mmHg will cause a reversal of $J_F$ from 75 ml/h to about -75 ml/h.

If the fluid absorbed in plasma is protein-poor then this translocation of fluid will cause the following sequential changes [Drucker et al., 1981]:
- decreases in the hematocrit, protein concentration and colloid osmotic pressure of the plasma compartment,
- decreases in the volume and hydrostatic pressure, and an increase in the colloid osmotic pressure of in the interstitial compartment, and
- decreases in the lymphatic return of fluid and solutes (proteins and small ions).

As fluid is continually absorbed into plasma, the capillary hydrostatic pressure increases until a new balance in Starling forces and a new steady state is achieved.

b) Plasma refill due to an increase in plasma osmolarity

More than a century ago, Claude Bernard observed that glucose concentrations increased in the blood of dogs subjected to hemorrhage [see Drucker et al., 1981]. This finding was confirmed later on for several other species including humans [Halmagyi et al., 1966; Carey et al., 1971; Friedman et al., 1982]. Independently of these observations, numerous other studies revealed that, after the onset of hemorrhage, there is an increase in the plasma osmolarity of humans, dogs, cats and rats [e.g., Baue et al., 1967; Boyd and Mansberger, 1968]. It was largely
due to the experimental effort of Jarhult [1975] and later Gann and his co-workers [Gann et al., 1981; 1982] that this increase in plasma osmolarity was directly linked to the increase in plasma glucose. These studies showed that hemorrhage was associated with an increase in blood osmolarity and that glucose, released mainly from the liver, accounts initially for more than 90% of the short-term osmolarity change. Additionally, these researchers found that hyperglycemia can lead to a transcapillary osmotic fluid absorption into plasma. Rather than being a sign of whole-body collapse post-hemorrhage, this mechanism is actually essential to the compensatory stage of hemorrhage [Jarhult, 1975; Gann et al., 1982; Pardon et al., 1983; Darlington et al., 1995]. Thus, in addition to the transcapillary refill caused by decreased capillary pressure, a second factor, the so-called glucose-osmotic mechanism of transcapillary refill [Jarhult, 1975; Gann et al., 1982; Darlington et al., 1995] may also be important. However, there still remains some controversy about how hyperglycemia causes transcapillary refill.

Jarhult [1975], based on his studies on hemorrhaged cats, proposed that the glucose released post-hemorrhage restores the vascular volume by causing an osmotic shift of water from the interstitium. He demonstrated that, once again, it is skeletal muscle, skin and intestinal tissues that are the principal participants in this type of fluid absorption. A summary of his experimental findings with respect to the role of plasma glucose and implicitly, plasma osmolarity, in restoring plasma volume is presented in Table 9-1.

Jarhult’s experiments consisted of short-duration (3 min.), large-volume hemorrhages of about 30 to 35% that were sufficient to reduce the arterial pressure to a level of about 40 to 50 mmHg in these animals. The reported data are for skeletal muscle tissue; however, similar observations were made by this author with respect to skin and intestinal tissues [Jarhult, 1975]. Some of these findings have been corroborated by other studies [e.g., Gann et al., 1981; Friedman et al., 1981; Kenney et al., 1983; Davies et al., 1984].

Jarhult attempted to quantify separately the effects of a decrease in the capillary hydrostatic pressure and the release of glucose on plasma fluid absorption. In his experiments, he eliminated the sympato-adrenal reflex mechanisms (by performing surgical and pharmacological sympathectomy) and, in some experiments, he eliminated alterations in capillary pressure by maintaining a constant venous flow. The resulting fluid absorption volumes and the contributions due either to hydrostatic or osmotic effects are summarized in Table 9-2. His data are representative of cat skeletal muscle. To gain a better idea about the magnitude of transcapillary refill in humans, the last three columns of the table show the volumes of fluid
absorbed when the cat results are extrapolated to a 70 kg 'Reference man'. For these calculations, it was assumed that the combined peripheral tissues constitute 60% of the total body weight. The results are only approximate since there likely are important interspecies differences in the hemodynamics of cats and humans.

A. Changes in plasma osmolarity

1. A change in plasma osmolarity (i.e., > 3mM) is observed within the first minute post-hemorrhage.

2. The most rapid increase in plasma osmolarity occurs within 10 min of hemorrhage.

3. Plasma osmolarity increases to a maximum of 25-30 mOsm/l after 20-30 min of arterial hypotension.

4. Hyperosmolarity remains at an elevated level between 1.5 and 2 h post-hemorrhage.

5. For other degrees of hemorrhage the increase in plasma osmolarity is related to the severity of blood loss.

6. An increase in plasma osmolarity is observed for a degree of hemorrhage as low as 10%.

B. Changes in plasma glucose

1. The increase in plasma osmolarity is closely paralleled by hyperglicemia up to 30 to 40 minutes post-hemorrhage (after this time the release of other substances or other mechanisms are responsible for the observed increase in plasma osmolarity).

2. After 1 hour post-hemorrhage, plasma glucose starts to decrease gradually.

C. Other Plasma Solutes

1. Plasma K⁺ and urea start to increase at 40 - 60 min post-hemorrhage. These solutes remain elevated even after glucose starts to decline, i.e., after 1 h post-hemorrhage.

2. Plasma sodium concentration starts to decrease slightly at 30-40 min post-hemorrhage.

Table 9-1. Summary of changes in plasma osmolarity, plasma glucose and other plasma solutes for a severe, non-resuscitated hemorrhage of 35% in cats [Jarhult, 1975].
Table 9-2. The rate of refill of plasma volume at different times after a non-resuscitated 35% hemorrhage in cats [Jarhult, 1975] and humans (estimated based on Jarhult's data). The total fluid absorption is divided between two contributions: that due to an increase in plasma osmolarity (osmotic contribution) and that due to a decrease in capillary pressure (hydrostatic contribution).

<table>
<thead>
<tr>
<th>Time after hemorrhage (min)</th>
<th>Fluid Absorption (ml/min-100g)</th>
<th>Osmotic Effect (%)</th>
<th>Hydrostatic Effect (%)</th>
<th>Total Fluid Absorbed (ml)</th>
<th>Osmotic Contribution (ml)</th>
<th>Hydrostatic Contribution (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>35</td>
<td>65</td>
<td>420</td>
<td>150</td>
<td>270</td>
</tr>
<tr>
<td>20</td>
<td>1.4</td>
<td>55</td>
<td>45</td>
<td>580</td>
<td>320</td>
<td>260</td>
</tr>
<tr>
<td>60</td>
<td>2.4</td>
<td>60</td>
<td>40</td>
<td>1000</td>
<td>600</td>
<td>400</td>
</tr>
</tbody>
</table>

For a 70 kg human, a 35% hemorrhage represents an external blood loss of approximately 2 l. As can be seen from Table 9-2, at 1 hour after this large hemorrhage, an estimated 1 l of fluid is absorbed into plasma at the expense of the neighboring interstitium (and possibly from the tissue cells). Based on Jarhult's experiments, therefore, about 50% of the plasma loss is replaced due to internal autotransfusion of fluids from neighboring compartments. According to Jarhult, the interstitium is the source of fluid mobilization into plasma.

The studies performed by Gann and his collaborators [Gann et al., 1981; Gann et al., 1982; Darlington et al., 1995] on dogs, corroborated Jarhult's main conclusions regarding the glucose-osmotic mechanism presented above. However, their results disagree with those of Jarhult in two respects: a) the degree of hemorrhage for which the glucose-osmotic mechanism is effective, and b) the proposed mechanism for and source of fluid absorption in plasma.

It was shown by Gann et al. [1981] that, for hemorrhages up to 25 to 30% of the initial blood volume, the increase in plasma osmolarity is effective in mediating fluid shifts into the vasculature. In agreement with other studies [e.g., Friedman et al., 1981], they have also shown that, up to this level of hemorrhage, the increase in plasma osmolarity is related to the extent of
the hemorrhagic insult; however, it levels off at higher degrees of hemorrhage. Additionally, above a certain threshold of blood loss (around 25 to 30% of the initial blood volume), the release of glucose into plasma fails to provide further significant transcapillary absorption.

This group also argued that the intracellular fluid is probably the main source of water mobilization into plasma by the glucose-osmotic mechanism. This conclusion is in contrast with the study by Jarhult [1975] who proposed an interstitial fluid mobilization. Gann and co-workers propose that the released glucose will dissipate rapidly into the interstitial space where it will exert its osmotic pressure at the cellular level. The fluid shifted from the cells, which increases the interstitial pressure, will subsequently be transferred into the vasculature. This mechanism has in its favor experimental reports showing that after the onset of hemorrhage there is no cellular uptake of glucose due to an epinephrine mediated insulin resistance [Bessey et al., 1983; Black et al., 1982]. As a result, the glucose that is retained extracellularly might exert an osmotic water shift at the cellular level. The intracellular fluid mobilized into the surrounding interstitial space by this mechanism, may bring about plasma refill by increased lymphatic drainage and/or transcapillary absorption.

Neither of the above-mentioned studies offers conclusive experimental evidence for their hypotheses about the precise plasma refill mechanism generated by the release of glucose. Additionally, there are two other ambiguous issues related to glucose. Although it has been suggested that the amount of glucose released as well as the gain in plasma osmolarity are related to the degree of blood loss, due to a paucity of systematic hemorrhage studies, the duration and the rate of post-hemorrhage glucose release has not yet been elucidated quantitatively. One of the difficulties in obtaining such information is associated with the fact that the nutritional status of the animal (and implicitly the liver glycogen reserves) will also influence the hyperglycemic and hyperosmolar responses observed after hemorrhage [e.g., Stone, 1977].

9.2.4 Hemoconcentration phase

If the hypovolemic insult is persistent and external treatment is not provided, the period of hemodilution (transcapillary refill) is followed by a period of progressive hemoconcentration. Several pathophysiological events were reported to be characteristic of this progressive stage (see Section 9.2.2), which is defined by a marked increase in both hematocrit and plasma protein concentration together with a decrease in plasma volume. As mentioned previously, progressive hemoconcentration is brought about by a failure of the compensatory mechanisms to sustain the
Chapter 9: Application of the model to hemorrhage

223

hemodynamic variables at the necessary levels required for survival. Deficits in energy and tissue hypoxia are probably factors that have a negative effect on the compensatory mechanisms. Accumulation of toxic metabolites and their inadequate clearance, initially from the tissues due to poor perfusion, and ultimately from the body, due to the reduced (or complete cessation) of renal excretion, are also important contributors to this decompensation. The exact mechanisms that bring about hemoconcentration as well as their timing and importance are still not well understood. Moreover, in addition to the 'altered' Starling forces that are responsible for a disturbed distribution and transport of fluid and solutes between the plasma and interstitial compartments, there is a superimposed generalized cellular transport defect. As a result of these latter disturbances, a maldistribution of fluid and solutes between the intra- and extracellular spaces also takes place. Based on experimental evidence reported by Shires et al. [1984], a cellular defect that characterizes and is responsible for the disturbances seen in the pre-terminal phase of hemoconcentration will be incorporated into the hemorrhage model developed below.

9.2.4.1 Cellular damage during shock

The intensity of the vasoconstriction after blood loss can be directly correlated with both the magnitude and the duration of the hypovolemic insult. A direct result of this vasoconstriction is an even further reduction of the blood flow to peripheral tissues in addition to the low blood flow caused by the hypovolemic insult. Experimental investigations were undertaken in order to establish whether the cells in these tissues (especially the skeletal muscle cells which constitute the highest cell mass in the body) are also affected by the hemorrhagic insult. More than 30 years ago, Hagberg et al. [1968] and Campion et al. [1969] demonstrated that the transport of ions across the cellular membrane of skeletal muscle cells was significantly altered in the hypotensive stage of hemorrhage. Their experiments, conducted in vivo, involved recording the transmembrane potential of skeletal muscle cells before, during and after hemorrhagic shock. It was found that, once hypotension was present (arterial pressure of about 50 mmHg), the cell membrane potential $V_m$, depolarized from a resting muscle value of about -90 mV to about -60 mV. Other studies on skeletal muscle cells corroborated these findings [Haljamae et. al, 1970; Cunningham et al., 1971(a); Shires et al., 1972; Jennische et al., 1978; Nakayama et al., 1985]. Post-shock muscle biopsies revealed that, depending on the magnitude of the depolarization, the intracellular amounts and concentrations of sodium and chloride are significantly increased while the intracellular potassium is greatly depressed. Experiments performed by Shires et al. [1972]
showed also that, corresponding to a depolarized transmembrane potential of about -60 mV, the skeletal muscle cells swell. Reported increases in muscle cell volume ranged from 2 - 3% [Illner and Shires, 1980] to as much as 12% [Nakayama et al., 1985]. The disturbed transport across the cellular membrane is reflected by the composition of the intracellular fluid. Shires et al. [1972] further showed that the skeletal muscle swelling correlates with a marked decrease in interstitial volume and also with an increase in the interstitial and plasma potassium concentrations and contents. A summary of the available information reported by Shires group [Cunningham et al., 1971; Shires et al., 1972; Shires et al., 1984] shows that the transmembrane depolarization is present only during shock producing hypotension, is closely correlated with the sustained fall in blood pressure, and is always associated with an increase in potassium concentration of the extracellular environment (both plasma and interstitium). A typical response in cell transmembrane potential that occurs during hemorrhagic shock is depicted in Fig. 9-2.

![Figure 9-2. Changes in blood pressure, transcellular membrane potential and plasma and interstitial potassium concentrations as a function of time post-hemorrhage. The experimental results are for rats subjected to shock-producing hemorrhage. (Note, in this figure only, PD is the cell membrane potential while BP is the blood pressure) From Cunningham et al. [1971].](image-url)
There are several studies for different animal species that corroborate the conclusions mentioned above. Due to their importance in the study of hemorrhagic shock, some of the results of these studies are summarized in Table 9-3. As is demonstrated in this table, there appears to be a direct correlation between the decrease in the blood pressure and the depolarization of the membrane potential at different times post-hypotension. A depolarization of the membrane potential for mild and moderate hemorrhages (i.e., blood volume losses of less than about 25%) is seldom reported.

It is interesting to note that the cellular defect occurs irrespective of the duration and rate of bleeding. Table 9-3 presents cases involving both short-duration hemorrhage (i.e., less than 15 to 30 min.) and long-duration hemorrhage (i.e., greater than 30 min.). Similar findings of a cellular defect associated with hypotension-producing hemorrhages were also reported to occur in RBCs, including those of humans [Cunningham et al., 1971(b)].

Despite the abundance of experimental data with respect to cell depolarization dating back more than 30 years, the factors that trigger this observed cellular defect are still poorly understood. The arterial pressure seems to be the only indicator of cellular malfunction post-hemorrhage. It is unclear if these low arterial pressures are a cause or an effect of the observed cellular depolarization. The magnitude of hemorrhage that generates such a defect as well as the post-hemorrhage time when it occurs, are also not clearly established. From the results presented in Table 9-3, it appears that a non-resuscitated blood loss of about 30% is needed to produce a defective cellular transport.

It is generally described in the literature that tissue hypoxia and low tissue perfusion are responsible for the depletion in cellular energy that occurs during a hemorrhagic event [see e.g., Haljamae, 1994]. A malfunctioning Na⁺-K⁺-pump and an increase in the cellular membrane permeability for sodium or possibly both factors, may be responsible for the defect observed in skeletal muscle, liver and possibly in red blood cells.

Based on experimental studies that show that the cellular ATP content is normal (or sometimes even increases) during shock [e.g., Illner et al., 1982; Peitzman et al., 1985; Gallagher and Shires, 1989], it is clear that the ATP production is not the source of Na⁺-K⁺-pump disturbance. It is possible that external factors produce a malfunction of the Na⁺-K⁺-pump such that ATP is not properly utilized.

A great deal of experimental effort has also been directed toward identifying the so-called "toxic-factors" which are produced during hemorrhage. The hunt for toxic factors has at its origin
<table>
<thead>
<tr>
<th>Type and degree of hemorrhage</th>
<th>Arterial Pressure at the end of hemorrhage (mmHg)</th>
<th>Transmembrane Potential (mV)</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_25%</td>
<td>C: 150&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: 60 &lt;br&gt;t&lt;sub&gt;30&lt;/sub&gt;: 50 &lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: 50 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: 30</td>
<td>C: -90&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: -80 &lt;br&gt;t&lt;sub&gt;30&lt;/sub&gt;: -65 &lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: -65 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: -55</td>
<td>Rat</td>
<td>[Cunningham et al., 1971(a)]</td>
</tr>
<tr>
<td>S_30%</td>
<td>C: 160&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: 60</td>
<td>C: -90&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: -70 &lt;br&gt;t&lt;sub&gt;20&lt;/sub&gt;: -68 &lt;br&gt;t&lt;sub&gt;30&lt;/sub&gt;: -55 &lt;br&gt;t&lt;sub&gt;45&lt;/sub&gt;: -65</td>
<td>Rat</td>
<td>[Campion et al., 1969]</td>
</tr>
<tr>
<td>S_30%</td>
<td>C: 90&lt;br&gt;t&lt;sub&gt;90&lt;/sub&gt;: 50</td>
<td>C: -84&lt;br&gt;t&lt;sub&gt;90&lt;/sub&gt;: 65</td>
<td>Rat</td>
<td>[Nakayama et al., 1985]</td>
</tr>
<tr>
<td>L_35%</td>
<td>C: 150&lt;br&gt;t&lt;sub&gt;Shock&lt;/sub&gt;: 50</td>
<td>C: -91.5&lt;br&gt;t&lt;sub&gt;Shock&lt;/sub&gt;: -70</td>
<td>Rat</td>
<td>[Illner and Shires, 1980]</td>
</tr>
<tr>
<td>S_45%</td>
<td>C: 140&lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: 90 &lt;br&gt;t&lt;sub&gt;240&lt;/sub&gt;: 80</td>
<td>C: -90&lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: -84 &lt;br&gt;t&lt;sub&gt;240&lt;/sub&gt;: -78</td>
<td>Cat</td>
<td>[Amundson et al., 1980]</td>
</tr>
<tr>
<td>L_40%</td>
<td>C: 95&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: 42</td>
<td>C: -93&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: -79</td>
<td>Rabbit</td>
<td>[Chiao et al., 1990]</td>
</tr>
<tr>
<td>S_40%</td>
<td>C: 130&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: 60</td>
<td>C: -90&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: -80 &lt;br&gt;t&lt;sub&gt;20&lt;/sub&gt;: -75 &lt;br&gt;t&lt;sub&gt;30&lt;/sub&gt;: -70 &lt;br&gt;t&lt;sub&gt;45&lt;/sub&gt;: -65</td>
<td>Dog</td>
<td>[Campion et al., 1969]</td>
</tr>
<tr>
<td>S_50%</td>
<td>C: 150&lt;br&gt;t&lt;sub&gt;30&lt;/sub&gt;: 30 &lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: 50 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: 40 &lt;br&gt;t&lt;sub&gt;180&lt;/sub&gt;: 30</td>
<td>C: -87&lt;br&gt;t&lt;sub&gt;30&lt;/sub&gt;: -82 &lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: -70 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: -65 &lt;br&gt;t&lt;sub&gt;180&lt;/sub&gt;: -60</td>
<td>Dog</td>
<td>[Arango et al., 1976]</td>
</tr>
<tr>
<td>S_25-30%</td>
<td>C: 160&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: 75 &lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: 75 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: 55 &lt;br&gt;t&lt;sub&gt;180&lt;/sub&gt;: 40</td>
<td>C: -90&lt;br&gt;t&lt;sub&gt;10&lt;/sub&gt;: -90 &lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: -85 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: -80 &lt;br&gt;t&lt;sub&gt;180&lt;/sub&gt;: -60</td>
<td>Primate</td>
<td>[Shires et al., 1972]</td>
</tr>
<tr>
<td>S_35%</td>
<td>C: 98&lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: 40 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: 38 &lt;br&gt;t&lt;sub&gt;180&lt;/sub&gt;: 34</td>
<td>C: -90&lt;br&gt;t&lt;sub&gt;60&lt;/sub&gt;: -85 &lt;br&gt;t&lt;sub&gt;120&lt;/sub&gt;: -65 &lt;br&gt;t&lt;sub&gt;180&lt;/sub&gt;: -65</td>
<td>Primate</td>
<td>[Trunkey et al., 1973]</td>
</tr>
</tbody>
</table>

Table 9-3: Experimental studies showing the direct correlation between the decrease in arterial pressure and cell membrane depolarization for different animal species. Data are shown for short (S) and long (L) hypotension-producing hemorrhages. The percentage of blood lost from normal is indicated in the brackets in the first column. The values for the arterial pressure and the cell membrane potential correspond to control (C) and several subsequent times post-hemorrhage (t/min).
the simple (but intriguing) experimental observation that the RBCs of normal animals incubated in plasma obtained from animals that suffered hemorrhagic shock, will start to manifest a cellular defect with its characteristic membrane potential depolarization [Illner, 1984]. Alternatively, the depolarized membrane potential of RBCs from an animal in shock, recovers if the RBCs are incubated in normal plasma.

It was hypothesized that certain endotoxin-like factors might be able either to bind chemically with the cellular membrane (thereby changing its properties) or to diffuse inside the cell (thus compromising the cell’s integrity). Although a multitude of substances have been found that cause cellular damage, none of these species seems to be produced during hemorrhage. Therefore, unequivocal experimental proof for the existence of such toxic factors has never been provided. Table 9-4 summarizes some recent studies concerning potential factors believed to be responsible for cellular membrane defects in several pathological conditions.

There is experimental evidence that, when proper external therapy is provided, the cellular damage can actually be reversed. For example, Nakayama et al. [1985] and Trunkey et al. [1973] showed that cell membrane depolarization and cellular swelling are reversible processes when hyperosmotic solutions are used for resuscitation. The reversibility of the cellular transport defect suggests that the cells are the final link in the entire syndrome of shock. Therefore, although other disturbances may speed the progression toward the state of shock, the cells might ultimately be the cause of the decompensated shock. Among other things, this will be one of the hypotheses that we will attempt to clarify with the predictions of the model described in the next section.

<table>
<thead>
<tr>
<th>Toxic Factor</th>
<th>Mechanism of Action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated plasma lysosomal enzymes</td>
<td>Increased cellular permeability for sodium, ( p_{Na} )</td>
<td>[Starling et al., 1973]</td>
</tr>
<tr>
<td>CSP70 (Circulatory shock protein)</td>
<td>Increased cellular permeability for sodium, ( p_{Na} ), decrease in ATPase activity and poisoning of the ( \text{Na}^+\text{-K}^+ )-pump.</td>
<td>[Borchelt et al., 1995]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Boulanger et al., 1993]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Evans et al., 1991]</td>
</tr>
<tr>
<td>Fe(^{2+})-CSP</td>
<td>Increased cellular permeability for sodium, ( p_{Na} ), and decrease in ATPase activity.</td>
<td>[Eastridge et al., 1994]</td>
</tr>
</tbody>
</table>

Table 9-4: Factors hypothesized to be responsible for cellular swelling, cellular sodium and chloride content increase, and cellular potassium decrease during hemorrhagic shock.
9.3 Application of the model to the study of hemorrhage

With some modifications, the four-compartment model that describes the distribution and transport of fluid and solutes within the body (see Chapters 4 and 8), can be applied to the study of hemorrhage. The following sections present some preliminary changes in the formulation of the model and the results of several simulations that attempt to describe various hemorrhage scenarios. All the cases simulated involve non-resuscitated blood losses, i.e., only the dynamic mass exchanges that result from the hemorrhagic insult are considered. This first application of the hemorrhage model is used to provide mainly (but not only) qualitative information.

9.3.1 Proposed time-course of events for modeling hemorrhage

The previous discussion classified hemorrhagic shock essentially according to the presence or the deterioration of the compensatory mechanisms that take place in response to the external loss of blood. Often, for a given stage of hemorrhage, the compensatory mechanisms overlap temporarily, or even totally (e.g., the decrease in the average capillary pressure and the glucose release). Moreover, the magnitude, rate of deterioration and, especially, the time-course of compensation are uncertain. Therefore, although helpful in providing a qualitative description of a hemorrhagic episode, this classification scheme is of little use when a more quantitative description, as required by a mathematical model, is desired. In this latter case, a clear delineation between the different stages of shock, based on reliable and easily measurable system variables that are accounted for by the model, is needed.

The present work uses the blood volume loss, expressed as percentage change from the normal blood volume, (i.e., the degree of hemorrhage, DegHEM) and the time of progression of an untreated hemorrhage, to delineate between its different stages. Additionally, the dynamics associated with the disturbed mass exchange that occurs after blood loss will be followed for a relatively short term, up to an arbitrary time of 6 hours post-hemorrhage. Based on these considerations, the hemorrhage and time-course of events it generates will be classified as follows.

A DegHEM ≤ 30%, even if untreated up to 6 h post-hemorrhage, will be considered a moderate, hemodilution type of hemorrhage. For this magnitude of blood loss, the various compensatory mechanisms described in the introductory sections (such as the release of glucose from internal sources within the body) are assumed to take place immediately after the onset of hemorrhage and up to at least 6 hours post-hemorrhage.
Chapter 9: Application of the model to hemorrhage

A DegHEM >30% is defined as a shock-producing hemorrhage. The progression of this type of hemorrhage is considered to occur in two stages: an initial hemodilution followed by a hemoconcentration. For this case, the initial compensatory mechanisms are assumed to take place only for the first 2 hours post-hemorrhage; this value was estimated based on measured data from Illner and Shires [1980] and Nakayama et al. [1985]. The remaining time up to six hours (the hemoconcentration stage) is defined as the period of shock and is characterized by a depolarization of the cell membrane potential due to a disturbed cellular transport of fluid and solutes.

The case of administration of resuscitants after hemorrhage is not simulated in the current study. However, if fluid therapy were to be considered, it would be assumed that the compensatory mechanisms associated with the release of solutes into plasma would cease. Recall that the various compensatory mechanisms attempt to produce an internal autotransfusion in order to diminish any disturbance in tissue perfusion and low vascular volume. It is envisaged therefore, that the external therapy would take over from these mechanisms.

Table 9-5 shows the percentage of blood loss expected to initiate a severe state of hypovolemic shock for several animal species including humans. As can be inferred from this table, the threshold value of DegHEM = 30% selected above, is a reasonable choice for delineating between the compensatory and decompensatory stages of shock.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percentage of normal blood loss that characterize a severe shock</th>
<th>Arterial Pressure (mmHg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>~ 35%</td>
<td>50-60</td>
<td>[Koller and Lund, personal communication]</td>
</tr>
<tr>
<td>Swine</td>
<td>40%</td>
<td>40</td>
<td>[e.g., Sondeen et al., 1990(b)]</td>
</tr>
<tr>
<td>Dog</td>
<td>40%</td>
<td>50</td>
<td>[e.g., Velasco et al., 1980]</td>
</tr>
<tr>
<td>Cat</td>
<td>30-35%</td>
<td>40</td>
<td>[e.g., Amundson et al., 1980]</td>
</tr>
<tr>
<td>Rat</td>
<td>34%</td>
<td>50</td>
<td>[e.g., Illner and Shires 1980]</td>
</tr>
</tbody>
</table>

Table 9-5: Literature information regarding the percentage of blood loss where hypovolemic shock was first detected.
In the absence of a specific information from an experimental study, the use of a threshold value for $\text{Deg}_{\text{HEM}}$ has two advantages: 1) it eliminates the need to directly link the hemorrhage model with the changes in systemic arterial pressure, and 2) it is relatively independent of the animal species.

9.3.2 Model description

The model uses the same set of equations described in Chapter 4 for the transport of fluid, proteins and the ionic species $\text{Na}^+$, $\text{K}^+$, $\text{C}_2^+$, $\text{Cl}^-$ and $\text{A}^-$. Additionally, the renal function is represented by the equations developed in Chapter 8. For the study of non-resuscitated blood losses, the rates of fluid and solute infusions were set to zero and thus there was no addition of material to the body. In addition to renal excretion, the losses from the body are sensible/insensible losses and hemorrhage. The blood lost during hemorrhage includes red blood cells and their contents, as well as plasma together with its proteins and small ions. The volume of RBCs relative to the volume of plasma lost at any the time is determined by the instantaneous blood Hct. The only modifications required by the model are those inherent in the descriptions of the compensatory and decompensatory stages of hemorrhage.

a) Changes of the model to account for the compensatory stage of hemorrhage

To describe the compensatory stage of hemorrhage, a new neutral species, glucose, must be accounted for in the model. Because the simulations are restricted to 6 hours post-hemorrhage, the model does not consider the complex glucose metabolism in the body. In addition, the following simplifications and assumptions related to glucose release and transport are employed:

- Glucose (MW = 180 g/mol) is normally present in both of the extracellular compartments. Its concentration, under normal conditions of hydration and nutrition, is around 100 mg/(100 ml of blood) or the equivalent of 160 mg/(100 ml of plasma) for the standard 70 kg reference man [Guyton, 1991]. The concentrations of glucose in plasma and interstitium are similar. Table 9-6 gives the normal concentrations and amounts of glucose in the plasma and interstitial fluid compartments. The algebraic equations describing the concentrations of glucose in plasma and interstitial fluid, together with the osmotic pressure exerted by this species, are similar to those for ions, i.e., Eqs [4-33], [4-34], [4-42] and [4-43], respectively.
At steady state, the production and consumption of glucose in the body take place at equal rates, i.e., the extracellular amount of glucose is constant. During hemorrhage, however, there is an increase of glucose in the body due to the excessive release of this solute from the liver. The rate of release of glucose, \( M_{\text{Glu, REL}} \), is described by an empirical relationship that will be discussed in a later section. It should be pointed out that, under normal conditions glucose is continuously metabolized by all the cells in the body. Experimental studies have shown, however, that after a hemorrhage, a mediated insulin resistance mechanism prevents the cellular transport of this species across the cell membranes [Black et al., 1982; Bessey et al., 1983]. Accordingly, the model assumes that there is no cellular uptake of glucose after the hemorrhage is initiated.

Because it is a neutral solute, glucose is not involved in the electrical neutrality requirements of the extracellular fluid nor with the Donnan partition across the capillary membrane. However, it contributes to the total osmolarity of both the plasma and interstitial fluid. As a consequence, the initial conditions that describe the normal partition of all the solutes accounted for by the model (i.e., small ions, proteins and glucose) between the four fluid compartments (i.e., plasma, interstitium, RBCs and tissue cells) include contributions due to glucose. These initial conditions are tabulated and presented in Appendix D.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concentration in plasma, ( c_{\text{Glu, PL}} )</td>
<td>9.0 mM</td>
<td>[Guyton, 1991]</td>
</tr>
<tr>
<td>Glucose amount in plasma, ( \text{Glu}_{\text{PL}} )</td>
<td>5.184 g</td>
<td>Calculated based on normal ( V_{\text{PL}} ) value of 70 kg reference man</td>
</tr>
<tr>
<td>Glucose concentration in interstitial fluid, ( c_{\text{Glu, IT}} )</td>
<td>8.99 mM</td>
<td>[Guyton, 1991]</td>
</tr>
<tr>
<td>Glucose amount in interstitium, ( \text{Glu}_{\text{IT}} )</td>
<td>13.6 g</td>
<td>Calculated based on normal ( V_{\text{PL}} ) value of 70 kg reference man</td>
</tr>
<tr>
<td>Permeability-surface area product, ( P_{\text{S, Glu}} )</td>
<td>886 ml/(kg-h)</td>
<td>[Altman and Dittmer, 1971]</td>
</tr>
<tr>
<td>Reflection coefficient, ( \sigma_{\text{Glu}} )</td>
<td>0.2</td>
<td>Estimated based on Perl [1971] and Section 6.3.3</td>
</tr>
</tbody>
</table>

Table 9-6. Initial glucose concentrations and amounts in the extracellular compartments and the glucose transport parameters of the capillary membrane.
Glucose participates in the transcapillary transport between the plasma and interstitium as well as in the lymphatic return from the interstitium to plasma. Similar to the transport of small ions, glucose transport across the capillary membrane is assumed to take place via both diffusive and convective pathways and is described by an equation of the form of Eq. [4-18]. The convective transport of this species in lymph is described by an equation similar to Eq. [4-25]. Two transport coefficients are required to describe the transcapillary exchange of glucose: the permeability-surface area product, \( \text{PS}_{\text{Glu}} \) and the reflection coefficient \( \sigma_{\text{Glu}} \). \( \text{PS}_{\text{Glu}} \) was obtained from Altman and Dittmer [1971], who estimated that the permeability of skeletal muscle endothelium for glucose was approximately 3 to 4 times lower than the corresponding value for small ions, or about \( 850\text{PS}_{\text{Alb}} \). This latter value is considered as the initial value for \( \text{PS}_{\text{Glu}} \). A review by Aukland and Nicolaysen [1981] suggests that the reflection coefficient for glucose is very close to that of small ions, i.e., \( \sigma_{\text{Glu}} = 0.1 \). However, Perl [1971] assigns a value of 0.2 to this parameter. Considering the \( \sigma_{\text{ION}} \) values estimated previously in this study (see Section 6.3.3), a value of 0.2 was assumed for \( \sigma_{\text{Glu}} \). The parameter values that characterize the transcapillary transport of glucose are also presented in Table 9-6.

Due to a paucity of data, it will be assumed that, although the glucose concentration increases from its baseline value during the compensatory phase, there will be no renal elimination of this species post-hemorrhage, at least for the relatively short times simulated in this work. This solute is, however, eliminated with plasma during the hemorrhage.

The schematic diagram of the four compartment model applied to study the hemorrhage is presented in Fig. 9-3. Further details of this diagram are presented in the legend of this figure. Based on Fig. 9-3, the glucose mass balances for the plasma and interstitial compartments are:

\[
\frac{dM_{\text{Glu,PL}}}{dt} = M_{\text{Glu,REL}} - M_{\text{Glu,IT}} + M_{\text{Glu,L}} - M_{\text{Glu,HEM}} \quad [9-1]
\]

\[
\frac{dM_{\text{Glu,IT}}}{dt} = M_{\text{Glu,IT}} - M_{\text{Glu,L}} \quad [9-2]
\]

where \( M_{\text{Glu}} \) and \( M_{\text{Glu}} \) represent the compartmental content and transport rate of glucose, respectively; while the subscripts IT, L, HEM stand for the interstitium, lymph and hemorrhage, respectively. \( M_{\text{REL}} \) is the internal rate of glucose release into plasma.
Figure 9-3. A schematic diagram depicting the compartments and flows, which comprise the hemorrhage model, is presented. The four compartments include the plasma and red blood cells as well as the interstitial fluid and its associated cells. J represents the flow of fluid, Q represents the transport of macromolecules (proteins), M represents the transport of small solutes and glucose and HEM refers to whole blood loss via hemorrhage. The losses through HEM are indicated on the right side of this symbol. The subscripts are as follows: IT represents the interstitial fluid, TC represents the tissue cells, RBC denotes the red blood cells, L corresponds to the lymph, U represents urine, ION represents the individual ionic solutes, Glu indicates glucose while PER and ISL corresponds to perspiration and insensible losses, respectively.
As suggested earlier, in addition to glucose, several ions are probably released from hepatic sources. To accommodate the internal release of these additional solutes, the plasma ion balance equations from Chapter 4 were modified accordingly to give:

\[
\frac{dM_{\text{ION,PL}}}{dt} = M_{\text{ION,IT}} + M_{\text{ION,L}} - M_{\text{ION,RBC}} - M_{\text{ION,UR}} + M_{\text{ION,REL}} \tag{9-3}
\]

where \( M \) is the compartmental ion content and \( M \) represents the rates of transport of small ions into or out of the compartment. The subscript \( \text{PL} \) denotes the plasma compartment while \( \text{IT, L, RBC, UR and REL} \) indicate transport associated with the capillary wall, lymph, the red blood cell membrane, urine and glucose release, respectively. \( \text{ION} \) is a generic term used to describe any of the ionic species (i.e., \( \text{ION} = \text{Na}^+, \text{K}^+, \text{C}_2^+, \text{Cl}^- \) or \( \text{A}' \)). Empirical relationships for the release rate term, \( M_{\text{ION,REL}} \), are given in Section 9.5.3.2.1

b) Changes of the model to account for the decompensatory stage of hemorrhage

Based on the general description of fluid and ion transport across the cellular membrane presented in Chapter 3, it can be seen that disturbances that occur due to traumas such as hemorrhage, i.e., increased intracellular sodium, chloride and fluid contents and decreased potassium content, can be the result of two possible causes:

1. an inability of the \( \text{Na}^-\text{K}^+ \)-pump to actively extrude \( \text{Na}^+ \) out of, and \( \text{K}^+ \) into the intracellular medium, i.e., a "poisoning" of the \( \text{Na}^-\text{K}^+ \)-pump.
2. an increase in the cellular permeability to \( \text{Na}^+ \).

Several authors [e.g., Nakayama et al., 1985], present experimental evidence that infusion of a hyperosmolar saline solution can completely restore the cellular damage which occurs during hemorrhage. It might be anticipated that a cell with an already increased permeability for \( \text{Na}^+ \) would be completely compromised if faced with such rise in external \( \text{Na}^+ \) concentration. Nevertheless, for completeness, both of the above cases will be tested using the hemorrhage model developed here. To account for a change in the rate of the pump, \( \text{RP} \), or, alternatively, for a change in sodium permeability, \( p_{\text{Na}} \), from normal, discrete increased or decreased values of these two parameters were simply imposed on the model at 2 hours after a severe hemorrhage (\( \text{DegHEM} > 30\% \)) had taken place.
9.3.3 Simulated hemorrhage scenarios

In this section, the applicability of the hemorrhage model is demonstrated for the following scenarios:

1) A first set of simulations explores the importance of a decreased capillary pressure on the fluid and solute redistribution following the removal of different volumes of blood from the plasma compartment. For each volume of blood lost, two cases were tested: a short hemorrhage period of 0.05 h and a long one of 1 h. For both cases, it is assumed that there was no release of glucose after the onset of hemorrhage. Drucker et al. [1981], for example, have shown that food deprived animals, with depleted liver glycogen stores, may lack the ability to mobilize glucose into plasma. However, the main reason for studying this decreased capillary pressure mechanism on its own is to allow for a better interpretation of later simulated scenarios where the hemorrhage-induced release of glucose and other solutes is also considered.

2) The second set of simulations will explore the compensatory effect of plasma hyperosmolarity on fluid mobilization into the plasma compartment from neighboring sources. As part of this study, empirical equations for the release of solutes (glucose and ions) into plasma after an external loss of blood will be proposed. The simulations for this case will be based on and compared with an experimental study on dogs reported by Gann et al. [1982].

3) The third set of simulations aims to provide a better understanding of the cellular dysfunctions associated with the state of shock. The predictions of the model will be compared with the results of an experimental study of hypotension-producing hemorrhage on rats reported by Illner and Shires [1980].

4) Finally, an example that demonstrates that the model can be applied to study the evolution of hemorrhagic shock will be provided. Specifically, the model will be used to simulate a hypothetical blood loss scenario where both the compensated and decompensated stages of shock can be clearly delineated.

9.3.3.1 Simulation of hemorrhage in the absence of glucose release

Model predictions are obtained for two different degrees of hemorrhage: a mild hemorrhage of 10% and fairly severe one of 30%. For both cases, a high and a low rate of blood removal are simulated. The initial compartmental conditions for a 70 kg 'Reference man' are those presented in Table 4-1, except for the recalculated initial concentrations of proteins, small ions and glucose which are given in Appendix F (see Section 9.3.2). The changes in several
variables of the system are followed up to 6 h after the onset of blood removal. The protocol used for the simulations is provided in Table 9-7.

a) Model predictions for 10% and 30% hemorrhages of long duration

Some of the model-predicted changes that correspond to either a 10% or 30%, 1-h hemorrhage, are shown in Figs. 9-4 to 9-7.

Figure 9-4 presents the predicted changes in the volumes of all four fluid compartments that make up this model for 10% (left panel) and 30% (right panel) hemorrhages. In these simulations, blood is removed between $t = 1$ and $t = 2$ h, as described in Table 9-7. Because the system is at steady state prior to the onset of hemorrhage, outputs of fluid and solutes through the kidney are only allowed between 1 and 6 h. Figure 9-4 shows that, for both degrees of hemorrhage, the plasma and red blood cell volumes decrease only for the duration of blood loss and reach a minimum value at the end of this period. Once the hemorrhage is terminated, $V_{RBC}$ remains relatively constant at its minimum value for the next 4 hours. Also, the tissue cell volumes are minimally affected throughout the simulation period. The predicted changes in the plasma and interstitial volumes, however, are more complex.

<table>
<thead>
<tr>
<th>Type of hemorrhage</th>
<th>Duration of hemorrhage</th>
<th>Corresponding clinical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DegHEM (%)</td>
<td>Volume removed (ml)</td>
<td>3 min</td>
</tr>
<tr>
<td>10</td>
<td>530 ml</td>
<td>60 min</td>
</tr>
<tr>
<td>30</td>
<td>1600 ml</td>
<td>3 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 min</td>
</tr>
</tbody>
</table>

Table 9-7. Conditions used for simulating mild and severe hemorrhages. Blood removal starts at $t = 1$ h.
Figure 9.4: Predicted changes in the fluid volumes of plasma, $V_{pl}$, interstitium, $V_{it}$, red blood cells, $V_{rbc}$, and tissue cells, $V_{tc}$, during and after external blood removal. The results correspond to a 10% blood removal (left panel) or a 30% blood removal (right panel) over 1 hour. Renal losses of fluid and small ions occur between $t = 1$ h and $t = 6$ h.
Figure 9-5. Model predictions for normalized transcapillary fluid flows (left panel) and lymphatic flows (right panel). Predicted results are presented for the 10% hemorrhage (solid line) and 30% hemorrhage (dotted line). In both scenarios the hemorrhage starts at $t = 1$ h and ends at $t = 2$ h; fluid and small ion losses take place through the kidney between $t = 1$ h to $t = 6$ h of simulations.
Figure 9-6. Model predictions for the capillary (left panel) and interstitial (right panel) hydrostatic pressures. Predicted results correspond to a 10% hemorrhage (solid line) and a 30% hemorrhage (dotted line). As indicated by the arrows on the time axis the hemorrhage starts at $t = 1$ h and ends at $t = 2$ h. Renal losses of fluid and small ions occur between $t = 1$ h and $t = 6$ h.
Figure 9-7. Model predictions for colloid osmotic pressure in plasma (left panel) and interstitium (right panel). The predicted results corresponds to a 10% hemorrhage (solid line) and a 30% hemorrhage (dotted line). As indicated by the arrows on the time axis the hemorrhage starts at $t = 1$ h and ends at $t = 2$ h. Renal losses of fluid and small ions occur between $t = 1$ h and $t = 6$ h.
Immediately after the hemorrhage stops, the plasma volume in both cases starts to increase from the minimum value reached at the end of hemorrhage. Since \( V_{\text{RBC}} \) is essentially unchanged, the source of the fluid mobilized into plasma is the interstitium. As shown in Fig. 9-4, \( V_{\text{IT}} \) continuously decreases for the entire period from 1 to 6 h. These predictions indicate that a transcapillary fluid absorption takes place immediately after the initialization of blood removal.

For the mild 10% hemorrhage, the model predicts that at \( t = 2 \) h (i.e., when the hemorrhage stops), the interstitial fluid volume decreases by about 115 ml. Of this volume, approximately 52% remains in the vasculature while the remainder is excreted through urine. For the period immediately following the hemorrhage and up to 6 h, an additional volume of 235 ml is lost from the interstitial compartment, of which about 15% remains in the vascular space while the rest is lost through the kidney.

In contrast, for the more severe case of 30% blood lost, about 86% and 72% of the absorbed interstitial fluid is retained within the vasculature during and after the hemorrhage, respectively, while the rest constitutes the urinary fluid output. The total of 440 ml absorbed from the interstitium at 1 h post-hemorrhage is in good agreement with the value of 400 ml estimated, based on Jarhult's [1975] cat study (see Table 9-2), to be due to a drop in capillary pressure alone.

The shift of interstitial fluid to the vascular compartment is also illustrated by the transcapillary fluid flows shown in left the panel of Fig. 9-5. The model predicts that, within 3 minutes after the onset of the severe hemorrhage and about 10 minutes after the mild one, transcapillary fluid filtration converts to absorption. For both degrees of blood loss, the absorption reaches a maximal value at the end of the hemorrhage. After the hemorrhage terminates, \( J_{\text{IT}} \) continues to be negative for another 30 and 60 minutes for the 10% and 30% hemorrhages, respectively. Eventually, the transcapillary flow turns into filtration again and reaches an apparent steady-state value at about 2 hours post-hemorrhage. For the two cases simulated, the steady-state \( J_{\text{IT}} \) values are about 20-30 ml/h, significantly below the normal value of 76 ml/h. The right panel of Fig. 9-5 shows a reduced lymphatic flow rate for both degrees of hemorrhage. The reduced lymphatic return of fluid to plasma is a direct reflection of the decreased interstitial volume and, implicitly, the reduced interstitial hydrostatic pressure.

The main driving force for the absorption of interstitial fluid into plasma is the decrease in the capillary pressure illustrated in the left panel of Fig. 9-6. The model predicts that \( P_{\text{C}} \) is decreased by about 2.5 and 7 mmHg from normal for the 10% and 30% hemorrhages,
respectively. As a direct consequence of fluid absorption to plasma, the colloid osmotic pressure in the plasma compartment decreases while that of the interstitial compartment increases, (see the two panels of Fig. 9-7). As will be shown later, a decrease Hct also occurs for both hemorrhages; a direct result of plasma hemodilution. For a complete representation of all Starling forces that govern fluid exchange across the capillary, the right panel of Figs. 9-6 illustrates the corresponding changes in the interstitial hydrostatic pressure.

b) Model predictions for short and long duration hemorrhages

Some responses of the system when blood is lost rapidly (i.e., over 0.05 h) or more slowly (i.e., over 1 h) are illustrated in Figs. 9-8 to 9-10.

The changes in Hct given in Fig. 9-8 along with the decrease in plasma osmotic pressure shown in Fig. 9-7, indicate a continuous hemodilution of the blood. As expected, the decrease in Hct is proportional to the decrease in capillary pressure (and implicitly with the degree of hemorrhage). Although the slope of the Hct decrease is steeper for the cases where blood is removed more rapidly, by t = 6 h, this variable reaches essentially the same value independent of the duration of the hemorrhage.

Similarly, as exemplified by the fluid volume changes shown in Fig. 9-9 for a 30% hemorrhage, the differences in the dynamics of fluid exchange between short- and long-duration hemorrhages are relatively short lived (i.e., about 2-3 hours after the onset of hemorrhage). By 6 h, there are no significant differences between any of the compartmental fluid variables including fluid volumes (Fig. 9-9), hydrostatic and colloid osmotic pressures and solute concentrations (not shown).

The model-predicted changes in the renal output for all four cases are shown in Fig. 9-10. For both the short and long duration hemorrhages from t = 1 to 6 h, the cumulative urinary outputs predicted by the model were about 245 ml and 156 ml for the 10% and 30% hemorrhages, respectively. As expected, both these values are less than the 300 ml of urine that is normally produced by a typical 70 kg human during a five-hour interval. As shown in the right panel of Fig. 9-10, corresponding to the sharp decrease in plasma volume at the end of the hemorrhage, the urinary output falls to rates as low as 0.15 ml/min. As discussed in Chapter 8, such low rates are expected when a severe blood loss of 30 to 40% occurs.
Figure 9-8. Model predictions for Hct changes after a 10% (solid line) or a 30% (dotted line) hemorrhage. The predictions presented in the left panel describe a long 1 h hemorrhage. The right panel presents the scenario of a short 0.05 h hemorrhage. As indicated by the arrows on the time axis, the hemorrhage starts at \( t = 1 \) h and ends at \( t = 2 \) h; fluid and small ion losses take place through the kidney between \( t = 1 \) h to \( t = 6 \) h of simulations.
Chapter 9: Application of the model to hemorrhage

Figure 9-9. Changes in the fluid volumes of plasma, $V_{\text{PL}}$, interstitium, $V_{\text{IT}}$, red blood cells, $V_{\text{RBC}}$ and tissue cells $V_{\text{TC}}$ during and after external blood removal. The results of the simulations correspond to a 30% blood removal of long (1 h) duration (left panel) or short (0.05 h) duration (right panel). The duration of the hemorrhage is indicated by the arrows on the time-axis.
Figure 9-10. Model predicted fluid excretion rates after a 10% (dotted line) or a 30% (solid line) hemorrhage. The results of the simulations correspond to long (1 h) duration (left panel) or short (0.05 h) duration (right panel) blood removal. The duration of the hemorrhage is indicated by the arrows on the time-axis.
Chapter 9: Application of the model to hemorrhage

The results of the simulations presented above help us to better understand the dynamics of fluid and protein exchanges that take place after different degrees and/or different rates of blood removal. None of the qualitative model predictions are in contradiction with common physiological knowledge, such as the occurrence of hemodilution and the presence of reduced renal excretion. Amongst the most important predictions of the model, which are very difficult to be measured clinically, are the perturbed rate and direction of transcapillary fluid flow, as well as the changes that occur in the intracellular compartments.

9.3.3.2 Simulations of glucose release and osmolarity increase after blood removal

In the absence of starvation, the release of glucose and/or other solutes after hemorrhage is an indisputable occurrence. It is now widely accepted that such releases help promote fluid mobilization into plasma in addition to the absorption caused by a decrease in capillary pressure. Despite current knowledge regarding post-hemorrhage hyperglycemia and hyperosmolarity, there is still no quantitative information about the amount and dynamics of solute released as a function of the degree of hemorrhage. Consequently, one of the objectives of the present study is to propose an empirical equation that describes the release of both glucose and other solutes following the onset of blood loss. After an initial discussion about the physiological basis of the proposed equation, an example comparison between the predictions of a model that incorporates this equation with a set of experimental data will be presented.

9.3.3.2.1 Empirical equations for release of glucose and small ions after hemorrhage

Several researchers have reported that plasma osmolarity increases in relationship to the severity of the traumatic insult [e.g., Pardon et al., 1983; Friedman et al., 1982]. However, the amount of glucose released in plasma does not seem to be proportional to the degree of hemorrhage. Furthermore, some researchers have found that, after large hemorrhages, the increase in plasma osmolarity levels off and fails to achieve an adequate restitution of the blood volume [Pirkle and Gann, 1976; Gann et al., 1982]. These facts suggest that, after a hemorrhage, two competing actions may take place: a) the vasoconstriction of the splanchnic bed, which is a direct consequence of the traumatic event, promotes glucose mobilization from the liver, while b) the reduced blood flow to the liver itself which is direct consequence of blood volume deficit, alters the ability of this organ to either produce glucose and/or to make it available to other peripheral vascular beds.
Therefore, it is hypothesized in this work that, the rate of glucose release is directly proportional to the vasoconstriction, reflected by the change in the capillary hydrostatic pressure produced by the hemorrhage, and inversely proportional to the blood volume deficit. Thus, the empirical equation that describes glucose release is expressed as

\[ M_{\text{Glu,REL}} = \frac{k_{\text{Glu,REL}}}{\text{Deg}_{\text{HEM}}} \cdot (P_{C,NL} - P_C) \]

where \( M_{\text{Glu,REL}} \) (g/h) represents the rate of internal glucose release, \( (P_{C,NL} - P_C) \) (mmHg) represents the instantaneous change in the capillary pressure produced by the hemorrhage, \( \text{Deg}_{\text{HEM}} \) is the degree of hemorrhage (%) while \( k_{\text{Glu,REL}} \) (g-%/h-mmHg) is a proportionality constant that was set to 100 after trials with several different values.

Equation [9-4] was incorporated into the program and simulations were performed for various degrees of hemorrhage. The model predictions showed (results not presented here) that, if only glucose were released during the hemorrhage, the fluid absorbed into plasma from the interstitium and tissue cells would cause a marked decrease in the concentration of all the plasma ions. Such decreases in the concentrations of plasma ions were beyond those normally reported to occur in clinical studies of hemorrhage. Additionally, according to Jarhult [1973] as well as of other [e.g., Ware et al., 1982; Friedman et al., 1982], glucose only accounts for the entire increase in plasma osmolarity up to about 20 to 30 min post-hemmorhage. After this time, in addition to glucose, other solutes such as potassium, chloride, urea, etc., contribute to the observed increased in plasma osmolarity.

In order to understand the mechanisms responsible for the release of solutes other than glucose from the liver and to gain an appreciation for the magnitude of this release, we looked for alternative conditions which may cause the liver to break down its glycogen reserves. Interestingly enough, it was found that some of the physiological changes that occur after hemorrhage closely resemble those that take place during moderate and heavy physical exercise. It was found that, even though different physiological mechanisms are involved, both the loss of blood and heavy physical exercise produce vasoconstriction of the splanchnic bed and a severe reduction in blood flow to the liver [Hultman, 1967; Jarhult, 1975; Sayeed et al., 1981]. Similar to hemorrhage, where an increase in extracellular lactic acid was reported [Amundson et al., 1980], during heavy physical exercise, there is a decrease in lactic acid uptake by the liver.
Chapter 9: Application of the model to hemorrhage

[Hultman, 1967]. Additionally, as in severe hemorrhage, during heavy physical exercise the glucose release into plasma manifests a saturation limit; probably reflecting the depletion of the internal reserves of glycogen. The most interesting finding reported in Hultman’s study, that is relevant to the work at hand, is the fact that there is a parallel release of both potassium and glucose from the liver. According to this author’s experimental results, for each g of glucose there is a release of 0.53 mEq of potassium.

Based upon the findings of Hultman [1967] and Jarhult [1975], as well as by Sayeed et al. [1981] and Bakker-Grunwald [1982], who showed that, after a severe hemorrhage, there is a disturbed cellular transport in the liver hepatocytes which causes an extracellular release of Na⁺, K⁺ and Cl⁻, the present work proposes the following hypothesis. Initially, after the onset of hemorrhage and up to about 0.5 h post-hemorrhage, there is a release of glucose that increases plasma osmolarity. For the subsequent post-hemorrhage period, the release of glucose is associated with a concurrent release of Na⁺, K⁺, Cl⁻ and A⁻ (to preserve electroneutrality) ions. To accommodate this hypothesis and the degree of hemorrhage, the proposed equations for solute release are as follows:

If \( \text{Deg}_{\text{HEM}} \leq 30\% \)

\[
M_{\text{Glu,REL}} = \frac{100}{\text{Deg}_{\text{HEM}}} (P_{c,\text{NL}} - P_c), t > t^0_{\text{HEM}}
\]

\[
M_{\text{ION,REL}} = 0, \quad t^0_{\text{HEM}} \leq t \leq (t^f_{\text{HEM}} + 0.5 \text{ h})
\]

\[
= k_{\text{REL}} \cdot M_{\text{Glu,REL}}, \quad t > (t^f_{\text{HEM}} + 0.5 \text{ h})
\]  \[9-5\]

If \( \text{Deg}_{\text{HEM}} > 30\% \)

\[
M_{\text{Glu,REL}} = \frac{100}{\text{Deg}_{\text{HEM}}} (P_{c,\text{NL}} - P_c), t^0_{\text{HEM}} \leq t \leq 2 \text{ h}
\]

\[
= 0, \quad t > 2 \text{ h}
\]

\[
M_{\text{ION,REL}} = 0, \quad t^0_{\text{HEM}} \leq t \leq (t^f_{\text{HEM}} + 0.5 \text{ h})
\]

\[
= k_{\text{REL}} \cdot M_{\text{Glu,REL}}, \quad (t^f_{\text{HEM}} + 0.5 \text{ h}) < t \leq 2 \text{ h}
\]

\[
= 0, \quad t > 2 \text{ h}
\]
Equation [9-5] shows that, for any hemorrhage that starts at $t = t_{\text{Hem}}^0$ and finishes at $t = t_{\text{Hem}}^f$, during the hemorrhage and for another 0.5 h post-hemorrhage, there is only glucose released into plasma. This glucose release is responsible for the increase in plasma osmolarity that occurs up to the first half-hour post-hemorrhage. After this time, for $\text{Deg}_{\text{HEM}} \leq 30\%$ (i.e., hemorrhages for which cellular dysfunction is absent), the release of glucose is accompanied by a concomitant release of $\text{K}^+$, $\text{Na}^+$, $\text{Cl}^-$ and $\text{A}^-$ ions. Due to a lack of alternative information, the proportionality constant, $k_{\text{REL}}$, that correlates the glucose and ION release rates, was assumed to be the same for all released ions and was assigned the value of 0.53 mEq/(g-glucose) reported in the study of Hultman [1967] for $\text{K}^+$ release during heavy physical exercise.

Studies by Nakayama et al. [1985] as well as Illner and Shires [1980] showed that hypotension-producing hemorrhages ($\text{Deg}_{\text{HEM}} > 30\%$) cause shock that is characterized by cellular dysfunction and cell membrane potential depolarization. The occurrence of shock in these studies was reported to occur at 1.5 to 2.5 h post-hemorrhage. Other studies done by Gann and his colleagues [e.g., Gann et al., 1981] show that for hemorrhages exceeding the threshold value of about 30%, the increase in blood osmolarity is not effective in restoring the blood volume. Accordingly, Eq. [9-5] assumes that for hemorrhages greater than 30%, the glucose and ion releases take place only up to 2 h post-hemorrhage.

Equation [9-5] was incorporated in the hemorrhage model, allowing a comparison between the model-predicted changes of plasma osmolarity and blood volume restitution after hemorrhage, with the experimental data of Gann et al. [1982]. This comparison is presented in the next section.

9.3.3.2 Experimental data of Gann et al. [1982]

Gann et al. [1982] performed hemorrhage experiments on awake (non-anesthetized), splenectomized dogs that had an intact renal function. The dogs were divided into three groups, each of which was subjected to three different degrees of hemorrhage, i.e., 10%, 20% and 30% of the estimated initial blood volume. The initial body weight of these dogs averaged 20 kg. The estimated normal blood volume was 7.5% of the body weight. Each dog that belonged to one of the groups was subjected to a short 3-minute hemorrhage. The hemorrhages were not followed by resuscitation. After the appropriate volume of blood was removed, the changes in plasma osmolarity and blood volume restitution were followed for a total of 6 h of post-hemorrhage.
Chapter 9: Application of the model to hemorrhage

The initial compartmental values for dogs used as initial conditions in the program are presented in Appendix G. These values were determined as described in Appendix C.

9.3.3.2.3 Simulation results

A comparison of the model predictions and the experimental results of Gann et al. [1982] for 10%, 20% and 30% hemorrhages are presented in Figs. 9-11, 9-12 and 9-13, respectively. The upper panel in each figure shows the change in plasma osmolarity, while the lower panel gives the blood volume restitution, BVR. BVR is defined as the net volume of fluid that is mobilized into the vasculature to help restore the volume of blood after the hemorrhage (i.e., the difference between the volume of fluid absorbed from the interstitium to plasma, and the loss of fluid from plasma through the kidney).

In Fig. 9-11, for a 10% acute hemorrhage, both the model predictions and the experimental results show that the plasma osmolarity (upper panel) starts to increase immediately after the onset of hemorrhage, rising about 2 mOsm/l within the first 10 min post hemorrhage. Over the next 6 hours, plasma osmolarity increases gradually as a result of the continuous release of solutes. At t = 6 h, in good agreement with the experiments, the model predicts an increase in plasma osmolarity of about 7 mOsm/l.

As a consequence of both the decrease in capillary pressure and the release of solutes, there is a continuous reabsorption of fluid into the plasma compartment as shown in the lower panel of Fig. 9-11. According to the simulations, the initial source of this fluid is the interstitial fluid and to some extent the red blood cells. However, within about 0.2 to 0.5 h, once the released solute begins to equilibrate across the capillary wall, fluid is shifted extracellularly from the tissue cells as well. At 6 hours after the hemorrhage, the model predicts that the tissue cell volume is reduced by 2.3% from normal. The model-predicted values of blood volume restitution are in good agreement with the experimental data for the first 2 to 3 hours after the blood removal.

The experiments of Gann et al. [1982] show that, by 6 hours, the entire blood volume removed has returned to normal. The predicted blood volume restitution at t = 6 h underestimates the experimental value by about 4.5 ml/kg. According to the results of the simulation, this difference corresponds to the cumulative urinary output predicted by the model, which at 6 h was 4.6 ml/kg. The only way the animals in the experimental study could have completely restored
Figure 9-11. Model-predicted results (solid line) and experimental data (symbols) for changes in plasma osmolarity (upper panel) and blood volume restitution, BVR (lower panel) for a 10% hemorrhage. The duration of hemorrhage is 3 minutes as indicated by the marker on the time axis.
Figure. 9-12. Model-predicted results (solid line) and experimental data (symbols) for changes in plasma osmolarity (upper panel) and blood volume restitution, BVR (lower panel) for a 20% hemorrhage. The duration of hemorrhage is 3 minutes as indicated by the marker on the time axis.
Figure 9-13. Model-predicted results (solid line) and experimental data (symbols) for changes in plasma osmolarity (upper panel) and blood volume restitution, BVR (lower panel) for a 30% hemorrhage. The duration of hemorrhage is 3 minutes as indicated by the marker on the time axis.
their blood volume would have been if, for the entire 6 hours of the experiment, there was essentially no urine elimination.

In order to estimate the contributions of the increase in plasma osmolarity vs. the decrease in capillary pressure on the overall restitution of blood, additional simulations were performed in which the solute release rate was set to zero while all other conditions were maintained the same. As illustrated in Fig. 9-14 for the 10% hemorrhage, the model predicts that, at $t = 6$ h, the increase in plasma osmolarity due to the release of solutes accounts for 47% of the total blood volume restituted. The remaining 53% restitution is contributed by the decrease in $P_c$.

A comparison between the model predictions and the experimental results for the 20% hemorrhage is illustrated by Fig. 9-12. In good agreement with the experimental data, the model predicts a sustained increase in the plasma osmolarity up to a maximum of about 9 mOsm/l at 6 h (upper panel). The increase in plasma osmolarity is paralleled by the restitution of blood volume illustrated in the lower panel of Fig. 9-12. As for the milder 10% hemorrhage, the rate of blood volume compensation is maximal during the first 30 - 40 minutes post-hemorrhage, a period that corresponds to the gradual reduction of the transcapillary solute concentration differences. At $t = 6$ h, the model predicts that the volume of tissue cells is decreased by 3% from normal, and that, of the total fluid absorbed into plasma during this period, approximately 77% takes place within the first hour post-hemorrhage. Again, the predicted and experimental results are in agreement for up to about 3 h post-hemorrhage; but, at $t = 6$ h, the predicted BVR is about 4.5 ml/kg less than the value reported by Gann et al. The total urinary output predicted for the 6-hour simulation period was 4 ml/kg, compared to the value of about 2 ml/kg calculated from the Gann et al. data. As shown in the middle panel of Fig. 9-14, for a 20% hemorrhage, 33% of the restored blood volume is due to the hyperosmolar state of plasma while the remainder is due to a decreased capillary pressure.

Comparisons between simulations and experiments for the highest degree of hemorrhage reported by Gann et al. [1982] are illustrated in Fig. 9-13, for the change in plasma osmolarity (upper panel) and the BVR (lower panel). A 30% blood loss borders on a hypotension-producing hemorrhage that eventually ends up in shock. However, according to Gann et al., all of the dogs subjected to a 30% hemorrhage survived for the 6 hour experimental procedure even though they were not resuscitated. It was inferred, therefore, that this hemorrhage did not produce shock and hence, the simulations did not take into account any cellular defects which can cause depolarization of the cell membrane potential.
Figure 9-14. Model predictions of BVR in the presence (solid line) and absence (dotted line) of solute release to plasma. The predictions correspond to degrees of hemorrhage of 10% (upper panel), 20% (middle panel) and 30% (lower panel).
Similar with the two lower degrees of hemorrhage, the model predictions for the 30% hemorrhage case approximate fairly well the measured data with respect to the trend and magnitude of both the plasma osmolarity and BVR changes. Again, although a gradual increase in plasma osmolarity takes place over the entire 6-hour post-hemorrhage period, the highest rates of increase in blood restitution are obtained during the first 30-40 minutes. In good agreement with the measured results, at \( t = 6 \) h, the model predicts a plasma osmolarity of about 10 mOsm/l and a final blood volume restitution of about 10 ml/kg. The simulations suggest that, 6 hours after a 30% hemorrhage, about 40% of the total blood lost is recovered into the vasculature at the expense of the interstitial and intracellular fluids. At this time, approximately 3.3% of the tissue cell volume is shifted to the extracellular compartments. Figure 9-14 shows that, for the 30% hemorrhage, the increased plasma osmolarity accounts for only about 26% of the total blood restituted by 6 h post-hemorrhage. This percentage is much lower than the values of 33% and 47% predicted for the 20% and 10% hemorrhages, respectively.

The fact that the model predicted much lower BVR values at 6 h than were obtained experimentally for the 10% and 20% hemorrhage cases, suggests that there might be a discrepancy between the simulated urinary outputs and those calculated from the data of Gann et al. [1982] for these two cases. This discrepancy is most likely due to the fact that these laboratory animals were deprived of water for 18 h prior to experimentation. Thus, there is a high probability that all of the dogs were in a slightly dehydrated state with impaired urine production rates right from the outset of the experiments.

Based on the comparison between the model predictions and experimental results presented above, it would appear that the equations for solute release (i.e., glucose and small ions) proposed here are reasonable. However, as a note of caution, it should be clearly stated that this work is merely putting forward a simple hypothesis to explain some of the observed physiological disturbances that follow a hemorrhage. Although it is reasonable in many respects, to this author's knowledge, there are no published reports that either confirm or deny this hypothesis. Thus, one important purpose of the model is to suggest directions for future experiments. Studies in which the releases of small ions as well as glucose from hepatic sources are measured following different degrees of hemorrhage, would be very useful in testing the solute release hypothesis proposed here.

In the next section, the hemorrhage model is applied to describe the dynamic changes that follow a severe hemorrhage (i.e., \( \text{Deg}_{\text{HEM}} > 30\% \)). For this scenario, the glucose and small ion
releases described by Eq. [9-5] take place only up to a maximum of 2 hours after blood removal, i.e., it is assumed that the liver exhausts its glycogen reserves within this time span.

9.3.3.3 Cellular defect in hemorrhagic shock

From an experimental point of view, the study of mass transport in hypovolemic shock is a difficult task. Following a severe untreated hemorrhage, the disturbances associated with shock shift from the plasma compartment, which is accessible to measurements, to the interstitium and tissue cells, which are difficult to assess by invasive methods. Hence, the interplay of disturbances, that either bring about the state of shock, or are generated by it, remains uncertain. The timing of these disturbed events is also unknown. Due to these uncertainties, mathematical models purporting to describe hypovolemic shock take the risk of entering an entirely speculative domain. The sections that follow attempt to use the hemorrhage model to investigate some aspects of the disturbed cellular mass exchange associated with hemorrhagic shock. To achieve this task, the only additional modifications required by the model relate to the type of perturbations applied to the cell membrane parameters. Two such parameters are investigated below: the rate of the \( \text{Na}^+\text{-K}^+\)-pump, \( \text{RP}_{TC} \), and the sodium permeability, \( \text{p}_{\text{Na},TC} \), of the tissue cells. For the purposes of this study, the experimental protocol of Illner and Shires [1980] was simulated. Whenever data were available, comparisons of model-predicted and experimental results are provided; otherwise only the results of the simulations will be discussed.

9.3.3.3.1 Experimental information and simulation protocol

In the study of Illner and Shires [1980], anesthetized rats having an intact renal function were bled until the arterial pressure reached 50 mmHg. This level of hypotension was maintained by subsequent bleeding. The total volume of blood removed over an estimated 0.2 h was 34% of the normal blood volume. The hemorrhage was left untreated until hypovolemic shock was produced. Shock was assumed to exist when the measured membrane potential of skeletal muscle cells decreased by about 20-25% (average 23.3% ± 2.5) corresponding to an average depolarization of \( V_m \) from -91 mV to -70 mV. This degree of depolarization occurred at about 165 min after the initial hemorrhage. Other measurements reported in this study include the concentration of \( \text{Na}^+, \text{K}^+, \text{Cl}^- \) ions in plasma and skeletal muscle cells, the concentration of \( \text{K}^+ \) within the interstitial fluid, and the intracellular volume of skeletal muscle cells. Due to experimental difficulties, the interstitial \( \text{K}^+ \) concentrations were measured at an advanced state of
shock, at about 225 min after the blood was removed, just prior to animal death. At this time, a muscle biopsy was also performed. The cell membrane potentials were recorded continuously for the entire experimental procedure. The measurements of all the other variables were reported for $t = 0$ (control) and $t = 165$ (initialization of shock).

The initial compartmental values for the rat were not provided by Illner and Shires. The average body weight of these animals was 190 g, a value comparable to the one of animals in the study by Onarheim [1995]. As initial conditions for the simulations, it was assumed, therefore, that the volume ratios between the different fluid compartments were the same as those measured by Onarheim (see Section 6.4.1). However, an appropriate scaling to the slightly lower body weight of Illner and Shires rats, was carried out. The initial conditions used in the model are presented in Appendix G.

The simulation protocol, including the times when certain perturbations associated with the hemorrhage occur, is illustrated in Fig. 9-15. Between $t = 0$ and $t = 0.2$ h, 34% of the estimated normal blood volume is removed from the body. Immediately after the onset of hemorrhage and for the next 30 min post-hemorrhage there is a release of glucose. This period is followed, from $t = 0.7$ to $t = 2$ h, by a further release of glucose as well as $\text{Na}^+$, $\text{K}^+$, $\text{Cl}^-$ and $\text{A}^-$ ions, as described by Eq. [9-5].

![Experimental Data Recorded](image)

Figure 9-15. Simulation protocol for a severe 34% hemorrhage based on the experiments of Illner and Shires [1980]. The perturbations post-hemorrhage include the release of glucose with or without small ions, followed by a perturbation of a cell membrane parameter.
From $t = 2\, h$ to $t = 4\, h$, a step change in one of the cellular membrane parameters, either $RP_{TC}$ or $p_{Na,TC}$, is imposed as an input to the program. The time $t = 2\, h$ when the cell membrane perturbation was applied was approximated based on literature reports. According to Illner and Shires [1980] a state of shock is observed by about 165 minutes post-hemorrhage. Nakayama et al. [1985], who also studied hemorrhagic shock, report a similar magnitude of muscle cell membrane potential depolarization at approximately 100 to 120 minutes after blood removal.

The cell membrane potential, $V_m$, can be depolarized by an increase in $p_{Na}$ or a decrease in $RP$. Since there is insufficient experimental information to allow a delineation between these two types of perturbations, either a step increase in $p_{Na,TC}$ or step decrease in $RP_{TC}$ was imposed separately. The magnitude of the imposed changes were chosen such that $V_{m,TC}$ depolarized by 23.3% immediately after the perturbation, in order to achieve the identical state of shock as that reported by [Illner and Shires, 1980]. Such a sudden perturbation in either $RP_{TC}$ or $p_{Na,TC}$ probably does not occur within the body. As demonstrated in Fig. 9-2, and also shown by other studies [e.g., Nakayama et al., 1985], the cell membrane potential decreases gradually after the end of a severe hemorrhage reaching a maximum depolarization just prior to death. Since it is not known what factors actually influence the depolarization process, the addition to the model of yet another empirical equation that accounts for a gradual depression of $V_m$ could not be justified at this time.

In what follows, therefore, only those changes in fluid and solute distribution that correspond to a pre-established degree of cell membrane potential depolarization of tissue cells, is analyzed. Additionally, in order to avoid confounding the results, the cellular transport parameters related to exchanges in RBCs are not altered from normal.

**9.3.3.3.2 Simulation results**

Figure 9-16 shows the change in tissue cell transmembrane potential that takes place following a step increase in $p_{Na,TC}$ or a step decrease in $RP_{TC}$. In order to achieve the 23.3% depolarization of $V_{m,TC}$, $p_{Na,TC}$ was increased by 210% from its normal value and $RP$ was reduced by 55%.

This depolarization of the cell membrane potential is accompanied by significant volume changes in the plasma, interstitium and tissue cell compartments as shown in Fig. 9-17. The changes in fluid volumes during the hemorrhage and up to 2 h post-hemorrhage were discussed in previous sections and will not be repeated here. Of greater interest now are the compartmental
changes that occur after the membrane potential is depolarized, i.e., at $t > 2 \text{ h}$. As a first observation, Fig. 9-17 shows that, as far as the partition of fluid between the four compartments is concerned, the means by which the transmembrane potential is depolarized is not important.

Figure 9-16. Changes in the membrane potential of tissue cells corresponding to either an increase in $p_{\text{Na,TC}}$ by $210\%$ (dotted line) or decrease in $R_{\text{PTC}}$ by $55\%$ (solid line) (The two lines are almost indistinguishable). The change in either of these parameters was imposed at $t = 2 \text{ h}$. The symbols correspond to the experimental data reported by Illner and Shires [1980]. The duration of hemorrhage is $0.2 \text{ h}$ as indicated by the arrows on the time axis.
Chapter 9: Application of the model to hemorrhage

Figure 9-17. Percentage changes in the volumes of plasma, $V_{PL}$, interstitial fluid, $V_{IT}$, tissue cells, $V_{TC}$, and RBCs, $V_{RBC}$, for the entire simulation period, from the onset of hemorrhage up to 4 h post-hemorrhage. The predicted changes correspond to either an increase in $p_{Na_{TC}}$ by 210% (dotted lines), or a decrease in RP by 55% (solid lines). The change in either of these parameters was imposed at $t = 2$ h. The symbols represent the percentage change in the volume of skeletal muscle cells reported by Illner and Shires [1980]. The duration of hemorrhage was 0.2 h and is indicated by the arrows on the time axis.
In agreement with the results of other experimental studies [e.g., Nakayama et al., 1985; Cunningham, 1971], Illner and Shires [1980] found that, during shock, at the time when cellular membrane depolarization is detected, the tissue cells swell. They report that corresponding to a 23.3% transmembrane potential depression, at 165 min post-hemorrhage (i.e., $t = 2.95$ h), the tissue cell volume increased by about 2 to 3 % from normal. In good agreement with these measurements, the model predicts an increase of $V_{TC}$ of about 2% from control. The simulations also predict that fluid withdrawn from both the plasma and interstitial compartments participates in cellular swelling.

Immediately after the membrane potential is depolarized, the net interstitial outflow that restitutes plasma volume post-hemorrhage turns into a net flow toward the interstitium. As illustrated in Fig. 9-18 for the conditions simulated here, the net interstitial inflow lasts for about 0.5 h. After this time, the plasma resumes.

**Figure 9-18.** The normalized transcapillary (solid line) and lymph (dashed line) flows after hemorrhage which corresponds to the experiment of Illner and Shires [1982]. The flows were normalized with respect to their initial values at $t = 0$ h. The simulated case corresponds to a poisoning of the Na$^+$/K$^+$-pump, i.e., RP is depressed 55% from normal (solid line) at $t = 2$ h. The duration of hemorrhage, of 0.2 h, is indicated by the arrows on the time axis.
Based on the predictions of the model at $t = 2.95$ h, the interstitial fluid volume is decreased by about 8% from normal. Of this decrease, about 62% was shifted into the tissue cells while the remainder contributed to the restitution of plasma volume. From the skeletal muscle biopsy data presented by Illner and Shires, it can be calculated that, at $t = 4$ h, about 67% of the decreased interstitial volume contributed to cellular swelling.

According to the model predictions, the depolarization of the tissue cells does not produce any significant changes in the volume or content of the RBCs. Nonetheless, as will be discussed shortly, the membrane potential of the RBCs also depolarizes as a direct consequence of the altered concentrations of small ions in plasma.

In addition to the compartmental volume changes, there are marked changes in the concentrations and amounts of all three ions that participate in transport across the cellular membrane, i.e., $Na^+$, $K^+$ and $Cl^-$. Again, these changes occur only in three of the fluid compartments: plasma, interstitium and tissue cells; there are no significant changes in the composition or concentration of ions in the RBCs. The model-predicted changes of $Na^+$, $K^+$ and $Cl^-$ in these three compartments, at 2.95 h post-hemorrhage, are provided in Table 9-8. The detailed changes associated with each of these three fluid compartments will be discussed separately below.

**a) Changes in concentrations of small ions within tissue cells**

Not only is the tissue cell volume expanded immediately after $V_m$ depolarizes, but also, as shown in Table 9-8, both the tissue cell chloride and sodium ion concentrations increase markedly. The increase of these quantities suggests an enhanced transport of both $Na^+$ and $Cl^-$ into the cells from the extracellular fluid. At the same time, the intracellular concentration of $K^+$ decreases. Model simulations show that the intracellular concentration of potassium is reduced more than can be accounted for by the increase in $V_{TC}$ alone. Hence, the cell membrane depolarization is accompanied by a loss of potassium from the tissue cells.

Figure 9-19 compares the model predictions and the experimental results of Illner and Shires [1980] for the changes in $Na^+$, $K^+$ and $Cl^-$ concentrations. In each panel of Fig. 9-19, two separate perturbations in cellular transport parameters were imposed, i.e., poisoning of the pump (solid line) and increase in $p_{Na,TC}$ (dotted line). At $t = 2.95$ h, the model predicts an increase of approximately 60% and 100% for $[Na^+]_{TC}$ and $[Cl^-]_{TC}$, respectively. These values compare well
### Table 9-8

Model predictions for small ion concentration in plasma, interstitial fluid and interstitial cells in control (t = 0) and during a shock characterized by a 23% $V_m$ depolarization (t = 2.95 h)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plasma (mEq/l)</th>
<th>Interstitial Fluid (mEq/l)</th>
<th>Interstitial Cells (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Na^+$</td>
<td>$K^+$</td>
<td>$Cl^-$</td>
</tr>
<tr>
<td>Control (t = 0.0 h)</td>
<td>141.9</td>
<td>4.1</td>
<td>105.4</td>
</tr>
<tr>
<td>Shock (t = 2.95 h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55% decrease in RP</td>
<td>131.7</td>
<td>9.9</td>
<td>102.4</td>
</tr>
<tr>
<td>210% increase in $P_{Na}$</td>
<td>133.1</td>
<td>8.1</td>
<td>102.3</td>
</tr>
</tbody>
</table>
Chapter 9: Application of the model to hemorrhage

Figure 9-19. Comparison of the model predictions (solid and dotted lines) with the experimental results of Illner and Shires [1980] (solid circles) for changes in the tissue cell concentrations of sodium (upper panel), potassium (middle panel) and chloride (lower panel). Experimental data are available for control (i.e., at $t = 0$ h) and shock at $t = 2.95$ h (i.e., 165 min past hemorrhage). For additional details see legend of Fig. 9-16.
the increases of 47% and 115% for sodium and chloride, respectively, reported by Illner and Shires [1980] for rat skeletal muscle cells. As shown in Table 9-8, there are no significant differences in the post-perturbation intracellular concentrations of Na\(^+\) and Cl\(^-\), obtained through either of the cell parameter perturbations.

Also in agreement with the experimental results, the model predicts about a 3% decrease in the intracellular concentration of K\(^+\) for both mechanisms of cell depolarization. According to the model the concentration of this ion decreases from a control value of 150.4 mEq/l to an average of about 145 mEq/l. The theoretical decrease in [K]\(_{TC}\) calculated by assuming that there is no excessive potassium loss from the cells would be about 147 mEq/l. As shown in Table 9-8, the amount of K\(^+\) lost from the cells contributes to about a 5 mEq/l increase in the extracellular concentration of this ion.

### b) Changes in concentration of small ions in interstitial fluid

The small ion concentration changes in the interstitial fluid during shock reflect the disturbed transport across the tissue cell membrane. As a result of cellular uptake of Na\(^+\) and Cl\(^-\) by the tissue cells, the extracellular concentrations of both of these ions is decreased (see Table 9-8). Thus, the results of the simulations show that, at t = 2.95 h, [Na]\(_{IT}\) decreases by 8.2 mEq/l from control when the pump is poisoned and by 6.8 mEq/l when pNa,\(_{TC}\) is increased. For the interstitial Cl\(^-\) concentration, the model predicts a decrease of about 4.3 mEq/l from control for both disturbances simulated. As a result of potassium loss from the tissue cells, the concentration of this ion outside the cells more than doubles. Table 9-8 shows that [K]\(_{IT}\) increases by 5.7 mEq/l when RP is poisoned and by 4.3 mEq/l when pNa,\(_{TC}\) is increased.

In their study, Illner and Shires [1980] where only able to measure the interstitial concentration of K\(^+\). According to their report, during shock, [K]\(_{IT}\) increased by 205% from normal (i.e., from a control value of about 5 mEq/l to 15.2 mEq/l). Their value is significantly higher than the model-predicted increase of 142% for this ion. Furthermore, these authors state that, during shock, and for the degree of hemorrhage investigated, there is a K\(^+\) concentration difference of about 6 mEq/l across the capillary membrane, i.e., between the plasma and interstitium. As will be shown later, the model does not corroborate this statement. Based on the high value of [K]\(_{IT}\) and the transcapillary difference for this ion, Illner and Shires [1980] hypothesized that, as a result of the reduced blood flow observed in shock, the potassium leaked from the cells does not equilibrate with the K\(^+\) in plasma. For the reasons explained below, there
is a possibility that the hypothesis proposed by Illner and Shires is in error. Furthermore, due to the manner in which their experiments were conducted, these authors may have misinterpreted their data and subsequently the dynamics of ion exchange during shock.

According to Illner and Shires, technical difficulties did not allow them to record simultaneously both the cellular membrane potential and the interstitial potassium concentration. Therefore, while the majority of the variables reported in this study were measured at $t = 2.95\ h$, the interstitial potassium concentration was measured near the end of the experiment at $t = 3.95\ h$ (i.e., 225 min after hemorrhage). At this later time, the membrane potential was found to be even further depolarized to a value of about 35% from normal (i.e., around -60 mV). Additional simulations were performed using the model for a case where $R_{PTC}$ was decreased such that $V_{m,TC}$ was depolarized to 35% from control. Corresponding to such a perturbation, the $[K]_{IT}$ predicted by the model reached 15.5 mEq/l, a value that is very similar to the one reported by Illner and Shires. It would seem that the interstitial potassium concentration reported by these authors is correct only in the context of a very advanced shock, in which the cell membrane depolarizes to about 35% from control. Furthermore, the change in this variable should not be analyzed in parallel with the changes in other variables measured at an earlier stage of shock corresponding to a cell membrane depolarization of 25%.

c) Changes in volume and composition of plasma

In parallel to the changes in skeletal muscle water and ion concentrations, the experimental study of Illner and Shires provides information about the concentration changes of sodium, potassium and chloride ions in plasma. Comparisons between the model-predicted changes and the experimental data for these three ions are shown in the three panels of Fig. 9-20. According to the experimental study, at $t = 2.95\ h$, the plasma sodium and chloride concentrations are decreased from normal by about 1% and 4%, respectively. At the same time, the plasma potassium concentration increases to a reported value of 133% from control. The model-predicted changes for $[K]_{PL}$ and $[Cl]_{PL}$ compare well with the measured results. At $t = 2.95\ h$, the simulated $[Cl]_{PL}$ decreases by about 3% or, as shown in Table 9-8, from a control value of 105.4 mEq/l to a shock value of about 102.4 mEq/l. At the same time, the potassium concentration is predicted to increase from the baseline value of 4.1 mEq/l to shock values of about 9.9 mEq/l (138% increase) and 8.1 mEq/l (100% increase) for the $R_{PTC}$ decrease and $p_{Na,TC}$ increase, respectively. Both percentage increases are in reasonable agreement with the
Figure 9-20. Comparison of the model predictions (solid and dotted lines) with the experimental results of Illner and Shires [1980] (solid circles) for changes in plasma concentrations of sodium (upper panel), potassium (middle panel) and chloride (lower panel). Experimental data are available for control (i.e., at t = 0 h) and shock at t = 2.95 h (i.e., 165 min past hemorrhage). For additional details see legend of Fig. 9-16.
experimental value mentioned above. However, the 6-7% model-predicted decrease in $[\text{Na}]_{\text{PL}}$ is significantly more than the 1% decrease reported to take place during shock. According to the model, $[\text{Na}]_{\text{PL}}$ decreases from a baseline value of 142 mEq/l to 131.7 and 133.1 mEq/l for the $R_{\text{TC}}$ decrease and $p_{\text{Na,TC}}$ increase, respectively. This change is a direct consequence of the massive Na$^+$ transport into the tissue cells, for which the model and experiments are in good agreement (see Fig. 9-19). This important discrepancy between the model and experiments cannot be entirely explained. The model predictions, however, corroborate other experimental studies that report decreases of plasma sodium concentration ranging from 3% to 8% during hemorrhagic shock. For example, studies in dogs by Day and Friedman [1980] report a 3.2% decrease in $[\text{Na}]_{\text{PL}}$ during shock while experiments with rabbits by Menguy and Masters [1979] as well as Mohsein and DuBois [1983] indicate 5% and 8% decreases, respectively. However, since none of these other studies measured the membrane potentials of tissue cells, it is not known if the reported measurements correspond to the dysfunctional cellular transport associated with hemorrhagic shock. However, in all of these studies, the degrees of hemorrhages, on the order of 30 to 45%, should have produced shock.

The hemorrhage model of Carlson et al. [1996] was formulated on the basis that Na$^+$ and K$^+$ ions might be released from the interstitial matrix into the interstitial fluid following a severe hemorrhage. Simulations carried out by Carlson et al. show that $[\text{Na}]_{\text{PL}}$ decreases only slightly (up to 2%) during and after the hemorrhage. However, since the model proposed by these authors was never fully validated against experimental data, and since there is not enough experimental evidence to clearly justify the assumed role of the interstitial matrix, it is difficult to know if their modelling approach is reasonable.

Conversely, one might speculate that, during shock, a blood depleted hypoxic liver might release much more sodium than we accounted for. Due to a paucity of data, it does not make sense at this time to reformulate the model to accommodate an additional release of Na$^+$. However, when more experimental information becomes available, the possible release of sodium from another internal source may have to be reconsidered. It is clear that much more experimental and modeling effort is needed to clarify this subject.

Two additional predictions of the model are worth noting. The first concerns the predicted RBC membrane potential values plotted in Fig. 9-21. As shown in this figure, although the two membrane parameters, $R_{\text{RBC}}$ and $p_{\text{Na,RBC}}$, were not altered, $V_{\text{m,RBC}}$ still depolarizes as a consequence of the increased plasma potassium concentration. Unlike in the
tissue cells, however, this depolarization did not produce any significant changes in either the volume or ion contents of the RBCs. The model predictions are in agreement with the experimental observations that show a depression of the RBCs cell membrane potential during severe hypovolemia [e.g., Day and Friedman, 1980; Illner et al. 1982]. In these reports, a disturbed transmembrane transport of RBCs was claimed as well. However, the intracellular concentrations of Na\(^+\), K\(^+\) and Cl\(^-\) during the shock state are changed only by 1 - 2 mEq/l from normal.

![Figure 9-21. Model-predicted changes of the cell membrane potential of RBCs that results from a hemorrhage followed by a 210% increase in $P_{Na,TC}$ (dotted line) or a 55% decrease in $RP_{TC}$ (solid line) at $t = 2$ h. The hemorrhage and subsequent system alterations follow the protocol shown in Fig. 9-15. The values of the transport parameters of RBCs are not altered from normal.](image)
The second aspect concerns the Donnan equilibrium during shock. The study of Illner and Shires [1980] was one of the few reports which measured the concentration of potassium in the interstitial fluid. Because of the experimental difficulty associated with this type of measurement, most researchers studying hemorrhagic shock calculate $[K^+]_{rt}$ based on Donnan constraints. Hence, an important question, that has never been answered, is whether the Donnan distribution is the same in the control and shock states [e.g., see discussion of Nakayama et al., 1985].

Figure 9-22 illustrates the model-predicted differences in the concentrations of $Na^+$, $K^+$ and $Cl^-$ or alternatively, the difference in total cations and anions across the capillary, due to the Donnan effect alone.

![Figure 9-22. Model predictions for the Donnan distributions across the capillary. $\Delta(Na)_D$, $\Delta(K)_D$ and $\Delta(Cl)_D$ are the transcapillary concentration differences of sodium, potassium and chloride ions, respectively, generated due to an effective immobilization of these ionic species by the plasma proteins. $\Delta(Cat)_D$ and $\Delta(An)_D$ represent the total cationic and anionic differences caused by the Donnan effect. The results presented were obtained by decreasing Rp$_{tc}$ by 55% from at $t = 2$ h; however, similar results were obtained when p$_{Na,tc}$ was increased by 210%.}
As shown in Fig. 9-22, the Donnan equilibrium is actually less important for the entire duration post-hemorrhage (including the shock period) than it is in the control state. The main cause for the minimized Donnan effect observed in this figure, is a decrease in the transcapillary protein concentration differences after the removal of blood. A marked decrease in this difference can be observed immediately after the onset of hemorrhage and is due to the plasma hemodilution that lasts from $t = 0$ to $t = 2$ h. After this time, the fluid mobilization into the tissue cells does not change significantly the transcapillary protein concentration differences, since, as mentioned previously, both the plasma and interstitial fluid are sources for cellular edema. Hence, the protein concentrations in the two compartments rise to almost the same extent. The model seems to suggest, therefore, that calculations of the interstitial concentration of $K^+$ (and generally of all small ions) based on Donnan constraints (or alternatively by ignoring this constraint completely) would provide fairly reasonable results in hemorrhage studies.

In conclusion, in agreement with the trends reported by Illner and Shires [1980], the hemorrhage model predicts that a 20 - 25% depolarization of $V_{m,TC}$ is accompanied by cellular swelling, and cellular uptakes of $Na^+$ and $Cl^-$ that result in increased concentrations and amounts of these ions in the tissue cells. The magnitudes of all the predicted changes are also in close agreement with the measured data of Illner and Shires. The model and experiments also showed that the cellular swelling is accompanied by a loss in intracellular potassium. In contrast to the conclusion of Illner and Shires, the model predicts that the concentration of this ion is increased to the same extent in both plasma and interstitium, i.e., there is no significant concentration difference of this ion across the capillary wall. The measured plasma concentrations of potassium and chloride were well predicted by the model. However, there remains an unexplained discrepancy between the model-predicted plasma sodium concentration and the experimental value.

In vivo experiments which measure the changes in cellular transport parameters during hemorrhagic shock have never been done. However, the numerous measurements of cell membrane potential depolarization found in the literature suggest that such changes are likely to occur. The results of the simulations presented in the previous section corroborate the hypothesis that altered cellular transport parameters (such as $RP$ and $p_{Na}$) can be responsible for the cellular changes observed in hypotension-producing hemorrhage [e.g., Nakayama et al., 1985; Cunningham et al., 1971(a and b); Campion et al., 1969]. The predictions demonstrate that a depolarized cell membrane potential can be produced by poisoning the $Na^+-K^+$-pump or by
increasing the cellular membrane permeability for sodium. Unfortunately, because of the limited experimental information available, it is not possible at this time to delineate between the two mechanisms. Furthermore, although the results are not illustrated here, the model can be used to show that there are an infinite number of combinations of $R_{PTC}$ and $p_{Na,TC}$ changes that can predict the same intracellular concentration behaviour described above.

### 9.3.3.4 Application of the model in identifying the hemodilution and hemoconcentration stages of hemorrhage

A final set of simulations presents a hypothetical scenario that better approximates the progression of hemorrhage from an initial compensatory hemodilution toward a decompensated hemoconcentration and ultimately toward vascular collapse.

For the same traumatic conditions used by Illner and Shires [1980], glucose and ions were released as described previously up to 2 h post-hemorrhage. After 2 h, $R_{PTC}$ was decreased linearly by 95% over the next 2 h such that the membrane potential, $V_{m, TC}$, decreased from its control value to a shock value of about -35 to -40 mV. The continuous decrease of the transmembrane potential achieved in this manner is more similar to the trend of this variable reported in various experimental studies (e.g., see Fig. 9-2).

Some relevant results of these simulations are shown in the three panels of Fig. 9-23, which depict the transmembrane potentials of interstitial and red blood cells (upper panel), the hematocrit (middle panel) and the change in plasma volume (lower panel). As can be seen from this figure, the model predicts the two distinct phases that are widely known to occur during shock: the compensatory stage and the decompensatory stage. The first phase (compensation) is characterized by a relatively stable transmembrane potential of all the cells in the body (upper panel), blood hemodilution as reflected by a decrease of the hematocrit (middle panel) and a restitution of the plasma volume toward normal values (lower panel). During the hemoconcentration phase, the gradual depolarization of the tissue cells causes progressive swelling of these cells as well a depolarization of the RBCs (upper panel), hemoconcentration as indicated by increased hematocrit (middle panel) and a continuous decrease of plasma volume that progresses toward vascular collapse (lower panel).

There is a striking similarity between the changes in plasma volumes during the decompensatory phase presented in Fig. 9-23, and the arterial pressures presented in Fig. 9-1, which shows the gradual vascular collapse that results after a severe hemorrhage.
Figure 9-23. Model predictions for a hypothetical scenario where a severe external loss (34%) of blood is followed by an initial 2 h period of compensation (in the form of release of glucose and ions from internal sources of the body) and a subsequent 2 h tissue cell perturbation (in the form of poisoning of the Na⁺-K⁺-pump). Model predictions are provided for the membrane potential of tissue cells (solid line) and RBCs (dotted line) in the upper figure, hematocrit (middle panel) and changes in plasma volume (lower panel). The duration of the hemorrhage is indicated by the arrows on the time axis.
It was hypothesized for a long time, that a severely depressed cellular function is responsible for the low plasma volume that triggers death in hypovolemic shock. Solid evidence to support this widely accepted hypothesis has never been provided. This last set of simulations shows the model-predicted consequences of a disturbed cellular transport. It seems, at least from the preliminary simulations, that the damaged cells are indeed the final link in the progression of shock.

Although this case involved blood removal in the absence of any type of fluid resuscitation, the model-predicted changes presented in Fig. 9-23, emphasize the importance of developing resuscitation protocols directed toward preventing cellular trauma.

The simulations presented in this chapter make a strong case for pursuing the improvement and development of this hemorrhage model further. Providing that its validation proves to be successful, such a model would be a valuable tool in helping to clarify the mechanisms and events leading to shock.
Chapter 10: CONCLUSIONS AND RECOMMENDATIONS

The primary objective of this work was to develop and validate an improved compartmental model of whole body fluid, protein and small ion distribution and transport. The goal in formulating such a model was to achieve an integrated description of cellular and capillary transport that can provide useful information regarding the mass-exchange related changes that can be expected after infusions of different resuscitants.

To meet this goal, previous models developed by our group were significantly extended to accommodate the mathematical description of transcapillary and cellular transports of small ions. In this first formulation of the model, we accounted for the three primary species that govern cell volume regulation, namely Na$^+$, K$^+$ and Cl$^-$. In order to meet additional criteria of intra- and extracellular electroneutrality and osmolarity, two other generic species were introduced: all positive ions other than Na$^+$ and K$^+$, referred to as C$^{2+}$, and all negative ions other than Cl$^-$, called A$^-$. Chapter 4 provides a detailed description of this model, which is based on 24 ordinary differential mass balance equations (when glucose and dextran are accounted for), 2 nonlinear cellular membrane potential equations and a large number of algebraic auxiliary equations.

The estimation of two uncertain transport parameters required for describing the transcapillary transport of small ions, namely $\sigma_{\text{ION}}$ and $\text{PS}_{\text{ION}}$, was presented as part of Chapter 6. Their values, obtained by statistical fitting of model predictions to experimental results, were found to be $\sigma_{\text{ION}} = 0.15$ and $\text{PS}_{\text{ION}} = 3000 \times \text{PS}$; where PS is the permeability-surface area product of plasma proteins. The estimated value for $\sigma_{\text{ION}}$ is higher than the value of 0.05 traditionally assumed for these ions [e.g., Curry, 1979]; however, it is not as high as the value of 0.5 suggested by Wolf and Watson [1989]. As far as $\text{PS}_{\text{ION}}$ is concerned, its estimated value lies approximately in the middle of the range of values suggested by an early study of Yudilevich [1968]. The animal study used for parameter estimation [Wolf, 1982] was chosen because it involved successive infusions of both NS and HS solutions, and it reported a large number of transient measurements. Parameter estimates based on this study give us confidence that the values are applicable to a wide range of osmolarity conditions.

The model is able to predict both experimentally measurable system variables, such as plasma volume, plasma osmolarity, etc., as well as inaccessible or difficult-to-measure quantities
including intracellular ion contents, cell membrane potentials, etc. With the two newly estimated parameters, the model was used to simulate cases of fluid administration that covered a wide range of osmolarities, from 270 mOsm/l in the case of NS solutions to as high as 2400 mOsm/l in the case of HS and HSD solutions. Thus, Chapter 6 presented an initial validation of the model, obtained by comparing the model-predicted results with data from animal studies involving large-volume NS and HS infusions in rats [Onarheim, 1995] and variable-volume RS infusions in dogs [Manning and Guyton, 1980]. These comparisons proved satisfactory for both the time-dependent plasma volumes and protein concentrations measured by Manning and Guyton, as well as for the final steady state fluid volumes and solute concentrations of Onarheim's study. Based on both the experimental data and the model predictions, the following conclusions about the comparative effectiveness of NS and HS infusates can be drawn:

- Administration of both NS and HS causes a transient, very short-lived increase in plasma volume; however, HS leads to a greater hemodynamic improvement than RS, as indicated by the degree values of plasma expansion at the end of infusion.

- The HS infusion is accompanied by extensive fluid mobilization into plasma from the interstitium and, more importantly, from the interstitial cell reservoirs. Thus, an autotransfusion of the internal body fluids is achieved. In agreement with the experimental data of Onarheim [1995], for the volume of infusion considered here, the interstitial cells lose as much as 10% of their fluid. Considering the normal $V_{TC}$ for a human, this is equivalent to about 2.8 l of fluid movement from the cells into the extracellular environment.

- In order to achieve a pre-established plasma volume improvement, the volume of HS that needs to be infused is much lower than that of its NS counterpart, and thus, its administration requires shorter times. This observation is of major importance in various traumatic circumstances, where the restoration of plasma volume should take place as rapidly as possible.

Chapter 7 presented a further validation of the model using data obtained from human studies. Since the two capillary transport parameters for small ions were determined from animal data, it was essential to prove that the model could also be applied to predict human results. Human data that involved both large volume NS infusions [Watenpaugh et al., 1992] and small volume HSD infusions [Tølløfsrud et al., 1997] were selected for validation purposes. As presented in Chapter 7, the model provided a good description of almost all the available
experimental information. Thus, model-predicted variables such as blood volumes, Hct and colloid osmotic pressures were, in the majority of cases, within the standard error of means, of either of the two studies.

All the animal studies presented above involved nephrectomized animals. For the two human studies, the model used the renal output rates of fluid and solutes measured during the experiments. Because many experimental studies do not report renal outputs, it was necessary to formulate a self-contained model of renal excretion. Such a kidney module was developed in Chapter 8. The kidney model, when incorporated into the whole-body fluid and solute exchange model, provided very good predictions of the experimental results from two infusion studies and an investigation involving an untreated severe hemorrhage. The main conclusions drawn from these kidney validation studies can be summarized as follows:

- For isotonic expansions, in agreement with the experimental results of Sondeen et al. [1990(a)] as well as of other investigators [e.g., Watenpaugh et al., 1992], the model predicted the enhanced diuresis and mild natriuresis that accompanies NS infusions.
- The increased natriuresis and diuresis associated with HSD infusions were also well predicted by the model for both the pigs in the study of Sondeen et al. [1990(a)] as well as the humans investigated by Tølløfsrud et al. [1997].
- When a severe hemorrhage was simulated, the renal output rates provided by the model simulated well the antidiuretic response of the kidney that accompanies this condition. The decrease in renal excretion and clearances of sodium and potassium measured by Sondeen et al. [1990(b)] for swine were also in excellent agreement with the model predictions.

The kidney plays an important role in controlling the fluid and solute levels in the body. The renal function and, implicitly, the mechanisms of urine formation are extremely intricate. It is probably due to this reason that several researchers have undertaken complex modeling exercises to describe specific mechanistic aspects of the renal function. To our knowledge, an approach, such as the one presented in Chapter 8, which describes the renal excretions of fluid and solutes of interest based on simple clinical concepts linking urine production with plasma compartment variables, had never before been attempted. Its relative simplicity and the excellent results it generates when they are directly compared with experimental data, make this model unique. However, a further validation of the renal model based on a larger collection of human and animal data is strongly recommended.
Chapter 9 demonstrates the applicability of this model to the study of hemorrhage. Although a proper validation of the hemorrhage model was not provided, in most cases, the model-predicted trends of a multitude of system variables were in agreement with what is known about the hemodynamic disturbances that take place during hemorrhagic shock. However, some of the quantitative changes predicted by the model are difficult to explain at this time. It is clear that the hemorrhage model needs further modifications, particularly in the way it describes some of the specific pathophysiological changes that occur post-hemorrhage (e.g., release of glucose/solutes or the depolarization of the cell membrane potential). However, even in this incipient form, the model was still able to shed light on the system behavior for each of the three hemorrhage scenarios studied.

Thus, for the case of a mild or severe hemorrhage that is not associated with the release of glucose (e.g., experiments in which the laboratory animals are deprived of food prior to the experiment), the simulations indicate the following:

- the decrease in the capillary hydrostatic pressure determines an imbalance in the transcapillary Starling forces that result in fluid absorption from the interstitium into plasma.
- the absorption lasts up to 30 to 60 min. post-hemorrhage depending on the severity of blood loss. After this time, transcapillary fluid filtration resumes, but at lower rates.
- as a result of fluid being mobilized into plasma, the interstitial volume decreases and correspondingly, the fluid transport via the lymphatics also decreases.
- the renal output rates of fluids and solutes are decreased.
- during the hemorrhage and for the entire duration thereafter, Hct is decreased.

All the above observations are characteristic of the hemodilution stage of hemorrhage. The only variable that triggers this compensatory behaviour is the drop in $P_c$.

In addition to a decrease in $P_c$, the release of glucose and/or other solutes is also characteristic of the compensatory stage of hemorrhage. By using empirical equations to describe the release rates of these materials, simulations were performed based on a study by Gann et al. [1982] involving hemorrhages in dogs. In agreement with the experimental findings of these authors, the model predicts the following changes:

- after an acute hemorrhage, plasma osmolarity starts to increase immediately and continues to increase for at least 6 h after the hemorrhagic event.
- the increase in plasma osmolarity is also accompanied by fluid absorption into plasma as indicated by the increasing values of the blood volume restitution.
• For the 10 and 20% hemorrhages of Gann et al., the model predictions underestimate the experimental BVR values after 3 h post-hemorrhage. Since the increases in plasma osmolarity predicted by the model parallel closely those found in the experiments, it is difficult to establish what other factors might be responsible for this discrepancy. This issue will have to be studied more thoroughly at a future time.

The third scenario explored in Chapter 9 yielded a comparison between the model predictions and the experimental data of Illner and Shires [1980] that involves an advanced state of shock characterized by a defective cellular transport. In the model, the transmembrane transport of the interstitial cells was altered by imposing a step change in the sodium permeability, $p_{Na,TC}$, or the rate of the $Na^+-K^+$-pump, $RP_{TC}$, at about 2 h after the hemorrhage started. The findings predicted by the model that were corroborated by the experiments are:

• a depolarization of the transmembrane potential of the interstitial cells is the primary cause of cellular swelling.

• either an increase in $p_{Na,TC}$ or a decrease in $RP_{TC}$, or both, can bring about a depolarization of $V_{m,TC}$.

• once depolarization takes place, there is an increase in the concentrations of sodium and chloride ions within the cells. At the same time potassium is lost from the cells, as reflected by the decreased intracellular concentration of this ion.

• contrary to what is proposed by Illner and Shires [1980], the model predictions indicate a rapid equilibration of small ions between the plasma and interstitial fluid; therefore, the concentration of small ions in these two media are similar. Furthermore, they are indicative of the disturbed transport across the membrane of the interstitial cells. Thus, $[Na]_{IT}$, $[Cl]_{IT}$, $[Na]_{PL}$, and $[Cl]_{PL}$ decrease while the plasma and interstitial concentrations of potassium increase.

With the exception of the sodium concentration in plasma, all of the predicted system variables are in reasonable agreement with the measured values of Illner and Shires [1980].

One of the most valuable predictions offered by the model refers to the delineation between the hemodilution and hemoconcentration stages of shock based on the magnitude of cellular dysfunction. The predicted results show that cellular edema is one of the probable causes of the severe hypovolemia observed near the end of a lethal hemorrhagic shock. Also interesting and in agreement with other experimental findings was the predicted depolarization of the RBC
membrane during shock (even though the membrane transport parameters of these cells were not altered).

As can be seen from the above discussion, the preliminary hemorrhage model suggests new avenues for modelling the events occurring after an external loss of blood. But, this modelling exercise also points out that certain mechanisms presumed to take place after severe blood loss may need further consideration. Possible suggestions for improving the hemorrhage model are:

1) to investigate in more detail the glucose metabolism during hypovolemic states,
2) to explore alternative empirical formulations for glucose/solute release after hemorrhage; among other trials, the constant \( \text{DegHEM} \) of Eq. [9-4] should be replaced by an alternative term that accounts for the instantaneous values taken by the blood volume deficit in the progression of hemorrhage.
3) to test the hypothesis put forward by Carlson et al. [1996] that the interstitial matrix may act as a source of solute release; this might explain why our model significantly underestimated the value of \([\text{Na}]_{pL}\), and
4) to establish an alternative relationship that accounts for the continuous depolarization of \( V_m \) after severe hemorrhage.

Once the hemorrhage model is satisfactorily validated for cases of non-resuscitated blood loss, it could be taken a step further to study the effect of different resuscitants and resuscitation protocols on the treatment of hypovolemia.

In addition to the study of hemorrhage, the model can be further modified to handle a multitude of other applications. For instance, in terms of volumes, rates and combinations of fluids to be administered, optimum fluid resuscitation protocols can be investigated through similar exercises as that exemplified near the end of Chapter 7. Additionally, the existing MVES-cellular exchange model can be directly applied to the study of cardiovascular by-pass. Finally, with appropriate modifications, this model can be extended even further to study other hypovolemic conditions such as those that take place following burn injuries.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>cellular membrane surface area</td>
<td>(cm²)</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
<td>(kg)</td>
</tr>
<tr>
<td>c</td>
<td>protein concentration</td>
<td>(g/l)</td>
</tr>
<tr>
<td>C₁,₁</td>
<td>renal clearance</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>C₂₃</td>
<td>renal water clearance</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>DegHEM</td>
<td>degree of hemorrhage</td>
<td>(%)</td>
</tr>
<tr>
<td>F</td>
<td>Faraday’s constant</td>
<td>(96485.3 Coulomb/mol)</td>
</tr>
<tr>
<td>[FA]</td>
<td>concentration of negative non-diffusible intracellular species FA</td>
<td>(mM)</td>
</tr>
<tr>
<td>[FC]</td>
<td>concentration of positive non-diffusible intracellular species FC</td>
<td>(mM)</td>
</tr>
<tr>
<td>FCOMPC</td>
<td>circulatory compliance</td>
<td>(ml/mmHg)</td>
</tr>
<tr>
<td>FCOMPI</td>
<td>interstitial compliance</td>
<td>(ml/mmHg)</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>Hct</td>
<td>hematocrit</td>
<td>(%)</td>
</tr>
<tr>
<td>HS</td>
<td>hypertonic saline solution</td>
<td></td>
</tr>
<tr>
<td>HSD</td>
<td>hypertonic/hyperoncotic saline solution</td>
<td></td>
</tr>
<tr>
<td>[ION]</td>
<td>ion concentration</td>
<td>(mM)</td>
</tr>
<tr>
<td>(ION)</td>
<td>ion concentration</td>
<td>(mEq/l)</td>
</tr>
<tr>
<td>J</td>
<td>rate of fluid transfer</td>
<td>(ml/h)</td>
</tr>
<tr>
<td>kF</td>
<td>fluid filtration coefficient</td>
<td>(ml/mmHg-h)</td>
</tr>
<tr>
<td>kGlu,REL</td>
<td>proportionality constant (used to calculate the rate of glucose release after the onset of hemorrhage)</td>
<td>(g-%/h-mmHg)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>(k_{\text{ION}})</td>
<td>constant for renal excretion</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>(k_{\text{Prot}})</td>
<td>proportionality constant (used to calculate Donnan distribution)</td>
<td></td>
</tr>
<tr>
<td>(k_{\text{U}}^D)</td>
<td>'volume-depleted' constant for renal excretion</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>(k_{\text{U}}^E)</td>
<td>'volume-elevated' constant for renal excretion</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>(\text{LS})</td>
<td>lymph flow sensitivity</td>
<td>(ml/mmHg-h)</td>
</tr>
<tr>
<td>(\text{NS})</td>
<td>normal saline solution</td>
<td></td>
</tr>
<tr>
<td>(M)</td>
<td>ion/glucose content</td>
<td>(mmol)</td>
</tr>
<tr>
<td>(\dot{M})</td>
<td>rate of ion/glucose transfer</td>
<td>(mmol/h)</td>
</tr>
<tr>
<td>(\text{MW})</td>
<td>molecular weight</td>
<td>(g/mol)</td>
</tr>
<tr>
<td>(\text{NS})</td>
<td>normal saline solution</td>
<td></td>
</tr>
<tr>
<td>(\text{Osm})</td>
<td>total number of mOsmoles</td>
<td>(mOsm \equiv \text{mmoles})</td>
</tr>
<tr>
<td>(p)</td>
<td>cellular membrane permeability</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>(P)</td>
<td>hydrostatic pressure</td>
<td>(mmHg)</td>
</tr>
<tr>
<td>(\text{PS})</td>
<td>permeability-surface area product</td>
<td>(ml/h)</td>
</tr>
<tr>
<td>(Q)</td>
<td>protein content</td>
<td>(g)</td>
</tr>
<tr>
<td>(\dot{Q})</td>
<td>rate of protein transfer</td>
<td>(g/h)</td>
</tr>
<tr>
<td>(R)</td>
<td>macromolecular species infused, e.g., Dextran 70</td>
<td>(g)</td>
</tr>
<tr>
<td>(\dot{R})</td>
<td>rate of macromolecular species transfer</td>
<td>(g/h)</td>
</tr>
<tr>
<td>(\text{RPF(RBF)})</td>
<td>Renal plasma/blood flow</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>(\text{RP})</td>
<td>rate of (\text{Na}^+ - \text{K}^+)-pump</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>(\text{RS})</td>
<td>Ringer's solution</td>
<td></td>
</tr>
<tr>
<td>(S)</td>
<td>osmolarity</td>
<td>(mOsm/l)</td>
</tr>
<tr>
<td>(t)</td>
<td>time</td>
<td>(h)</td>
</tr>
<tr>
<td>(T)</td>
<td>temperature</td>
<td>(K)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
<td>Unit</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>U</td>
<td>urinary output</td>
<td>(ml/min)</td>
</tr>
<tr>
<td>V</td>
<td>volume</td>
<td>(ml)</td>
</tr>
<tr>
<td>V&lt;sub&gt;m&lt;/sub&gt;</td>
<td>cellular transmembrane electrical potential</td>
<td>(mV)</td>
</tr>
<tr>
<td>z</td>
<td>valence</td>
<td></td>
</tr>
</tbody>
</table>

**Greek Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔION&lt;sub&gt;D&lt;/sub&gt;</td>
<td>capillary transmembrane concentration difference</td>
<td>(mEq/l)</td>
</tr>
<tr>
<td>Δφ</td>
<td>dimensionless cellular transmembrane potential</td>
<td></td>
</tr>
<tr>
<td>Φ</td>
<td>osmotic coefficient</td>
<td></td>
</tr>
<tr>
<td>π</td>
<td>colloid osmotic pressure</td>
<td>(mmHg)</td>
</tr>
<tr>
<td>σ</td>
<td>reflection coefficient</td>
<td></td>
</tr>
<tr>
<td>ρ</td>
<td>ratio of the Na&lt;sup&gt;+&lt;/sup&gt;- K&lt;sup&gt;+&lt;/sup&gt;-pump</td>
<td></td>
</tr>
</tbody>
</table>

**Subscripts**

<table>
<thead>
<tr>
<th>Subscript</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV</td>
<td>available volume</td>
</tr>
<tr>
<td>C</td>
<td>capillary</td>
</tr>
<tr>
<td>COMP</td>
<td>compliance</td>
</tr>
<tr>
<td>D</td>
<td>Donnan effect</td>
</tr>
<tr>
<td>ECV</td>
<td>extracellular medium/(volume)</td>
</tr>
<tr>
<td>EX</td>
<td>excluded</td>
</tr>
<tr>
<td>FA</td>
<td>negative non-diffusible intracellular species</td>
</tr>
<tr>
<td>HEM</td>
<td>hemorrhage</td>
</tr>
<tr>
<td>ICV</td>
<td>intracellular medium/(volume)</td>
</tr>
<tr>
<td>ION</td>
<td>ION = Na&lt;sup&gt;+&lt;/sup&gt;, K&lt;sup&gt;+&lt;/sup&gt;, C&lt;sup&gt;2+&lt;/sup&gt;, Cl&lt;sup&gt;-&lt;/sup&gt;, A&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>ISL</td>
<td>insensible losses</td>
</tr>
<tr>
<td>IT</td>
<td>interstitium</td>
</tr>
<tr>
<td>L</td>
<td>lymph</td>
</tr>
<tr>
<td>NL</td>
<td>normal steady-state value</td>
</tr>
<tr>
<td>P</td>
<td>protein</td>
</tr>
<tr>
<td>PER</td>
<td>perspiration</td>
</tr>
<tr>
<td>PL</td>
<td>plasma</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cells</td>
</tr>
<tr>
<td>RES</td>
<td>infusion</td>
</tr>
<tr>
<td>TC</td>
<td>tissue cells</td>
</tr>
<tr>
<td>U</td>
<td>urine</td>
</tr>
<tr>
<td>UR</td>
<td>urinary loss</td>
</tr>
<tr>
<td>W</td>
<td>cellular water shift</td>
</tr>
</tbody>
</table>


References


References


Weisberg H.F.: Water, Electrolyte and Acid-Base Balance: Normal and pathological physiology as a basis for therapy; Chapt. 2, pp. 31-64., The Williams & Wilkins Company, Baltimore, 1962.


APPENDIX A: Derivation of the Electrodiffusional and Membrane Potential Equations

As a consequence of ions crossing the cellular membrane, a separation of charges occurs, causing the appearance of a membrane potential. Therefore, the membrane potential is a function of the ion concentrations outside and inside the cell. The first equation to describe this function is the Goldman [1943] equation, which is based on the 'constant electric field' assumption, i.e., the electric potential is assumed to vary linearly with the distance across the membrane.

The membrane potential equation can be derived by employing the following basic electrochemical transport principle: the flux of ionic species \( i \) across the membrane is governed by two driving forces, 1) a potential gradient causing the transport of ions by migration, and 2) a concentration gradient bringing about the transport of ions by diffusion. Mathematically, the ion flux is expressed as:

\[
T_{d,i} = z_i u_i F C_i \left( \frac{dV_m}{dx} \right) + D_i \left( \frac{dC_i}{dx} \right) \tag{A-1}
\]

where \( z_i \) is the charge carried by the ionic species \( i \), \( u_i \) is the ionic mobility (i.e. the rate of ion migration under an electric field of 1 V/cm), \( F \) is Faraday's constant, \( C_i \) is the concentration of ionic species \( i \), \( V_m \) is the membrane potential, \( D_i \) is the diffusion coefficient of ionic species \( i \) (note: \( D_i = u_i RT/zF \) according to the Nernst-Einstein equation), and \( x \) is the distance across the membrane.

The constant field assumption can be expressed as:

\[
\frac{dV_m}{dx} = \frac{V_m}{d_m} \tag{A-2}
\]

where \( d_m \) is the membrane thickness. By substituting Eq. [A-2] into Eq. [A-1], and solving analytically the first order differential equation for \( C_i \), the following expression for the ion flux is obtained:

\[
T_{d,i} = \frac{u_i z_i F V_m}{RT d_m} \left[ C_{i,d} \cdot e^{z_i F V_m / RT} - C_{i,0} \right] \tag{A-3}
\]
where $C_{i,d}$ is the ion concentration just within the membrane at $x = d_m$ and $C_{i,0}$ is the ion concentration just within the membrane at $x = 0$.

The ion concentrations inside the membrane are not measurable. They can be replaced by the ionic concentrations outside and inside the cell, $C_{ECV}$ and $C_{ICV}$, which are experimentally available. Thus, the ionic concentrations within the membrane can be written as:

$$C_{i,d} = k_{ION} C_{ICV} \quad \text{and} \quad C_{i,0} = k_{ION} C_{ECV},$$

[A-4]

where $k_{ION}$ is the partition coefficient between the oil (i.e., lipid membrane) and water (i.e., extra or intracellular medium).

Substituting Eq. [A-4] into Eq. [A-3] and defining the permeability constant of the ionic species $i$ as $p_i = k_{ION} u_i / d_m$, one obtains the following expression for the ion flux as a function of the membrane potential:

$$T_{d_i} = \frac{p_i z_i F V_m}{RT} \left[ \frac{C_{i,ICV} \cdot e^{z_i F V_m/RT} - C_{i,ECV}}{e^{z_i F V_m/RT} - 1} \right].$$

[A-5]

Usually there are only three ions, $Na^+$, $K^+$ and $Cl^-$, which are considered to move across the cell membrane and carry ionic currents. Therefore, Eq. [A-5] can be easily written for each of these three ions, by making $z_{Na} = z_K = +1$ and $z_{Cl} = -1$. At this point a very important assumption has to be made concerning the total (net) flux of ions across the membrane. In the original Goldman approach, the net flux of ions is considered to be zero, in other words, the ions are crossing the membrane at rates which respect the electroneutrality condition, i.e.,

$$T_{d,Na} + T_{d,K} = T_{d,Cl}.$$  

[A-6]

By replacing the equations for the ion fluxes in Eq. [A-6] and rearranging the terms, one obtains the well-known *Goldman equation* for the membrane potential:

$$V_m = \frac{RT}{F} \ln \left( \frac{p_K [K]_{ECV} + p_{Na} [Na]_{ECV} + p_{Cl} [Cl]_{ECV}}{p_K [K]_{ICV} + p_{Na} [Na]_{ICV} + p_{Cl} [Cl]_{ICV}} \right).$$

[A-7]
However, because of the existence of the Na\(^+\)-K\(^+\)-pump (see Sections 3.5.2 and 3.5.3), it is obvious that there is a net efflux of monovalent cations from the cell at a rate of \(T_a\). Thus, in this case, instead of Eq. [A-6] one has:

\[
T_{d,Na} + T_{d,K} - T_{d,Cl} = T_a .
\]  

[A-8]

By substituting the ion flux equations into Eq. [A-8] and rearranging the terms, the Moreton equation [1969] is obtained:

\[
V_m = \frac{RT}{F} \ln \left( \frac{P_K [K]_{ECV} + P_{Na} [Na]_{ECV} + P_{Cl} [Cl]_{ECV} + T_a/FV_m}{P_K [K]_{ECV} + P_{Na} [Na]_{ECV} + P_{Cl} [Cl]_{ECV} + T_a/FV_m} \right) .
\]  

[A-9]

As can be seen, the Moreton equation differs from the Goldman equation by the presence of the term \(T_a\), which represents the contribution of the electrogenic Na\(^+\)-K\(^+\)-pump to the membrane potential.

The net efflux of cations from the cell \(T_a\), can be expressed as [Jakobsson, 1980]:

\[
T_a = \frac{F}{RT} \cdot RP \cdot \frac{[Na]_{ICV}}{[Na]_{ECV}} \cdot ([Na]_{ECV} - [K]_{ICV}) = \frac{F}{RT} [Na]_{ICV} \cdot RP \cdot (1 - 1/p);
\]  

[A-10]

where \(RP\) is the pump rate, and \(p = \frac{[Na]_{ECV}}{[K]_{ICV}}\) is the pump ratio.

By substituting Eq. [A-10] into Eq. [A-9], the modified Moreton equation is obtained, which was employed in the present study:

\[
V_m = \frac{RT}{F} \ln \left( \frac{P_K [K]_{ECV} + P_{Na} [Na]_{ECV} + P_{Cl} [Cl]_{ECV} + RP[Na]_{ICV}(1 - 1/p)/(FV_m/RT)}{P_K [K]_{ECV} + P_{Na} [Na]_{ECV} + P_{Cl} [Cl]_{ECV} + RP[Na]_{ICV}(1 - 1/p)/(FV_m/RT)} \right) .
\]  

[A-11]

I. Steady-state case:

At steady-state, the total flux of each permeant species across the membrane must equal zero. The total flux is composed of the flux due to electrodiffusion plus the flux due to active transport.

**Na flux:**

\[
\frac{p_{Na} \cdot \Delta \phi \cdot ([Na]_{ECV} - [Na]_{ICV} \exp(\Delta \phi))}{(\exp(\Delta \phi) - 1)} - RP \cdot [Na]_{ICV} = 0 \quad [B-1]
\]

**K flux:**

\[
\frac{p_{K} \cdot \Delta \phi \cdot ([K]_{ECV} - [K]_{ICV} \exp(\Delta \phi))}{(\exp(\Delta \phi) - 1)} + \frac{RP[Na]_{ICV}}{\rho} = 0 \quad [B-2]
\]

**Cl flux:**

\[
[Cl]_{ICV} = [Cl]_{ECV} \exp(\Delta \phi) \quad [B-3]
\]

**Isotonicity equation:**

\[
[S]_{ECV} = [Na]_{ICV} + [K]_{ICV} + [Cl]_{ICV} + [FC^{z+}] + [FA^{z-}] \quad [B-4]
\]

where \([S]_{ECV}\) is the total solute concentration in the extracellular space; while \([FC^{z+}]\) and \([FA^{z-}]\) are the fixed cation and anion concentrations in the intracellular space, respectively (i.e., ions other than Na\(^+\), K\(^+\) and Cl\(^-\)).

**Internal electroneutrality equation:**

\[
[Na]_{ICV} + [K]_{ICV} + z^+ [FC^{z+}] = [Cl]_{ICV} + z^- [FA^{z-}] \quad [B-5]
\]
II. Time-dependent case

For the non-steady state case, Jakobsson [1980] developed the following set of four differential equations coupled with one non-linear algebraic equation to describe the interactions of cell volume, intracellular ion content, membrane potential and membrane transport parameters:

Membrane potential (modified Moreton equation, see Appendix A):

\[
\Delta \phi = \ln \left( \frac{p_{Na}[Na]_{ECV} + p_{K}[K]_{ECV} + p_{Cl}[Cl]_{ICV} + RP[Na]_{ICV}(1-1/\rho)/\Delta \phi}{p_{Na}[Na]_{ICV} + p_{K}[K]_{ICV} + p_{Cl}[Cl]_{ECV} + RP[Na]_{ECV}(1-1/\rho)/\Delta \phi} \right)
\]  \[B-6\]

\[Na\] flux:

\[
\frac{d[Na]_{ICV}}{dt} = A \cdot \left[ \frac{p_{Na} \cdot \Delta \phi \cdot ([Na]_{ECV} - [Na]_{ICV} \exp(\Delta \phi))}{\exp(\Delta \phi) - 1} - RP[Na]_{ICV} \right]
\]  \[B-7\]

\[K\] flux:

\[
\frac{d[K]_{ICV}}{dt} = A \cdot \left[ \frac{p_{K} \cdot \Delta \phi \cdot ([K]_{ECV} - [K]_{ICV} \exp(\Delta \phi)) + RP[Na]_{ICV}}{\rho \exp(\Delta \phi) - 1} \right]
\]  \[B-8\]

\[Cl\] flux:

\[
\frac{d[Cl]_{ICV}}{dt} = \frac{d[Na]_{ICV}}{dt} + \frac{d[K]_{ICV}}{dt}
\]  \[B-9\]

Water flux:

\[
\frac{dV_{ICV}}{dt} = \frac{1}{S_{ECV}} \left( \frac{d[Cl]_{ICV}}{dt} + \frac{d[Na]_{ICV}}{dt} + \frac{d[K]_{ICV}}{dt} + \frac{dFC_{ICV}}{dt} + \frac{dFA_{ICV}}{dt} \right)
\]  \[B-10\]

where the terms \( \frac{dFC_{ICV}}{dt} \) and \( \frac{dFA_{ICV}}{dt} \) are zero, i.e., the contents of the intracellular negative and positive anions other than \( Na^+ \), \( K^+ \) and \( Cl^- \) do not change from their initial values.
APPENDIX C: Scaling Procedure for Determination of the Initial Compartmental Values

The scaling procedure employed in this work makes use of two sets of initial values: one corresponding to the 70-kg 'Reference man', and one reported for the experimental animal in a specific study under investigation. The two sets are combined to determine a new set of compartmental values that correspond to what will be referred to as a 'Scaled Animal', and is used as input to the model. The steps undertaken to determine these initial conditions are exemplified for the study of Wolf [1982] that involves dogs as experimental animals.

Wolf's study [1982] reports the following initial average values for dogs:

- Body weight, $BW_{Dog} = 26$ kg
- Red Blood Cell Volume, $V_{RBC,Dog} = 0.8$ l
- Plasma Volume, $V_{PL,Dog} = 1.02$ l
- Interstitial Volume, $V_{IT,Dog} = 4.14$ l
- Total Body Water, $TBW_{Dog} = 15.5$ l

The compartmental values for fluid and solutes are determined by scaling these experimental animal values, to the appropriate human values through the following steps.

**Step 1: Initial values for $V_{PL}$ and the intensive physiological parameters of the 'Scaled Dog'

The plasma volume value together with all the intensive physiological parameters of the 'Scaled Dog' are considered to be the same as those of the 'Reference man' and are presented in Table C-1. The hematocrit value for the 'Scaled Dog' is the same as the Hct of the experimental dog. This variable is calculated based on the known plasma and red blood cell volume reported by Wolf [1982] and can be used further to determine $V_{RBC}$ of the 'Scaled Dog'. The partition of solutes (i.e., proteins and small ions) between plasma, interstitium and the two types of cellular compartments is the same as presented in Table 4-2 of Chapter 4. Additionally, the cellular membrane parameters for RBCs and tissue cells are maintained the same as the values presented in Table 4-3 of Chapter 4.
### Variable Values for 'Reference man' and/or 'Scaled Dog'

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value for 'Reference man' and/or 'Scaled Dog'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Volume, $V_{PL}$</td>
<td>3.2 l</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>43.9 %</td>
</tr>
<tr>
<td>Red Blood Cell Volume, $V_{RBC}$</td>
<td>2.5 l</td>
</tr>
<tr>
<td>Plasma Colloid Osmotic Pressure, $\pi_{PL}$</td>
<td>25.9 mmHg</td>
</tr>
<tr>
<td>Plasma Hydrostatic Pressure, $P_C$</td>
<td>11.0 mmHg</td>
</tr>
<tr>
<td>Plasma Protein Concentration, $c_{P,PL}$, g/l</td>
<td>70.0 g/l</td>
</tr>
<tr>
<td>Vascular Compliance, $P_{C,COMP}$</td>
<td>0.096 mmHg/l</td>
</tr>
<tr>
<td>Interstitial Colloid Osmotic Pressure, $\pi_{PL}$</td>
<td>14.7 mmHg</td>
</tr>
<tr>
<td>Plasma Hydrostatic Pressure, $P_C$</td>
<td>-0.7 mmHg</td>
</tr>
<tr>
<td>Interstitial Protein Concentration, $c_{P,IT}$</td>
<td>29.8 g/l</td>
</tr>
<tr>
<td>Interstitial Protein Concentration in the Available Volume, $c_{P,IT,AV}$</td>
<td>39.7 g/l</td>
</tr>
</tbody>
</table>

Table C-1: Initial compartmental values for the vascular volume and some of the intensive physiological parameters corresponding to the 'Scaled Dog' and the 'Reference man'.

### Step 2: Scaling of the interstitial and tissue cell volumes

The value for the interstitial volume for the 'Scaled Dog' is determined by using a scaling factor, $R(PL)$, given by the ratio of the plasma volume of the normal human to that of the experimental dog:

$$R(PL) = \frac{V_{PL,Reference\ man}}{V_{PL,Dog}} = \frac{3.2 \text{ l}}{1.020 \text{ l}} = 3.14$$  \[C-1\]

where the subscript 'Dog' refers to the value of the experimental dog provided by Wolf [1982] while the subscript, 'Reference man' has its usual meaning.

Therefore, the interstitial volume of the 'Scaled Dog', $V_{IT}$, is calculated as,

$$V_{IT} = R(PL) \cdot V_{IT,Dog} = 3.14 \cdot 4.14 \text{ l} = 12.9 \text{ l}$$  \[C-2\]
As discussed in Chapter 4, the excluded interstitial volume of the 'Scaled Dog', $V_{IT,EX}$ is calculated as 25% of $V_{IT}$. Thus, the interstitial volume available for proteins that corresponds to the 'Scaled Dog', $V_{IT,AV}$, can be calculated as,

$$V_{IT,AV} = \frac{75}{100} \cdot 12.9 \text{ l} = 9.7 \text{ l} \quad [C-3]$$

The tissue cell volume for the experimental dog is assumed to be 40% of BW$_{Dog}$ (i.e., it is assumed that the average whole-body density is 1 kg/l). In order to determine the initial value for the tissue cell volume of the 'Scaled Dog', $V_{TC}$, the calculated cell volume corresponding to the dog is multiplied by the scaling factor R(PL) to give:

$$V_{TC} = 3.14 \cdot 26 \cdot \frac{40}{100} = 32.6 \text{ l} \quad [C-4]$$

**Step 3: Determination of the interstitial compliance segments**

The interstitial volume values, needed for the intermediate interpolation range of the interstitial compliance, are determined by using the scaling factor R(IT) defined as the ratio of the interstitial volume of the 'Scaled Dog' by that of the normal human:

$$R(IT) = \frac{V_{IT, 'Scaled Dog'}}{V_{IT, 'Reference man'}} = \frac{12.9 \text{ l}}{8.4 \text{ l}} = 1.54 \quad [C-5]$$

Thus, any intermediate interstitial volume of the 'Scaled Dog' in the range of moderate tissue hydration is:

$$V_{IT} = R(IT) \cdot V_{IT, 'Reference man'} \quad [C-6]$$

The range of $V_{IT}$-$P_{IT}$ pairs for the 'Scaled Dog' that are obtained by using Eq. [C-6] together with the interstitial values for moderate tissue hydration representative of the 'Reference man', are presented in Table C-2.

The two slopes of the linear segments corresponding to either the dehydration region, AS, and the overhydration region, BS, of the interstitial compliance are calculated by using Eqs. [4-31] and [4-32] (see also Chapter 4). By using the two extreme values of the interpolation range for the 'Scaled Dog', these two equation can be simply re-written as,
Appendix C

<table>
<thead>
<tr>
<th>Values for $V_{IT}$, 'Reference man' (l)</th>
<th>Values for $V_{IT}$, 'Scaled Dog' (l)</th>
<th>Values for $P_{IT}$ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.4</td>
<td>12.9</td>
<td>-0.7</td>
</tr>
<tr>
<td>8.92</td>
<td>13.7</td>
<td>0.32</td>
</tr>
<tr>
<td>9.45</td>
<td>14.6</td>
<td>0.86</td>
</tr>
<tr>
<td>9.97</td>
<td>15.4</td>
<td>1.15</td>
</tr>
<tr>
<td>10.5</td>
<td>16.2</td>
<td>1.37</td>
</tr>
<tr>
<td>11.2</td>
<td>17.2</td>
<td>1.56</td>
</tr>
<tr>
<td>11.55</td>
<td>17.8</td>
<td>1.69</td>
</tr>
<tr>
<td>12.07</td>
<td>18.6</td>
<td>1.8</td>
</tr>
<tr>
<td>12.6</td>
<td>19.4</td>
<td>1.88</td>
</tr>
</tbody>
</table>

Table C-2. The ranges of interstitial volume and hydrostatic pressure values used for determination of the intermediate segment of the interstitial compliance.

Dehydration segment:

$$AS = \frac{P_{IT} + 0.7}{(V_{IT, \text{'Scaled Dog'}} - 12.9)}$$  \[C-7\]

Overhydration segment:

$$BS = \frac{P_{IT} - 1.88}{(V_{IT, \text{'Scaled Dog'}} - 19.4)}$$  \[C-8\]

The values for AS and BS obtained by using Eqs. [C-7] and [C-8] for the 'Scaled Dog' are presented in second column of Table C-3. For completeness the AS and BS values for the 'Reference man', and the experimental dog [Wolf, 1982] are also included in this table.
Step 4: Determination of the transport parameters

All the transport parameters other than the reflection coefficients for proteins and small ions, $\sigma$ and $\sigma_{\text{ion}}$, respectively, are also obtained by making use of the corresponding human values and the scaling factor $R(IT)$ as follows:

$$\text{Transport Parameter}_{\text{'Scaled Dog'}} = R(IT) \cdot \text{Transport Parameter}_{\text{'Reference man'}}$$  \hfill [C-9]

The values for the transport parameters obtained by employing Eq. [C-9] are presented in Table C-4. The reflection coefficients for proteins and small ions are maintained the same as presented in Table 4-1 for all the simulations done in this work; that is, $\sigma = 0.9888$ and $\sigma_{\text{ion}} = 0.15$, respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>'Reference man'</th>
<th>'Scaled Dog'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration coefficient, $k_F$</td>
<td>120.64 ml/mmHg-h</td>
<td>185.7 ml/mmHg-h</td>
</tr>
<tr>
<td>Permeability-surface area product for proteins, PS</td>
<td>72.98 ml/h</td>
<td>112.4 ml/h</td>
</tr>
<tr>
<td>Permeability-surface area product for small ions, $P_{\text{ion}}$</td>
<td>218940.0 ml/h</td>
<td>337200.0 ml/h</td>
</tr>
<tr>
<td>Lymph flow-sensitivity, $L_S$</td>
<td>43.08 ml/mmHg-h</td>
<td>66.3 ml/mmHg-h</td>
</tr>
<tr>
<td>Lymph flow rate, $J_L$</td>
<td>75.46 ml/h</td>
<td>116.2 ml/h</td>
</tr>
</tbody>
</table>

Table C-4. Transport parameters for 'Reference man' and for 'Scaled Dog'.
Step 5: Determination of the body weight of the 'Scaled Dog'

The body weight of the 'Scaled animal' is estimated by making use of the known percentage of total body weight accounted for by water in the experimental animal, i.e., the experimental dog. Thus, if the total body water for the experimental dogs used by Wolf [1982] represents 59.6% of BW \(_{Dog}\), then, corresponding to the total volume of fluids yielded after scaling the body weight of the 'Scaled Dog' is estimated to be 85.9 kg, i.e.,

\[
\text{TBW } \text{'Scaled Dog'} = V_{RBC} + V_{PL} + V_{IT} + V_{TC} = 2.5 \text{ (l)} + 3.2 \text{ (l)} + 12.9 \text{ (l)} + 32.6 \text{ (l)} = 51.2 \text{ (l)}
\]

\[
\text{BW } \text{'Scaled Dog'} = 51.2 \times 100/59.6 = 85.9 \text{ kg}
\]
APPENDIX D: Initial Distribution of Proteins, Small Ions and Glucose between Red Blood Cells, Plasma, Interstitium and Tissue Cells

The distribution of solutes between the compartments under study was recalculated to account for compartmental small ion values that are more descriptive of humans [Tølløfsrud et al., 1997] as well as to incorporate the presence of glucose as a contributor to the total plasma and interstitial osmolarities. Although the new values obtained do not differ significantly from the original set of initial conditions (see Tables 4-2 and 4-3), they are presented below for completeness. Thus, Table D-1 gives the new steady-state cellular membrane parameters that were obtained as part of these new calculations, while the partition of proteins, small ions and glucose between the intra- and extracellular fluids are presented in Table D-2. The values presented in Tables D-1 and D-2 were maintained unchanged for all the cases simulated in Chapters 7-9.

<table>
<thead>
<tr>
<th>Membrane Parameter</th>
<th>Red Blood Cells</th>
<th>Muscle Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{Na}$ (cm/sec)</td>
<td>$4 \times 10^{-10}$</td>
<td>$8.48 \times 10^{-7}$</td>
</tr>
<tr>
<td>$P_{K}$ (cm/sec)</td>
<td>$3.81 \times 10^{-10}$</td>
<td>$5 \times 10^{-5}$</td>
</tr>
<tr>
<td>$P_{Cl}$ (cm/sec)</td>
<td>$10^{-8}$</td>
<td>$5 \times 10^{-5}$</td>
</tr>
<tr>
<td>$RP$ (cm/sec)</td>
<td>$6.4 \times 10^{-9}$</td>
<td>$3.99 \times 10^{-5}$</td>
</tr>
<tr>
<td>$V_m$ (mV)</td>
<td>-9.43</td>
<td>-87.75</td>
</tr>
</tbody>
</table>

Table D-1 Calculated values for steady-state cellular membrane parameters.
<table>
<thead>
<tr>
<th>Species</th>
<th>Red Blood Cells (RBC)</th>
<th>Plasma (PL)</th>
<th>Interstitium (IT)</th>
<th>Interstitial Cells (TC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>z mEq/l</td>
<td>mM</td>
<td>Ref.</td>
<td>z mEq/l</td>
</tr>
<tr>
<td>Positive Charges</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>+1</td>
<td>10.0</td>
<td>10.0</td>
<td>R1</td>
</tr>
<tr>
<td>K</td>
<td>+1</td>
<td>140.0</td>
<td>140.0</td>
<td>R2</td>
</tr>
<tr>
<td>C or FC</td>
<td>+2</td>
<td>39.5</td>
<td>19.75</td>
<td>C</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>189.5</td>
<td>169.75</td>
<td></td>
</tr>
<tr>
<td>Negative Charges</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>-1</td>
<td>74</td>
<td>74</td>
<td>R1</td>
</tr>
<tr>
<td>A or FA</td>
<td>-2</td>
<td>115.5</td>
<td>57.75</td>
<td>C</td>
</tr>
<tr>
<td>Proteins</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>-17</td>
</tr>
<tr>
<td>Glucose</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>9.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>189.5</td>
<td>131.75</td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL OSMOLARITY**  
301.5 301.5 300 300

Table D-2 Steady-state values for proteins, small ions, and glucose showing their partition between intra- and extracellular compartments. The values are obtained from: R - literature references, R1 - [Hoffman, 1987], R2 - [Kleinman and Lorentz, 1984], R3 - [Tølløfsrud et al., 1997], R4 -[Guyton, 1991] and C - calculations.
APPENDIX E: Compartmental Values for Swine

The initial values presented in this appendix were used for validation of the kidney model presented in Chapter 8 and are based on information given in the studies of Sondeen et al. [1990(a), 1990(b)]. Since these two experimental studies do not include data on the compartmental volumes of pigs, these values were obtained from additional literature information on normal pigs provided by the same group of authors as part of another study [Hannon et al., 1990]. Exceptions were made with respect to the Hct and the initial body weight values of the experimental pigs which were obtained from the original studies of Sondeen et al. [1990(a) and 1990(b)]. The scaling procedure for obtaining the initial set of data that constitutes the input to the model was similar to that exemplified in Appendix C for dogs.

Table E-1 presents the initial compartmental volume values together with the transport parameters estimated to be representative of Sondeen et al.'s experimental animals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Swine [Hannon et al. 1990]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, BW*</td>
<td>21.2 kg</td>
</tr>
<tr>
<td>Plasma volume, V_{PL}</td>
<td>47.1 ml/kg</td>
</tr>
<tr>
<td>Hematocrit, Hct</td>
<td>30.0%</td>
</tr>
<tr>
<td>Red blood cell volume, V_{RBC}</td>
<td>20.2 ml/kg</td>
</tr>
<tr>
<td>Interstitial volume, V_{IT}</td>
<td>171.7 ml/kg</td>
</tr>
<tr>
<td>Excluded interstitial volume, V_{IT,EX}</td>
<td>42.9 ml/kg</td>
</tr>
<tr>
<td>Tissue cell volume, V_{TC}</td>
<td>400.0 ml/kg</td>
</tr>
<tr>
<td>Fluid filtration coefficient, k_F</td>
<td>2.67 ml/kg-mmHg-h</td>
</tr>
<tr>
<td>Permeability-surface area product for protein, PS</td>
<td>1.62 ml/kg-h</td>
</tr>
<tr>
<td>Permeability-surface area product for small ions, PS_{ION}</td>
<td>4860.0 ml/kg-h</td>
</tr>
<tr>
<td>Lymph flow sensitivity, LS</td>
<td>0.95 ml/kg-mmHg-h</td>
</tr>
<tr>
<td>Normal lymph flow rate, J_{L, NL}</td>
<td>1.67 ml/kg-h</td>
</tr>
</tbody>
</table>

Table E-1. Initial compartmental values for pigs used to simulate the experimental results of Sondeen et al. [1990(a), 1990(b)].

* Values obtained directly from Sondeen et al. [1990(a)].
The concentrations of solutes (i.e., proteins and small ions) in plasma, interstitium and the two types of cellular compartments, as well as the cellular membrane parameters for RBCs and tissue cells, are the same as those presented in Appendix D. The relationships for the three segments descriptive of the swine interstitial compliance are presented in Table E-2.

<table>
<thead>
<tr>
<th>Region of Curve</th>
<th>Range of $V_{IT}$</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>$V_{IT} \leq 3640$ ml</td>
<td>$P_{IT} = -0.7 + 4.527 \times 10^{-3} (V_{IT} - 3640$ ml)</td>
</tr>
<tr>
<td>Moderate Hydration</td>
<td>$3640$ ml $\leq V_{IT} \leq 5460$ ml</td>
<td>Mathematical interpolation for $P_{IT}$ and $V_{IT}$ experimental data</td>
</tr>
<tr>
<td>Overhydration</td>
<td>$V_{IT} \geq 5460$ ml</td>
<td>$P_{IT} = 1.88 + 2.423 \times 10^{-4} (V_{IT} - 5460$ ml)</td>
</tr>
</tbody>
</table>

Table E-2. Interstitial compliance relationship for swine [Sondeen et al., 1990 (a) or 1990 (b)]; $P_{IT}$ is expressed in mmHg, while $V_{IT}$ is expressed in ml.

The initial urinary output and sodium excretion rates were maintained the same as the values reported in the experimental study. The initial renal conditions used for simulations that involve NS and HSD fluid infusions, based on the study of Sondeen et al. [1990(a)], are given in Table E-3. The initial renal excretion values of all other ions eliminated from plasma are the same as those corresponding to the normal human (see Table 8-4, Chapter 8). In order to account for a different initial urinary output rate than that representative of a normal human, i.e., 60 ml/h, the model incorporates a modified version of Eq. [8-5] (see Chapter 8) given as,

$$J_U = (k_U \cdot \frac{(V_{PL} - V_{PL,NL})}{V_{PL,NL}} + J_{U,NL}) \cdot \frac{J_{U,IN}}{J_{U,NL}}$$

[E-1]

where $J_{U,NL}$ is the normal urinary output considered for humans, $J_{U,IN}$ is the initial value reported by Sondeen et al. [1990(a)] for pigs, while all the other symbols were defined in Chapter 8. The initial clearance value of sodium was calculated from Eq. [8-2], based on known initial values for the plasma sodium concentration and the excretion rates of fluid and sodium. The value obtained in this manner is also given in Table E-3. For comparison purposes, the mean together
with the normal range of values, measured for normal pigs by Hannon et al. [1990] are also included in the last column of Table E-3.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Swine [Sondeen et al., 1990 (a)]</th>
<th>Mean value and Normal Physiological Range [Hannon et al. 1990]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary output, ( J_{U,IN} )</td>
<td>78.6 ml/h</td>
<td>63.6 ml/h (12.72 - 203.52)</td>
</tr>
<tr>
<td>Urinary sodium excretion, ( M_{Na, U, IN} )</td>
<td>3.0 mEq/h</td>
<td>3.9 mEq/h (0.25 - 8.13)</td>
</tr>
<tr>
<td>Sodium concentration in urine, ( [Na]_{U,IN} )</td>
<td>38.6 mEq/l</td>
<td>45.8 mEq/l (5 - 162.6)</td>
</tr>
<tr>
<td>Calculated Sodium Clearance, ( C_{l,Na} )</td>
<td>21.2 ml/h</td>
<td>20.3 ml/h (2.54 - 73.7)</td>
</tr>
</tbody>
</table>

Table E-3. Initial fluid and sodium excretion rates used in the simulations of the experimental results of Sondeen et al. [1990(a)] and normal physiological values for renal excretion of fluid and sodium measured in pigs by Hannon et al. [1990].

A similar approach was undertaken to determine the initial renal conditions necessary for simulations of the study that involves graded hemorrhage in otherwise normal pigs by Sondeen et al. [1990(b)]. The experimental values for urinary output, sodium excretion rate and urinary sodium concentration together with the calculated renal clearance of sodium that constitute inputs to the model are presented in Table E-4.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Swine [Sondeen et al., 1990(b)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary output, ( J_{U,IN} )</td>
<td>92.4 ml/h</td>
</tr>
<tr>
<td>Urinary sodium excretion, ( M_{Na, U, IN} )</td>
<td>5.88 mEq/h</td>
</tr>
<tr>
<td>Sodium concentration in urine, ( [Na]_{U,IN} )</td>
<td>63.6 mEq/l</td>
</tr>
<tr>
<td>Calculated Sodium Clearance, ( C_{l,Na} )</td>
<td>41.4 ml/h</td>
</tr>
</tbody>
</table>

Table E-4. Initial fluid and sodium excretion rates used in the simulations of the experimental results of Sondeen et al. [1990(b)].
APPENDIX F: Initial Values Used for the Study of Gann et al. [1982]

The initial values presented in this appendix were used when the model was applied to the study of hemorrhage. These values constitute the inputs to the model for the simulations based on the study of Gann et al. [1982]. Table F-1 presents the initial compartmental volume values together with the transport parameters. The partition of solutes (i.e., proteins, small ions and glucose) between plasma, interstitium and the two types of cellular compartments, together with the cellular membrane parameters for RBCs and tissue cells are the same as the information presented in Appendix D. The relationships for the three segments descriptive of the dog interstitial compliance are presented in Table F-2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dog [Gann et al. 1982]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, BW</td>
<td>20.0 kg</td>
</tr>
<tr>
<td>Plasma volume, ( V_{PL} )</td>
<td>42.9 ml/kg</td>
</tr>
<tr>
<td>Hematocrit, Hct</td>
<td>40.0 %</td>
</tr>
<tr>
<td>Red blood cell volume, ( V_{RBC} )</td>
<td>28.6 ml/kg</td>
</tr>
<tr>
<td>Interstitial volume, ( V_{IT} )</td>
<td>150.0 ml/kg</td>
</tr>
<tr>
<td>Excluded interstitial volume, ( V_{IT,EX} )</td>
<td>37.5 ml/kg</td>
</tr>
<tr>
<td>Tissue cell volume, ( V_{TC} )</td>
<td>400.0 ml/kg</td>
</tr>
<tr>
<td>Fluid filtration coefficient, ( k_F )</td>
<td>2.15 ml/kg-mmHg-h</td>
</tr>
<tr>
<td>Permeability-surface area product for protein, ( PS )</td>
<td>1.30 ml/kg-h</td>
</tr>
<tr>
<td>Permeability-surface area product for small ions, ( PS_{ION} )</td>
<td>3900.0 ml/kg-h</td>
</tr>
<tr>
<td>Lymph flow sensitivity, ( LS )</td>
<td>0.77 ml/kg-mmHg-h</td>
</tr>
<tr>
<td>Normal lymph flow rate, ( J_{L,NL} )</td>
<td>1.35 ml/kg-h</td>
</tr>
</tbody>
</table>

Table F-1. Initial compartmental values for dogs used to simulate the experimental results of Gann et al. [1982].

<table>
<thead>
<tr>
<th>Region of Curve</th>
<th>Range of ( V_{IT} )</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>( V_{IT} \leq 3000 \text{ ml} )</td>
<td>( P_{IT} = -0.7 + 5.492 \times 10^{-4} \ (V_{IT} - 3000 \text{ ml}) )</td>
</tr>
<tr>
<td>Moderate Hydration</td>
<td>3000 \text{ ml} \leq V_{IT} \leq 4500 \text{ ml}</td>
<td>Mathematical interpolation for ( P_{IT} ) and ( V_{IT} ) experimental data</td>
</tr>
<tr>
<td>Overhydration</td>
<td>( V_{IT} \geq 4500 \text{ ml} )</td>
<td>( P_{IT} = 1.88 + 2.94 \times 10^{-4} \ (V_{IT} - 4500 \text{ ml}) )</td>
</tr>
</tbody>
</table>

Table F-2. Interstitial compliance relationship for dog [Gann et al., 1982]; \( P_{IT} \) is expressed in mmHg, while \( V_{IT} \) is expressed in ml.
APPENDIX G: Initial Values Used for the Study of Illner and Shires [1980]

The initial values presented in this appendix were used when the model was applied to the study of cellular depolarization after a severe hemorrhage. These values constitute the inputs to the model for the simulations based on the studies of Illner and Shires [1980] presented in Chapter 8. Table G-1 shows the initial compartmental volume values together with the transport parameters for rats. Since the original study did not provide enough information to allow calculation of the fluid partition between the different body compartments, Table G-1 was put together based on additional data for rats provided by Onarheim [1995]. This latter study involved experimental rats with body weights comparable to those used by Illner and Shires [1982]. Thus, for example, the volume of the interstitial compartment was calculated based on the assumption that the total body water represents 62.3% of BW [Onarheim, 1995]. The partition of solutes (i.e., proteins, small ions and glucose) between plasma, interstitium and the two types of cellular compartments, together with the cellular membrane parameters for RBCs and tissue cells, are the same as the data presented in Appendix D. The relationships for the three segments descriptive of the rat interstitial compliance are presented in Table G-2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, BW*</td>
<td>0.190 kg</td>
</tr>
<tr>
<td>Plasma volume, V_P</td>
<td>26.0 ml/kg</td>
</tr>
<tr>
<td>Hematocrit, Hct*</td>
<td>48.0 %</td>
</tr>
<tr>
<td>Red blood cell volume, V_RBC</td>
<td>24.0 ml/kg</td>
</tr>
<tr>
<td>Interstitial volume, V_IT</td>
<td>173.0 ml/kg</td>
</tr>
<tr>
<td>Excluded interstitial volume, V_IT,EX</td>
<td>43.25 ml/kg</td>
</tr>
<tr>
<td>Tissue cell volume, V_TC</td>
<td>400.0 ml/kg</td>
</tr>
<tr>
<td>Fluid filtration coefficient, k_F</td>
<td>2.48 ml/kg-mmHg-h</td>
</tr>
<tr>
<td>Permeability-surface area product for protein, PS</td>
<td>1.5 ml/kg-h</td>
</tr>
<tr>
<td>Permeability-surface area product for small ions, PSION</td>
<td>4509.1 ml/kg-h</td>
</tr>
<tr>
<td>Lymph flow sensitivity, LS</td>
<td>0.88 ml/kg-mmHg-h</td>
</tr>
<tr>
<td>Normal lymph flow rate, J_L,NL</td>
<td>1.55 ml/kg-h</td>
</tr>
</tbody>
</table>

Table G-1. Initial compartmental values for rats used to simulate experimental results of Illner and Shires [1980].
* Values obtained directly from the study of Illner and Shires [1980].
### Table G-2. Interstitial compliance relationship for rat [Illner and Shires, 1980]; $P_{IT}$ is expressed in mmHg, while $V_{IT}$ is expressed in ml.

<table>
<thead>
<tr>
<th>Region of Curve</th>
<th>Range of $V_{IT}$</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>$V_{IT} \leq 32.87$ ml</td>
<td>$P_{IT} = -0.7 + 0.5013 \left( V_{IT} - 32.87 \text{ ml} \right)$</td>
</tr>
<tr>
<td>Moderate Hydration</td>
<td>$32.87$ ml $\leq V_{IT} \leq 49.3$ ml</td>
<td>Mathematical interpolation for $P_{IT}$ and $V_{IT}$ experimental data</td>
</tr>
<tr>
<td>Overhydration</td>
<td>$V_{IT} \geq 49.3$ ml</td>
<td>$P_{IT} = 1.88 + 2.6833 \times 10^{-3} \left( V_{IT} - 49.3 \text{ ml} \right)$</td>
</tr>
</tbody>
</table>
Appendix H : Program L i s t i n g

HH

<
o
o
z
o

HH

oo

321

00

C3
HH

H
<

O

a

o
>

w
w
p
o

JL,

a

JL

s ^ '^ '

b

o

PH

II

cn

w w ww
P D Q D
o o o o

w w ww
DDDQ
o o o o

EC w w
13
cd
CO

Q Q
O O

o

6 2

« -—'
ftM
CO 2

5

8
*

o

PH
PH

•7

~

a xT " •
w
OO

-JPX5>-

Xf

"

in

Oi
O

cd
OO

co

U
D
J

w
o
X
w

i ^3

60

e

G
s/

O ^
OH
VO

cd

oo H
ON

vq

»

vq

PH

O A

rf> j E H H ; ^ U
ug^.sIxTS

1

uW
HH
HH

OO

PH

O

o

z

T>

ALL
FIT

U u

•c
2 + +"+ + + + +
o
PH

<
Q

Xf !> ^

tf $ ^ =-

CJ

S

<:

•rEO
5-X
co H v i

UH

O

*


*......Define variables in the interstitium:
* Ions in 'Tissue Cell' compartment
  YMODEL(14)=CNAINI*CVITO
  YMODEL(15)=CKINI*CVITO
  YMODEL(16)=CCLIINI*CVITO

*TCs and Interstitial volumes
  YMODEL(17)=CVITO
  YMODEL(18)=VITO

* Ions and proteins in Interstitial fluid compartment
  YMODEL(19)=CNAOUTI*VITO
  YMODEL(20)=CKOUTI*VITO
  YMODEL(21)=CCLOUTI*VITO
  YMODEL(22)=QITO
  YMODEL(23)=FAEI*VITO
  YMODEL(24)=FCEI*VITO

*......Define variable for resuscitation w/Dextran or any other
* macromolecular species:
  YMODEL(25)=DEXPLO
  YMODEL(26)=DEXITO

*......Define variable for Glucose:
  YMODEL(27)=GLUPLO
  YMODEL(28)=GLUITO

*......Solve model equations
  IPERO=1
  IP=0
  J=1
  TIME=0.
  BV=BVO
  HCT=HCTO
  CVPL=CVPLO
  PLOST=0.0

CALL PUMP(TIME,TEND(IP),TSTART(IP))
CALL MODEL(TIME,YMODEL)
CALL MEMBRP(YMODEL)

*......Output the parameters at t=0

CALL ASSIGN(IP,YMODEL,SOLNON)
CALL OUTPUT(IP,TIME,HCT,SOLNON,BV)

*......Loop over the periods

DO 1000 l=1,NRES
  RINC=TEND(IP)-TSTART(IP)
  RNTIME=RINC/DT
  NTIME=INT(RNTIME)

IPERO=IP

CALL PUMP(TIME,TEND(IP),TSTART(IP))

DO 2000 l=1,NTIME

  TIME=TIME
  TIME=TIME+DT
  TMIN=1D-10*DT
  TMAX=DT*1.0
  TSTRT=1D-2*DT

*......Apply Ion balance

CALL HEM (IP)
CALL ODEINT(YMODEL,NEQ,TIMEIN,TIME,EPS,TSTRT,TMIN)

BV=YMOLD(6)+YMOLD(7)

CALL BALANCE(YMODEL)
CALL MEMBRP(YMODEL)
CALL MODEL(TIME,YMODEL)
*......OUTPUT results
   CALL ASSIGN(IP, YMODEL, SOLNON)

*...... WRITE results
   IF (DABS(TIME-TI(J)).LE.1.D-5) THEN
      J=J+1
      CALL OUTPUT(IP, TIME, HCT, SOLNON, BV)
   ENDF

2000 CONTINUE
1000 CONTINUE

STOP
END

*______________________________
* BLOCK DATA : Data to be passed to the MODEL
*______________________________

BLOCK DATA
IMPLICIT REAL*8 (A-H,O-Z)
INCLUDE 'C:\FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:\FORTRAN\CRISTINA\CELPARAM.FOR'

*_____ I. DATA FOR FLUID COMPARTMENTS:

*Normal Human Values
   DATA WT,HT/70.D0,170.D0/
*Compartmental volumes
   DATA CVITNL/28000.D0/
   DATA VEITNL/2100.D0/
   DATA VITNL/8400.D0/
   DATA VPLNL/3200.D0/
*Pressures
   DATA PCCOMP/0.009659D0/
   DATA PCNL/11.D0/

   DATA PITNL/14.7D0/
   DATA PIPLNL/25.9D0/
   DATA PITEX/-13.05772D0/
   DATA PITNL/-0.7D0/

*Proteins
   DATA QITNL/250.047D0/
   DATA QPLNL/223.776D0/

*Transport parameters
   DATA XLSNL/43.1D0/
   DATA XJLNL/75.7D0/
   DATA PSNL/73.0D0/

*Neutral Solutes
   DATA DEXPLNL/0.D0/
   DATA DEIXTNL/0.D0/
   DATA GLUPLNL/3.168D0/
   DATA GLUTNL/8.316D0/

*Molecular weights, g/mmol
   DATA WMALB/67.D0/
   DATA NALB/17/
   DATA WMDEX/70.D0/
   DATA WMGLU/180.D-3/

*Reflection coefficients
   DATA SGNL/0.9888D0/
   DATA SIGDEX/0.5D0/
   DATA SIGGLU/0.2D0/
   DATA SIGION/0.15D0/

*Osmotic coefficients
   DATA OSMCOP/2.7D-3/
   DATA OSMGLU/19300.D0/

*Constants
   DATA ALBKO/0.56668/
   DATA ASTC,VSTC/8.D-6,1.D-8/
   DATA AS,BS/1.96154D-3,1.05D-4/
   DATA GREL/0.53/
   DATA MP,MPM/9.8/
   DATA RT/19300.D0/
   DATA CT/3600.D0/
* KIDNEY CONSTANTS:
  DATA UKD/168.2D0/
  DATA UKE/1248.7D0/
* Human values
  DATA URINNL/60.D0/
  DATA URINLN/60.D0/
  DATA CNATRES/146.D-3/
  DATA UKNA/3080.D0/
  DATA CLRNA/54.D0/
  DATA UKK/0.48D0/
  DATA AKURO/3.D0/
  DATA UKCL/1.28D0/
  DATA ALCURO/8.2D0/
  DATA UKC/0.23D0/
  DATA ACURO/0.8D0/

** II. DATA FOR INTERPOLATION (Interstitial values)

  DATA VSP/8.4D+3,8.92D+3,9.45D+3,9.97D+3,1.05D+3,1.02D+3,
  + 11.5D+3,12.07D+3,12.60D+3/
  DATA PSP/0.7D0,0.32D0,0.86D0,1.15D0,1.37D0,1.56D0,1.69D0,
  + 1.8D0,1.88D0/

** III. DATA FOR ION CONCENTRATIONS

** A: VASCULAR - CELLULAR COMPARTMENT (RBC):

* a) sodium, (mmol/ml)
  DATA CNAINP/10.004D-3/

* b) potassium, (mmol/ml)
  DATA CKINP/139.9705D-3/

* c) chloride, (mmol/ml)
  DATA CCLINP/74.0028D-3/

* d) Fixed anions, cations (mmol/ml) and their charges
  DATA FAIP,FCIP/55.984D-3,17.9948D-3/
  DATA ZFAIP,ZFCIP/-2.D0,2.D0/

** B: VASCULAR - PLASMA COMPARTMENT:

* a) sodium, (mmol/ml)
  DATA CNAOUTP/ 141.9633D-3/

* b) potassium, (mmol/ml)
  DATA CKOUTP/4.1678D-3/

* c) chloride, (mmol/ml)
  DATA CCLOUTP/105.3701D-3/

* d) other mobile anions, cations (mmol/ml), and their charges
  DATA FAEP,FCEP/34.2734D-3,5.6378D-3/
  DATA ZFAEP,ZFCEP/-2.0012,2.D0/

* Total extracellular osmolarity (including proteins and glucose)
  DATA SOUTP/ 297.9561D-3/

** C: INTERSTITIUM - CELLULAR COMPARTMENT (TC):

* a) sodium, (mmol/ml)
  DATA CNAINI/9.989D-3/

* b) potassium, (mmol/ml)
  DATA CKINI/150.0195D-3/

* c) chloride, (mmol/ml)
  DATA CCLINI/4.0333D-3/

* d) Fixed anions, cations (mmol/ml), and their charges
  DATA FAII,FCII/105.2833D-3,27.267D-3/
  DATA ZFAII,ZFCII/-2.0012,2.D0/

Appendix H. Program Listing
Appendix H: Program Listing

*......D: INTERSTITIUM - INTERSTITIAL COMPARTMENT (VIT):

* a) sodium, (mmol/ml)
   DATA CNAOUTI/138.0417D-3/

* b) potassium, (mmol/ml)
   DATA CKOUTI/4.0527D-3/

* c) chloride, (mmol/ml)
   DATA CCLOUTI/107.8716D-3/

* d) other mobile anions, cations (mmol/ml), and their charges
   DATA FAEI,FCEI/35.0516D-3,5.4821D-3/
   DATA ZFAEI,ZFCEI/-1.0D0,2.0D0/

* Total extracellular solute concentration (osmolarity)
   DATA SOUTI/296.5921D-3/

* Residence time

*......Actual number of resuscitation intervals

   DATA NRES/7/
   DATA WEIGHT,HCTO/70.0D0,0.40D0/
   DATA DEGHEM/0.0D0/

*......Starting time, hrs
   DATA TSTART/0.0,0.5,1.0,1.5,2.0,2.5,3.0/0/

*......Ending time, hrs
   DATA TEND/0.5,1.0,1.5,2.0,2.5,3.0,6.0/0/

*.....RESUSCITATION:

*Equivalent plasma volume, ml/h
*(fluid with PROTEIN/ALBUMIN)

   DATA XEQPLV/7*0.0D0/

*PROTEIN/ALBUMIN concentration, g/ml

   DATA CRES/7*0.0D0/

*NEUTRAL SOLUTE Dextran) containing fluid flow rate, ml/h

   DATA XJNSCF/7*0.0D0/

*DEXTRAN concentration in resuscitant (g/ml)

   DATA DEXCRES/7*0.0D0/

*Ion containing fluid flow rate, ml/h

   DATA XJICF/7*0.0D0/

*IONS concentration in resuscitant

*Na, mEq/ml
   DATA CNARES/7*0.0D-3/

*K, mEq/ml
   DATA CKRES/7*0.0D-3/

*Cl, mEq/ml
   DATA CCLRES/7*0.0D-3/

*C(2+), mEq/l
   DATA CFRES/7*0.0D-3/

*A(-), mEq/l
   DATA CFARES/7*0.0D-3/
*TOTAL Fluid added by infusion with either ions
*or neutral solutes, XJCLF

DATA XJCLF/7*0.D0/

*___END RESUSCITATION

*PERSPIRATION
*Fluid perspiration rate, ml/h

DATA XIPER/7*0.D0/

*Ions lost with perspiration, mEq/ml

DATA CNAPER/7*0.D-3/
DATA CKPER/7*0.D-3/
DATA CCLPER/7*0.D-3/
DATA CFAPER/7*0.D-3/
DATA CFCPER/7*0.D-3/

*EVAPORATION RATE (SENSIBLE LOSSES)
*Fluid lost with evaporation, mEq/ml

DATA XJEVIT/7*0.D0/

*BLOOD LOSS
*Intervals of blood loss

DATA BLSTIM/0.0,0.5,1.0,1.5,2.0,2.5,3.0/
DATA BLEND/0.5,1.0,1.5,2.0,2.5,3.0,6.0/

*Volume of Blood Lost, ml
DATA BLOST/7*0.D0/

END

*SUBROUTINE PUMP: Provides the cellular membrane parameters

SUBROUTINE PUMP (TIME,TF,TIN)

IMPLICIT REAL*8 (A-H,O-Z)

INCLUDE 'C:\FORTRAN\CRISTINA\MKIDGLU.FOR'
INCLUDING 'C:\FORTRAN\CRISTINA\CELPARAM.FOR'

IF (DEGHEM.EQ.0.OR.TIME.LE.2) THEN

*Normal values
*.....Plasma
PNAP=4.1D-10
PKP=3.81D-10
PCLP=1.1D-8
ROP=1.5D0
RPP=6.4D-9

*.....Tissue Cells
PNAI=8.48D-7
PKI=5.5D-5
PCLI=5.5D-5
ROI=1.5D0
RPI=3.99D-5

ELSE

*Gradual depolarization of the RP,TC (95% the initial value over 2h
*post-hemorrhage)
*.....Plasma
PNAP=4.1D-10
PKP=3.81D-10
PCLP=1.1D-8
**Appendix H: Program Listing**

```
ROP=1.5D0
RPP=6.4D-9

*...Tissue Cells
PNA1=8.48D-7
PK1=5.D-5
PCLI=5.D-5
ROI=1.5D0
RPIO=3.99D-5

RP1=RP10*(1-0.95*(TIME-TIN)/(TF-TIN))
ENDIF
RETURN
END

**
**
** Subroutine CONS: Calculates/Assigns various constants required
** by the MODEL and scales the compartmental
** fluid volumes by a weight ratio
**
**
SUBROUTINE CONS

IMPLICIT REAL*8 (A-H,O-Z)

INCLUDE 'C:\FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:\FORTRAN\CRISTINA\CELPARAM.FOR'

*...Calculates scaling factor for weight
WTR=WEIGHT/WT

*...Calculates the surface/volume ratio for the two types of cells
*RBC
APVRP=ASRBC/VSRBC

*TC
APVRT=ASTC/VSTC

*...Adjust compartmental values as a function of WTR
* 1.Plasma

VPLO=VPLNL*WTR
QPLO=QPLNL*WTR
DEXPLO=DEXPLNL*WTR
GLUPLO=GLUPLNL*WTR

* 2. Interstitium

VITO=VITNL*WTR
VEITO=VEITNL*WTR
CVITO=CVITO*WTR
QITO=QITNL*WTR
DEXITO=DEXITNL*WTR
GLUITO=GLUITNL*WTR

*...Recalculate Transport Parameters:
XJLO=XLNL*WTR
PSO=PSNL*WTR
XLSO=XLSNL*WTR

RETURN
END

**
**
** Subroutine INIT: Calculate initial values to be used
**
**
SUBROUTINE INIT

IMPLICIT REAL*8 (A-H,O-Z)

INCLUDE 'C:\FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:\FORTRAN\CRISTINA\CELPARAM.FOR'
```

---

327
* **I. VASCULAR COMPARTMENT**

* Calculate initial blood volume, BVO  
  \[
  BVO = \text{VPLO}/(1.00 - \text{HCT})
  \]

* Calculate initial intracellular volume in plasma CVPLO,  
  * and initial cellular area available for transport, ACELP  
  \[
  \text{CVPLO} = \text{BVO} - \text{VPLO}  
  \text{ACELP} = \text{CVPLO} \times \text{APVRP} \times \text{CT}
  \]

* Calculate albumin concentration, in plasma, g/ml  
  \[
  \text{CPLO} = \text{QPLO}/\text{VPLO}
  \]

  * [Alb], mmol/ml  
    \[
    \text{CPLOM} = \text{CPLO} / \text{WMALB}
    \]

  * [Alb], mEq/ml  
    \[
    \text{CPLOE} = \text{CPLOM} \times \text{NALB}
    \]

* Calculate DEXTRAN concentration in plasma, g/ml  
  \[
  \text{CDEXPLO} = \text{DEXPLO}/\text{VPLO}
  \]

* Calculate GLUCOSE concentration in plasma, g/ml and mmol/ml  
  \[
  \text{CGLUPLOM} = \text{CGLUPLO}/\text{WMGLU}
  \]

* Calculate the initial colloid osmotic pressure given by albumin  
  \[
  \text{PIPO} = \text{CPLO}/(\text{OSMCOF})
  \]

* Calculate the initial colloid osmotic pressure given by dextran  
  *(Webb, 1992)*  
  \[
  \text{PIPLDEX} = 0.2688D0 \times \text{CDEXPLO} + 1.101D-2 \times (\text{CDEXPLO}^2)
  \]

* Calculate the initial colloid osmotic pressure given by glucose  
  \[
  \text{PIPLGLU} = \text{OSMGLU} \times \text{CGLUPLOM}
  \]

* Calculate the initial total colloid osmotic pressure given by albumin  
  * and dextran  
    \[
    \text{PIPLT} = \text{PIPLO} + \text{PIPLDEX}
    \]

* Calculate the initial capillary pressure  
  \[
  \text{PCO} = \text{PCNL}
  \]

* **II. Interstitium, IT**

* Calculate initial intracellular volume of tissue cells CVITO,  
  * and initial cellular area available for transport, ACEL1  
  \[
  \text{CVITO} = \text{CVITNL}  
  \text{ACEL1} = \text{CVITO} \times \text{APVRT} \times \text{CT}
  \]

* Calculation for albumin concentration in interstitial volume g/ml  
  \[
  \text{CITO} = \text{QITO}/\text{VITO}
  \]

* Calculation for albumin concentration in the available interstitial  
  * volume expressed in g/ml, mmol/ml and mEq/ml  
    \[
    \text{CITA} = \text{QITO}/(\text{VITO} - \text{VETO})  
    \text{CITAVO} = \text{CITA} / \text{WMALB}  
    \text{CITAVE} = \text{CITA} \times \text{NALB}
    \]

* Calculation for DEXTRAN concentration in g/ml  
  \[
  \text{CDEXIT} = \text{DEXITO}/\text{VITO}  
  \text{CDEXAV} = \text{DEXITO}/(\text{VITO} - \text{VETO})
  \]

* Calculation for GLUCOSE concentration in g/ml, (mmol/ml)  
  \[
  \text{CGLUITO} = \text{GLUITO}/\text{VITO}  
  \text{CGLUITOM} = \text{GLUITO}/\text{WMGLU}
  \]

* Calculate initial colloid osmotic pressure of albumin  
  \[
  \text{PIITO} = \text{CITA}/(\text{OSMCOF})
  \]

* Calculate initial colloid osmotic pressure of dextran  
  \[
  \text{PIITDEX} = 0.2688D0 \times \text{CDEXAVI} + 1.101D-2 \times (\text{CDEXAVI}^2)
  \]
*Calculate initial colloid osmotic pressure of glucose
   PIITGLU=OSMGlu*CGLUTOM

*Calculate the total initial colloid osmotic pressure given by albumin and
dextran
   PIITTOT=PIITO+PIITDEX

*Calculate the hydrostatic pressure
   PIITO=FCOMP(VITO)

*Calculate DONNAN contribution

*(+-)CATIONS:

   DELNA=ALBKO*(CPLOE*CNAOUTP/(CNAOUTP+CKOUTP+ZFCEP*FCEP)-CITAVOE*CNAOUTI/
      (CNAOUTI+CKOUTI+ZFCEI*FCEI))

   DELK=ALBKO*(CPLOE*CKOUTP/(CNAOUTP+CKOUTP+ZFCEP
      +FCEP*CITAVOE*CKOUTI/
      (CNAOUTI+CKOUTI+ZFCEI*FCEI))

   DELFC=ALBKO*(CPLOE*ZFCEP*FCEP/(CNAOUTP+CKOUTP+ZFCEP
      +ZFCEP*FCEP)-CITAVOE*ZFCEI*FCEI/
      (CNAOUTI+CKOUTI+ZFCEI*FCEI))

DALB=(CPLOE-CITAVOE)

*(-)-ANIONS:

   DELCL=(ALBKO-1)*(CPLOE*CCLOUTP/(CCLOUTP+FAEP)-
      CITAVOE*CCLOUTI/(CCLOUTI+FAEI))

   DELFA=(ALBKO-1)*(CPLOE+FAEP/(CCLOUTP+FAEP)-
      CITAVOE*FAEI/(CCLOUTI+FAEI))

DAND=(ALBKO-1)*DALB-DCLD

*Check Normal filtration coefficient, XKFO (Starling equation)

   DELP=(PCO-PIITO)-SIGNL*(PIITLO-PIIITO)-
      SIGDEX*(PIITDEX-PIIITDEX)-
      SIGGLU*(PIITGLU-PIIITGLU)-
      SIGION*RT*((CNAOUTP-CNAOUTI-DELNA)+
         (CKOUTP-CKOUTI-DELK)+
         (CCLOUTP-CCLOUTI-DELCL)+
         (FCEP-FCEI-DELFC)+
         (FAEP-FAEI-DELFA))

   XKFO=XJLO/DELP
   XJFO=XKFO*DELP

   PRINT 111, XKFO,XJFO,XJLO
   111 FORMAT(1X,'K,FO=',F12.8,X,'J,FO=',F12.8,
      'J,LO=',F12.8)

   RETURN
   END

* Subroutine MODEL: Supplies the equations to be solved

* SUBROUTINE MODEL(X,Y)

IMPLICIT REAL*8 (A-H,O-Z)

INCLUDE 'C:FORTRAN\CRISTINA\MVWIDGLU.FOR'
INCLUDE 'C:FORTRAN\CRISTINA\CELPARAM.FOR'

DIMENSION Y(NMAX)

EXTERNAL FCOMP
* Calculates the fractional areas available for exchange
  FAIT = (Y(7)/VPLO)

* _I.PLASMA

* Calculate the albumin concentration in plasma in g/ml, mmol/ml, mEq/l
  CPL = Y(13)/Y(7)
  CPLM = CPL/WMALB
  CPLE = CPLM*NALB

* Calculate DEXTRAN concentration in plasma, g/ml
  CDEXPL = Y(25)/Y(7)

* Calculate GLUCOSE concentration in plasma, g/ml, (mmol/ml)
  CGLUPL = Y(27)/Y(7)
  CGLUPLM = CGLUPL/WMGLU

* Calculate ion concentrations in plasma, (mmol/ml) and mEq/L
  CNAPL = Y(10)/Y(7)
  CKPL = Y(11)/Y(7)
  CCLPL = Y(12)/Y(7)
  CFAPL = Y(8)/Y(7)
  CFCPL = Y(9)/Y(7)
  CFAPLEX = CFAPL
  CFCPLEX = ZFCEP*CFCPL

* Calculate colloid osmotic pressure in plasma
  PIPL = CPL/(OSMCOF)
  PDEXPL = 0.2688D0*CDEXPL + 1.101D-2*(CDEXPL**2)
  PIGLUPL = CGLUPLM*OSMGLU
  PITOTPL = PIPL + PDEXPL

* Calculate capillary pressure
  PC = PCO + PCOMP*(Y(7)-VPLO)
  IF (PC.LT.3.D0) PC = 3.D0

* Release of glucose
  IF (DEGHEM.EQ.0.D0.OR.IPEROD.EQ.1) THEN
    GLUREL = 0.D0
    AKREL = 0.D0
    ANAREL = 0.D0
    ACLREL = 0.D0
    AFAREL = 0.D0
    GOTO 77
  ENDIF

  IF (BLOST(IPOD).GT.0) THEN
    THEM0 = TSTART(IPOD)
    THEMF = TEND(IPOD)
  ENDIF

  IF (DEGHEM.LE.30) THEN
    GLUREL = 100*(PCO-PC)/DEGHEM
  ELSEIF (X.LE.(THEMF+0.5)) THEN
    AKREL = 0.D0
    ANAREL = AKREL
    ACLREL = AKREL
    AFAREL = AKREL
  ELSEIF (X.GT.(THEMF+0.5)) THEN
    AKREL = GKREL*GLUREL
ANAREL=AKREL
ACLREL=AKREL
AFAREL=AKREL

ENDIF
ENDIF

IF (DEGHEM.GT.30) THEN
  IF (X.LE.2) THEN
    GLUREL=100*(PCO-PC)/DEGHEM
  ENDIF
  IF (X.LE.(THEMF+0.5)) THEN
    AKREL=0.D0
    ANAREL=AKREL
    ACLREL=AKREL
    AFAREL=AKREL
  ENDIF
  ELSEIF (X.GT.(THEMF+0.5).AND.X.LE.2) THEN
    AKREL=AKREL*GLUREL
    ANAREL=AKREL
    ACLREL=AKREL
    AFAREL=AKREL
  ENDIF
ELSE
  GLUREL=0
  AKREL=0.D0
  ANAREL=AKREL
  ACLREL=AKREL
  AFAREL=AKREL
ENDIF
ENDIF

*___ KIDNEY

* A. Fluid

77 IF (Y(7).LT.VPLO) THEN

  *dehydration segment
  UK=UKD

  ELSE

  *normal or overhydration segment
  UK=UKE

  ENDIF

*Set the Urine = 0 for the first time interval (steady state)

  IF (IPEROD.EQ.1) THEN

    XJURINE=0.D0
    XNAUR=0.D0
    XKUR=0.D0
    XCUR=0.D0
    XCLUR=0.D0
    XAUR=0.D0
    F_U=0

    GO TO 66

  ELSE

    XJURINE=(UK*(Y(7)/VPLO-1)+URIN_NL)*
                (URIN_IN/URIN_NL)

  ENDIF
* B. URINARY IONS (mmol/h)

IF (CNAPL.LT.CNATRES) THEN
    \( F_U = (XJURINE/URIN\_IN) \)
ELSE
    \( F_U = 1.0 \)
ENDIF

\[ \text{XNAUR} = (UKNA*(CNAPL/CNAOUTP)*(CNAPL-CNAOUTP) + CLRNA*CNAPL)*F_U \]

IF (XNAUR.LE.0.D0) THEN
    \( \text{XNAUR} = 0.0 \)
ELSE
    \( \text{DNAUR} = (XNAUR-CLRNA*CNAOUTP) \)
ENDIF

\[ \text{XKUR} = \text{AKURO}+\text{UKK}^*\text{DNAUR} \]
IF (XKUR.LE.0.D0) XKUR=0.0

\[ \text{XCUR} = \text{ACURO}+\text{UKC}^*\text{DNAUR} \]
IF (XCUR.LE.0.D0) XCUR=0.0

\[ \text{XCLUR} = \text{ACLURO}+\text{UKCL}^*\text{DNAUR} \]
IF (XCLUR.LE.0.D0) XCLUR=0

\[ \text{XAUR} = \text{XNAUR}+\text{XKUR}+2^*\text{XCUR}^*\text{XCLUR} \]

* K

\[ \text{CKUR} = \text{XKUR}/\text{XJURINE} \]
\[ \text{CL}_K = \text{XKUR}/\text{CKPL} \]

* Other Cations, C

\[ \text{CCUR} = \text{XCUR}/\text{XJURINE} \]
\[ \text{CL}_C = \text{XCUR}/\text{CFCPL} \]

* Cl

\[ \text{CCLUR} = \text{XCLUR}/\text{XJURINE} \]
\[ \text{CL}_\text{CL} = \text{XCLUR}/\text{CCLPL} \]

* Cl

\[ \text{CAUR} = \text{XAUR}/\text{XJURINE} \]
\[ \text{CL}_\text{A} = \text{XAUR}/\text{CFAPL} \]

* Concentration and clearance of Total Solute eliminated in urine

\[ \text{CUR} = \text{XUR}/(\text{CNAPL}+\text{CKPL}+\text{CFCPL}+\text{CCLPL}+\text{CFAPL}) \]

* __INTERSTITIUM

* Calculate the albumin concentration in g/ml; (mmol/ml), mEq/L

\[ \text{CIT} = \text{Y}(22)/\text{Y}(18) \]
\[ \text{CITAV} = \text{Y}(22)/(\text{Y}(18)\_\text{VEITO}) \]
\[ \text{CITAVM} = \text{CITAV}/\text{WMALB} \]
\[ \text{CITAVE} = \text{CITAVM}^*\text{NALB} \]

* Calculate dextran concentration in g/ml

\[ \text{CDEXIT} = \text{Y}(26)/\text{Y}(18) \]
\[ \text{CDXAVTT} = \text{Y}(26)/(\text{Y}(18)\_\text{VEITO}) \]
*Calculate glucose concentration in g/ml, (mmol/ml)

\[
\text{CGLUIT} = \frac{Y(28)}{Y(18)} \\
\text{CGLUITM} = \frac{\text{CGLUIT}}{\text{WMGLU}}
\]

*Calculate ion concentrations in interstitial tissue

\[
\text{CNAIT} = \frac{Y(19)}{Y(18)} \\
\text{CKIT} = \frac{Y(20)}{Y(18)} \\
\text{CCLIT} = \frac{Y(21)}{Y(18)} \\
\text{CFAIT} = \frac{Y(23)}{Y(18)} \\
\text{CFCIT} = \frac{Y(24)}{Y(18)} \\
\text{CFAITE} = \text{CFAIT} \\
\text{CFCITE} = \frac{\text{ZFCEI}}{\text{CFCIT}}
\]

*Calculate the concentration for fixed charges inside the cells(TT)

\[
\text{CFAITC} = \text{FAII} \times \text{CVITO} / Y(17) \\
\text{CFCITC} = \text{FCII} \times \text{CVITO} / Y(17)
\]

*Calculate hydrostatic pressure for interstitial tissue

\[
\text{PIT} = \text{FCOMP} / Y(18) \\
\text{PITEX} = \text{FCOMP} / \text{VEITO}
\]

*Calculate colloid osmotic pressure for interstitial tissue

\[
\text{PIT} = \text{CITAV} / (\text{OSMCOF}) \\
\text{PDEXIT} = 0.2688 \times 10^{-5} \times \text{CDXAVIT} + 1.101 \times 10^{-5} \times (\text{CDXAVIT} \times 10^{-2}) \\
\text{PIGLIT} = \frac{\text{CGLUITM} \times \text{OSMGLU}}{\text{PITOTT}} \\
\text{PITOTT} = \text{PIIT} + \text{PDEXIT}
\]

*DONNAN CONSTRAINTS:

*(+)-CATIONS:

\[
\text{DNAD} = \text{ALBKO} \times (\text{CPLEX} - \text{CNAPL} + \text{CKPL} + \text{CFCPLE}) + \text{CITAVE} \times \text{CNAIT} / (\text{CNAIT} + \text{CKIT} + \text{CFCITE})
\]

\[
\text{DKD} = \text{ALBKO} \times (\text{CPLEX} \times \text{CKPL} + \text{CNAPL} + \text{CKPL} + \text{CFCPLE}) + \text{CITAVE} \times \text{CKIT} / (\text{CNAIT} + \text{CKIT} + \text{CFCITE})
\]

*ANIONS:

\[
\text{DCLD} = (\text{ALBKO} - 1) \times (\text{CPLEX} \times \text{CCLPL} + \text{CCLPL} + \text{CAFAPL}) + \text{CITAVE} \times \text{CCLIT} / (\text{CCLIT} + \text{CFAITE})
\]

\[
\text{DAND} = (\text{ALBKO} - 1) \times (\text{CPLEX} \times \text{CAFAPL} + \text{CCLPL} + \text{CAFAPL}) + \text{CITAVE} \times \text{CFAITE} / (\text{CCLIT} + \text{CFAITE})
\]

*Lymph fluid flow

\[
\text{AJLITO} = \text{XJLO} + \text{XLSO} \times (\text{PIT-PIITO}) \\
\text{BJLITO} = \text{XJLO} \times ((\text{PIT-PITEX}) / (\text{PITO-PITEX})) \\
\text{IF} (\text{PIT.GE.PITO}) \text{ THEN} \\
\text{XJLIT} = \text{AJLITO} \\
\text{ELSEIF} (\text{PIT.GE.PITEX.AND.PIT.LT.PITO}) \text{ THEN} \\
\text{XJLIT} = \text{BJLITO} \\
\text{ELSEIF} (\text{PIT.LT.PITEX}) \text{ THEN} \\
\text{XJLIT} = 0.1 \\
\text{ENDIF}
\]

*Calculate fluid flowrate

\[
\text{SIGNL} = \text{SIFG} \\
\text{XKFIT} = \text{XKFO} \times \text{FAIT}
\]

\[
\text{XJFIT} = \text{XKFIT} \times ((\text{PC-PIT}) \times \text{SIGNL} \times (\text{PIPI-PIITO})) + \text{SIGDEX} \times (\text{PIDEXP} - \text{PDEXIT}) + \text{SIGGLU} \times (\text{PIGLUPL} - \text{PIGLU}) + \text{SIGIONR*RT} \times ((\text{CNAPL} - \text{CNAIT} - \text{DNAD}) + (\text{CKPL} - \text{CKIT}) - \text{DKD}) + (\text{CFCPLE} - \text{CFCITE} - \text{DFCD}) / 2 + (\text{CCLPL} - \text{CCLIT}) - \text{DCLD}) + (\text{CAFAPL} - \text{CFAITE} - \text{DAND}))
\]
Appendix H: Program Listing

*CALCULATE PROTEIN FLUXES ACROSS THE CAPILLARY
PSIT=PSO*FAIT

*Permeability-Surface Area Product for:
*  a)DEXTRAN (PS)alb /4 (assumed)
    PSDEX=PSIT/4.D0
*  b)ION (from sensitivity analysis)
    PSION=3000.D0*PSIT
*  c)GLUCOSE (assumed)
    PSGLU=850.D0*PSIT

*Peclet number for
*  a)albumin
    PEIT=(1.D0-SIGIT)*XJFIT/PSIT
*  b)ions
    PEION=(1.D0-SIGION)*XJFIT/PSION
*  c)dextran
    PEDEX=(1.D0-SIGDEX)*XJFIT/PSDEX
*  d)glucose
    PEGLU=(1.D0-SIGGLU)*XJFIT/PSGLU

*Calculation for ratios for transmembrane transport:Albumin, Dextran and
*optional, glucose
RATIT=(CPL-CITAV*DEXP(-PEIT))/(1.D0-DEXP(-PEIT))
DEXRAT=(CDEXPL-CDXAVIT*DEXP(-PEDEX))/
        (1.D0-DEXP(-PEDEX))

*Transmembrane protein flow
    QSIT=(1.D0-SIGIT)*XJFIT*RATIT
    QSITE=QSIT*NALB/WMALB

*Transmembrane dextran flow
    RDEXIT=(1.D0-SIGDEX)*XJFIT*DEXRAT

*Transmembrane glucose flow
    RGLUIITM=XJFIT*(1-SIGGLU)*(CGLUPLM+CGLUITM)/
              + 2.D0+PSGLU*(CGLUPLM-CGLUITM)
    RGLUIT=RGLUIITM*WMGLU

*Transcapillary ion flow:
*  _____Na: Diffusive-convective transport, (mmol/h)
    XNAIT=XJFIT*(1-SIGION)*(CNAPL+CNAIT)/2.D0+
             PSION*(CNAPL-CNAIT-DNAD)

*  _____K: Diffusive-convective transport, (mmol/h)
    XKIT=XJFIT*(1-SIGION)*(CKPL+CKIT)/2.D0+
             PSION*(CKPL-CKIT-DKD)

*  _____C: Diffusive-convective transport, (mmol/h)
    XFCIT=XJFIT*(1-SIGION)*(CFCPL+CFCIT)/2.D0+
             PSION*(CFCPL-CFCIT-(DFCD/2))

*  _____Cl: Diffusive-convective transport
    XCLIT=XJFIT*(1-SIGION)*(CCLPL+CCLIT)/2.D0+
             PSION*(CCLPL-CCLIT-DCLD)
* A is transported such that electro-neutrality is respected across the capillary
XFAIT=XNAIT+XKIT+ZFCEP*XFCIT-XCLIT-QSITE

*Lymph flow (convection only for all species):
*PROTEIN flow
QLIT=XJLIT*CIT
QLITE=QLIT*NALB/WMALB

*DEXTRAN flow
RDEXLIT=XJLIT*CDEXIT

*GLUCOSE flow
RGLULITM=XJLIT*CGLUINM
RGLULIT=RGLULITM*WMGLU

*ION flow
*Na
XNALIT=XJLIT*CNAIT

*K
XKLIT=XJLIT*CKIT

*C
XFCLIT=XJLIT*CFCIT

*Cl
XCLLIT=XJLIT*CCLIT

*A (respects electroneutrality in the transport across lymphatics)
XFALIT=XNALIT+XKLIT+ZFC2*XFCIT-XCLLIT-QLITE

RETURN
END

*--------------------------------------------------------
* HEM: Calculates the PLASMA Volume and RBC's lost
* with blood through hemorrhage
*--------------------------------------------------------

SUBROUTINE HEM(IP)
IMPLICIT REAL*8 (A-H,O-Z)
INCLUDE 'C:FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:FORTRAN\CRISTINA\CELPARAM.FOR'

PLOST=BLOST(IP)*(1-HCT)/(BLEND(IP)-BLSTIM(IP))
CVLOST=BLOST(IP)*HCT/(BLEND(IP)-BLSTIM(IP))

RETURN
END

*--------------------------------------------------------
* BALANCE: Calculates the water shifted across the cellular membrane
*--------------------------------------------------------

SUBROUTINE BALANCE(Y)
IMPLICIT REAL*8 (A-H,O-Z)
INCLUDE 'C:FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:FORTRAN\CRISTINA\CELPARAM.FOR'
DIMENSION Y(NMAX)

*Hematocrit calculations
   HCT=Y(6)/(Y(6)+Y(7))

*Calculations for the nr. of mOsm in the vascular compartment:
   ASOUTP=Y(8)+Y(9)+Y(10)+Y(11)+Y(12)+(Y(13)/WMALB)+
   (Y(25)/WMDEX)+(Y(27)/WMGLU)
   ASINP=Y(1)+Y(2)+Y(3)+Y(4)+Y(5)

*Fluid shifted between RBC and Plasma:
   WSHIFTF=(ASOUTP*Y(6)-ASINP*Y(7))/(ASINP+ASOUTP)

*Update the Compartmental Volumes:
   Y(6)=Y(6)-WSHIFTF
   Y(7)=Y(7)+WSHIFTF

*Calculate the Plasma Osmolarity:
   SOUTP=ASOUTP/Y(7)
   SINP=ASINP/Y(6)

*Calculations for the nr. of mOsm (=mM) in the interstitial compartment:
   FV=1-(VEITO/Y(18))
   ASOUTI=Y(19)+Y(20)+Y(21)+Y(23)+Y(24)+(Y(28)/WMGLU)
   AMOLEC=(Y(22)/WMALB)+(Y(26)/WMDEX)
   ASINI=Y(14)+Y(15)+Y(16)+(FAII+FCII)*CVITO

* Fluid shifted between TC and Interstitial Fluid:
   WSHIFTI=(Y(17)*(ASOUTI+AMOLEC/FV)-Y(18)*ASINI)/
   + (ASINI+ASOUTI+AMOLEC/FV)

*Update the Compartmental Volumes:
   Y(17)=Y(17)-WSHIFTI
   Y(18)=Y(18)+WSHIFTI

*Calculate the Interstitial Osmolarity:
   SOUTI=(ASOUTI/Y(18))+(AMOLEC/(Y(18)-VEITO))
   SINI=ASINI/Y(17)

RETURN
END

SUBROUTINE DERIVS (X,Y,DYDX)
IMPLICIT REAL*8 (A-H,O-Z)
INCLUDE 'C:\FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:\FORTRAN\CRISTINA\CELPARAM.FOR'
DIMENSION Y(NMAX),DYDX(NMAX)
CALL BALANCE(Y)
CALL MODEL(X,Y)
CALL MEMBRP (Y)
*DIFFERENTIAL EQUATION FOR:

I. VASCULAR COMPARTMENT:

* A: Red Blood Cells

*1) Fixed anions, FAIP:

\[ \text{DYDX}(1) = -\text{CVLOST} \cdot Y(1)/Y(6) \]

*2) Fixed cations, FCIP:

\[ \text{DYDX}(2) = -\text{CVLOST} \cdot Y(2)/Y(6) \]

*3) Na\(_{RBC}\), CNAINP:

\[ \text{DYDX}(3) = \text{ACELP} \cdot ((\text{PNAP} \cdot \text{DFIP}) \cdot (Y(10)/Y(7) - (Y(3)/Y(6))) \]
\[ + \text{DEXP}((\text{DFIP})/(\text{DEXP}(\text{DFIP}-1)) \cdot \text{RPP} \cdot Y(3)/Y(6)) \]
\[ + \text{CVLOST} \cdot Y(3)/Y(6) \]

\[ \text{ANAP} = \text{ACELP} \cdot ((\text{PNAP} \cdot \text{DFIP}) \cdot (Y(10)/Y(7) - (Y(3)/Y(6))) \]
\[ + \text{DEXP}((\text{DFIP})/(\text{DEXP}(\text{DFIP}-1)) \cdot \text{RPP} \cdot Y(3)/Y(6)) \]

*4) K\(_{RBC}\), CKINP:

\[ \text{DYDX}(4) = \text{ACELP} \cdot ((\text{PKP} \cdot \text{DFIP}) \cdot (Y(11)/Y(7) - (Y(4)/Y(6))) \]
\[ + \text{DEXP}((\text{DFIP})/(\text{DEXP}(\text{DFIP}-1)) \cdot \text{RPP} \cdot Y(3)/(\text{ROP} \cdot Y(6))) \]
\[ + \text{CVLOST} \cdot Y(4)/Y(6) \]

\[ \text{AKP} = \text{ACELP} \cdot ((\text{PKP} \cdot \text{DFIP}) \cdot (Y(11)/Y(7) - (Y(4)/Y(6))) \]
\[ + \text{DEXP}((\text{DFIP})/(\text{DEXP}(\text{DFIP}-1)) \cdot \text{RPP} \cdot Y(3)/(\text{ROP} \cdot Y(6))) \]

*5) Cl\(_{RBC}\), CCLINP:

\[ \text{DYDX}(5) = (\text{DYDX}(3) + \text{DYDX}(4) + \text{ZFAIP} \cdot \text{DYDX}(1) + \text{ZFCIP} \cdot \text{DYDX}(2)) \]

\[ \text{ACL} = \text{ANAP} + \text{AKP} \]

*6) Cellular Volume, RBC:

\[ \text{DYDX}(6) = -\text{CVLOST} \]

*7) Plasma Volume:

\[ \text{DYDX}(7) = \text{XJLIT} \cdot \text{XJFIT} + \text{XJRES} \cdot \text{IPEROD} \cdot \text{XJURINE} \cdot \text{PLOST} \]

*8) Anions, FAEP:

\[ \text{DYDX}(8) = \text{XFALIT} \cdot \text{XFAIT} + \text{XJICF} \cdot \text{IPEROD} \]
\[ + \text{CFARES} \cdot \text{IPEROD} \cdot \text{PLOST} \cdot Y(8)/Y(7) - \text{XAUR} + \text{AFAREL} \]

*9) Cations,FCEP:

\[ \text{DYDX}(9) = \text{XFCLIT} \cdot \text{XFCIT} + \text{XJICF} \cdot \text{IPEROD} \]
\[ + \text{CFCRES} \cdot \text{IPEROD} \cdot \text{PLOST} \cdot Y(9)/Y(7) - \text{XCUR} \]

*10) Na\(_{Pl}\), CNAOUTP:

\[ \text{DYDX}(10) = \text{XNALIT} \cdot \text{XNAIT} - \text{ANAP} + \text{XJICF} \cdot \text{IPEROD} \]
\[ + \text{CNARES} \cdot \text{IPEROD} - \text{XNAUR} \cdot \text{PLOST} \cdot Y(10)/Y(7) + \text{ANAREL} \]

*11) K\(_{Pl}\), CKOUTP:

\[ \text{DYDX}(11) = \text{XKLIT} \cdot \text{XKIT} - \text{AKP} + \text{XJICF} \cdot \text{IPEROD} \]
\[ + \text{CKRES} \cdot \text{IPEROD} - \text{XKUR} \cdot \text{PLOST} \cdot Y(11)/Y(7) + \text{AKREL} \]

*12) Cl\(_{Pl}\), CCLOUTP:

\[ \text{DYDX}(12) = \text{XCLLIT} \cdot \text{XCLIT} - \text{ACL} + \text{XJICF} \cdot \text{IPEROD} \]
\[ + \text{CCLRES} \cdot \text{IPEROD} - \text{XCLUR} \cdot \text{PLOST} \cdot Y(12)/Y(7) + \text{ACLREL} \]

*13) Protein (Albumin) Content (g), QPL:

\[ \text{DYDX}(13) = \text{QLIT} \cdot \text{QSI} + \text{CRES} \cdot \text{IPEROD} \cdot \text{XEQPLV} \cdot \text{IPEROD} - \text{PLOST} \cdot Y(13)/Y(7) \]
**II. INTERSTITIUM**

**A: Tissue Cells**

*14) Na,TC, CNAINI:
\[ \text{DYDX}(14) = \text{ACELI}^*((\text{PNAI}^*\text{DFII}^*\text{Y}(19)/\text{Y}(18)-(\text{Y}(14)/\text{Y}(17))) + \text{*DEXP}(\text{DFII})/(\text{DEXP}(\text{DFII}-1))-\text{RFI}^*\text{Y}(14)/\text{Y}(17)) \]

*15) K,TC, CKINI:
\[ \text{DYDX}(15) = \text{ACELI}^*((\text{PKI}^*\text{DFII}^*\text{Y}(20)/\text{Y}(18)-(\text{Y}(15)/\text{Y}(17)) + \text{*DEXP}(\text{DFII})/(\text{DEXP}(\text{DFII}-1))+\text{RFI}^*\text{Y}(14)/(\text{ROI}^*\text{Y}(17))) \]

*16) Cl,TC, CCLINI:
\[ \text{DYDX}(16) = \text{DYDX}(14)+\text{DYDX}(15) \]

*17) Cellular volume, TC,
\[ \text{DYDX}(17) = 0.00 \]

**B: Interstitial Fluid**

*18) Interstitial Volume:
\[ \text{DYDX}(18) = \text{XJFIT-XLIT-XJEVIT}(\text{IPEROD})-\text{XJPER}(\text{IPEROD}) \]

*19) Na,IT, CNAOUTI:
\[ \text{DYDX}(19) = \text{XNAIT-XNALIT-XJPER}(\text{IPEROD})* + \text{CNAPI}(\text{IPEROD})-\text{DYDX}(14) \]

*20) K,IT, CKOUTI:
\[ \text{DYDX}(20) = \text{XKIT-XKLIT-XJPER}(\text{IPEROD})^* \text{CKPER}(\text{IPEROD})-\text{DYDX}(15) \]

*21) Cl,IT, CCLOUTI:
\[ \text{DYDX}(21) = \text{XCLIT-XCLLIT-XJPER}(\text{IPEROD})^* + \text{CCLPER}(\text{IPEROD})-\text{DYDX}(16) \]

*22) Proteins (g/ml) (Albumin), QIT:
\[ \text{DYDX}(22) = \text{QSIQ-QLIT} \]

*23) Other Anions, FAEI:
\[ \text{DYDX}(23) = \text{XFAIT-XFALIT-XJPER}(\text{IPEROD})^* + \text{CFAPER}(\text{IPEROD}) \]

*24) Other Cations, FCEI:
\[ \text{DYDX}(24) = \text{XFCT-XFCLIT-XJPER}(\text{IPEROD})^* + \text{CFAPER}(\text{IPEROD}) \]

**III. DEXTRAN given with resuscitation**

*25) Dextran balance (g) in Plasma, DEXPL
\[ \text{DYDX}(25) = \text{DEXCRES}(\text{IPEROD})^* \text{XJNSCF}(\text{IPEROD})-(\text{RDEXIT-RDEXLIT})-\text{PLOST}^*\text{Y}(25)/\text{Y}(7) \]

*26) Dextran balance (g) in Interstitium, DEXIT
\[ \text{DYDX}(26) = \text{RDEXIT-RDEXLIT} \]

**IV. GLUCOSE**

*27) Glucose balance (g) in Plasma, GLUPL
\[ \text{DYDX}(27) = \text{GLUREL-RGLUIT}+\text{RGLUIT-PLOST}^*\text{Y}(27)/\text{Y}(7) \]

*28) Glucose balance (g) in Interstitium, GLUIT
\[ \text{DYDX}(28) = \text{RGLUIT-RGLUIT} \]

RETURN
END
SUBROUTINE FIT
IMPLICIT REAL*8 (A-H,O-Z)
COMMON/BKLB/QSP(100),RSP(101),SSP(100)
DIMENSION VCOMIT(100)
INCLUDE 'C:\FORTRAN\CRISTINA\MKIDGLU.FOR'

*....Interstitial tissue
DO 10 I=1,MP
    VCOMIT(I)=VSP(I)*WTR
10  CONTINUE

ISTART=2
IFINIS=2

CALL SPLINE(VCOMIT,PSP,MP,MPM,ISTART,IFINIS)

DO 20 I=1,MP
    XIT(I)=VCOMIT(I)
    YIT(I)=PSP(I)
20  CONTINUE

DO 25 I=1,MPM
    QIT(I)=QSP(I)
    RIT(I)=RSP(I)
    SIT(I)=SSP(I)
25  CONTINUE

NIT=MP
NMIT=MPM

RETURN
END

* Subroutine SPLINE

SUBROUTINE SPLINE (XSP,YSP,N,NM,ISTART,IFINIS)
IMPLICIT REAL*8 (A-H,O-Z)
INCLUDE 'C:\FORTRAN\CRISTINA\MKIDGLU.FOR'

PARAMETER (M=4)
COMMON/BKLB/QSP(100),RSP(101),SSP(100)
DIMENSION XSP(N),YSP(N),H(100)
DIMENSION A(101),B(101),C(101),D(101)

MM=M-1

*....Calculate H(I)

DO 20 I=1,NM
    H(I)=XSP(I+1)-XSP(I)
20  CONTINUE

*...SET COEFFICIENTS OF TRIDIAGONAL EQUATIONS:

*...(1).Set the boundary conditions for the starting point

GO TO (100,200,300), ISTART

* A. NATURAL

100  A(1)=0.0
    B(1)=1.0
    C(1)=0.0
    D(1)=0.0
    GO TO 400
* B.CLAMPED

200  A(1)=0.0
     B(1)=2.0*H(1)
     C(1)=H(1)
     GSTART=0.0
     D(1)=3.0*((YS(2)-YS(1))/H(1)-GSTART)
     GO TO 400

* C.FITTED

300  A4=0.0

   DO 310 I=1,M
       TERM=YS(I)
       DO 320 J=1,M
           IF (J.NE.I) THEN
               TERM=TERM/(XS(I)-XS(J))
           ENDIF
       320  CONTINUE

   A4=A4+TERM
   310  CONTINUE

   A(1)=0.0
   B(1)=H(1)
   C(1)=H(1)
   D(1)=3.0*H(1)*H(1)*A4

*(2). Set the boundary conditions for the end point

400  GO TO (600,700), IFINIS

* A) Natural

600  A(N)=0.0
     B(N)=1.0
     C(N)=0.0
     D(N)=0.0

GO TO 900

*B) Fitted

700  B4=0.0
     NL=N-NM
     DO 810 I=NL,N
         TERM=YS(I)
         DO 820 J=NL,N
             IF (J.NE.I) THEN
                 TERM=TERM/(XS(I)-XS(J))
             ENDIF
         820  CONTINUE
     B4=B4+TERM
     810  CONTINUE

     A(N)=H(NM)
     B(N)=-H(NM)
     C(N)=0.0
     D(N)=-3.0*H(NM)*H(NM)*B4

900  DO 910 I=2,NM
     IM=I-1
     A(I)=H(IM)
     B(I)=2.0*(H(IM)+H(I))
     C(I)=H(I)
     D(I)=3.0*((YS(I+1)-YS(I))/H(I)-(YS(I)-YS(IM))/H(IM))
     910  CONTINUE

* Call the THOMAS algorithm to solve tridiagonal set

     CALL TDMA (A,B,C,D,RSP,N,NM)

* Determine QSP(I) and SSP(I)

     DO 920 I=1,NM
         IP=I+1
         QSP(I)=(YS(IP)-YS(I))/H(I)-H(I)*RSP(I)+
+ RSP(IP)/3.0

SSP(I)=(RSP(IP)-RSP(I))/(3.0*H(I))

920 CONTINUE

RETURN
END

* FUNCTION FCOMP: Interpolation function for interstitial tissue
* COMPLIANCE relationship
*

DOUBLE PRECISION FUNCTION FCOMP(Z)

IMPLICIT REAL*8 (A-H,O-Z)
INCLUDE 'C:\FORTRAN\CRISTINA\MKIDGLU.FOR'

EXTERNAL FCIT

IF (Z.LT.WTR*VITNL) THEN
   FCOMP=-0.70+AS/WTR*(Z-WTR*VITNL)
ELSEIF (Z.GT.WTR*1.5*VITNL) THEN
   FCOMP=1.88*D+BS/WTR*(Z-WTR*1.5*VITNL)
ELSEIF (Z.LE.WTR*VITNL.AND.Z.LE.WTR*1.5*VITNL) THEN
   FCOMP=FCIT(Z)
ENDIF

RETURN
END

* FUNCTION FCIT: Interpolation for interstitial tissue
*

DOUBLE PRECISION FUNCTION FCIT(Z)

IMPLICIT REAL*8 (A-H,O-Z)
COMMON/BLKIT1/X(101),Y(101),N,NM
COMMON/BLKIT2/Q(100),R(101),S(100)
CALL BISX(Z,J)

DX=Z-X(I)
FCIT=Y(I)+DX*(Q(I)+DX*(R(I)+DX*S(I)))

RETURN
END

**********************************************************************
* RKF ALGORITHM
* (C) Copr. 1986-92 Numerical Recipes Software "17K.
**********************************************************************

SUBROUTINE ODEINT(ystart,nvar,x1,x2,eps,h1,hmin)

INTEGER nbad,nok,nvar,KMAXX,MAXSTP,NMAX
REAL*8 eps,h1,hmin,x1,x2,ystart(nvar),TINY
EXTERNAL derivs,rkqs
PARAMETER (MAXSTP=30000,NMAX=50,KMAXX=200,TINY=1.e-30)
INTEGER i,kmax,kount,nstp
REAL*8 dxsav,h,hdid,hnext,x,xsav,dydx(NMAX),xp(KMAXX),y(NMAX),
+*yp(NMAX,KMAXX),yscal(NMAX)
COMMON /path/ kmax,kount,dxsav,xp,yp
x=x1
h=sign(h1,x2-x1)
nok=0
nbad=0
kount=0
do 11 i=1,nvar
   y(i)=ystart(i)
11 continue
if (kmax.gt.0) xsav=x-2.*dxsav
do 16 nstp=1,MAXSTP
   call derivs(x,y,dydx)
   do 12 i=1,nvar
      yscal(i)=abs(y(i))+abs(h*dydx(i))+TINY
   12 continue
if(kmax.gt.0)then
   if(abs(x-xsav).gt.abs(dxsav)) then
      if(kount.lt.kmax-1)then
         kount=kount+1
         xp(kount)=x
         do 13 i=1,nvar
            yp(i,kount)=y(i)
         13 continue
         xsav=x
      endif
   endif
   endif
   if((x+h-x2)*(x+h-x1).gt.0.) h=x2-x
   call rkqs(y,dydx,nvar,x,h,eps,yscal,hdid,hnext,derivs)
   if(hdid.eq.h)then
      nok=nok+1
   else
      nbad=nbad+1
   endif
   IF((X-X2)*(X2-X1).GE.0.)THEN
      do 14 i=1,nvar
         ystart(i)=y(i)
14 continue
   if(kmax.ne.0)then
      kount=kount+1
      xp(kount)=x
      do 15 i=1,nvar
         yp(i,kount)=y(i)
15 continue
   endif
   return
endif
   if(abs(hnext).lt.hmin) pause
*'steps' smaller than minimum in odeint'
h=hnext
16 continue
pause 'too many steps in odeint'
RETURN
END
SUBROUTINE rkqs(y,dydx,n,x,htry,eps,yscale,hdid,hnext,derivs)

(C) Copr. 1986-92 Numerical Recipes Software "17K.

INTEGER n,NMAX
REAL*8 eps,hdid,hnext,htry,x,dydx(n),y(n),yscale(n)
EXTERNAL derivs
PARAMETER (NMAX=50)
CU USES derivs,rkck

INTEGER i
REAL*8 errmax,h,xnew,yerr(NMAX),ytemp(NMAX),SAFETY,
+ PGROW,PSHRNK,*ERRCON
PARAMETER (SAFETY=0.9,PGROW=-.2,PSHRNK=-.25,
+ ERRCON=1.89e-4)

h=htry
1 call rkck(y,dydx,n,x,ytemp,yerr,derivs)
errmax=0.
do 11 i=1,n
errmax=max(errmax,abs(yerr(i)/yscale(i)))
11 continue
errmax=errmax/eps
if(errmax.gt.1.)then
  h=SAFETY*h*(errmax**PSHRNK)
if(h.lt.0.1*h)then
  h=1.*h
endif
xnew=x+h
if(xnew.eq.x)pause 'stepsize underflow in rkqs'
goto 1
else
if(errmax.gt.ERRCON)then
  hnext=SAFETY*h*(errmax**PGROW)
else
  hnext=5.*h
endif
hdid=h
x=x+h
do 12 i=1,n
y(i)=ytemp(i)
12 continue
return
endf
RETURN
END

SUBROUTINE rkck(y,dydx,n,x,yout,yerr,derivs)

INTEGER n,NMAX
REAL*8 h,x,dydx(n),y(n),yerr(n),yout(n)
EXTERNAL derivs
PARAMETER (NMAX=50)
CU USES derivs

INTEGER i
REAL*8 ak2(NMAX),ak3(NMAX),ak4(NMAX),ak5(NMAX),
+ k6(NMAX),*ytemp(NMAX),A2,A3,A4,A5,A6,B21,B31,B32,B41,
+ B42,B43,B51,B52,B53,*B54,B61,B62,B63,B64,B65,C1,C3,C4,C6,
+ DC1,DC3,DC4,DC5,DC6
PARAMETER (A2=.2,A3=.3,A4=.6,A5=1.,A6=.875,B21=.2,
+ B31=3./40.,*B32=9./40.,B41=.3,B42=-.9,B43=1.2,B51=11./54.,
+ B52=2.5,
+ B61=1631./55296.,B62=175./512.,
+ B63=575./13824.,B64=44275./110592.,B65=253./4096.,C1=37./378.,
+ B66=16384./131072.,C2=256./621.)

endf
x=x+h
y(i)=yout(i)
errmax=max(errmax,abs(yout(i)-y(i))
errmax=errmax/eps
x=x+h
if(xnew.eq.x)pause 'stepsize underflow in rkck'
goto 1
else
if(errmax.gt.ERRCON)then
  hnext=SAFETY*h*(errmax**PGROW)
else
  hnext=5.*h
endif
hdid=h
x=x+h
do 12 i=1,n
y(i)=yout(i)
12 continue
return
endf
RETURN
END
Appendix H: Program Listing

```
*.....Find the interpolation interval for the interstitial tissue

SUBROUTINE BISX(XARG,I)
*

IMPLICIT REAL*8 (A-H,O-Z)
COMMON /BLKIT1/X(101),Y(101),N,NM

IF (XARG.LT.X(1)) THEN
  I=1
  WRITE (10,10) XARG
ELSEIF (XARG.GT.X(N)) THEN
  I=N
  WRITE (10,10) XARG
ELSE
  I=1
  J=N
20  K=(I+J)/2
    IF (XARG.LT.X(K)) THEN
      J=K
    ELSE
      I=K
    ENDFI
    IF (J.GT.(I+1)) GO TO 20
ENDIF

10  FORMAT(2X,'WARNING- ITS NOT in the INTERPOLATION RANGE')

RETURN
END
```

```
* Subroutine for calculating the MEMBRANE POTENTIAL:
  * - DFIP-for RBC
  * - DFII-for Tissue Cells

SUBROUTINE MEMBRP(Y)

IMPLICIT REAL*8 (A-H,O-Z)
PARAMETER(NBMAX=20,N=100,FI1=-7.D0,FI2=-0.1D0)

INCLUDE 'C:\FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:\FORTRAN\CRISTINA\CELPARAM.FOR'

DIMENSION FIB1(NBMAX),FIB2(NBMAX),Y(NMAX)
EXTERNAL PFUNC,ZBRENT

IF(ROP.EQ.1.D0) THEN

* The membrane potential is given by a generalized Goldman equation

DFIP=DLOG((PNAP*Y(10)/Y(7)+PKP*Y(11)/Y(7)+PCLP*
  + Y(5)/Y(6))/(PNAP*Y(3)/Y(6)+PKP*Y(4)/Y(6)+
  + PCLP*Y(12)/Y(7)))

DFII=DLOG((PNAI*Y(19)/Y(18)+PKI*Y(20)/Y(18)+PCLI*Y(16)/
  + Y(17))/(PNAI*Y(14)/Y(17)+PKI*Y(15)/Y(17)+PCLI*Y(21)/Y(18))

ELSE

* The membrane potential is given by a NON-LINEAR equation

NB=NBMAX

DO 20 J=1,2
CALL ZBRAK(PFUNC,J,Y,FI1,FI2,N,FIB1,FIB2,NB)
IF(NB.GT.1) THEN

PRINT 15, NB

15 FORMAT(1X,'There are ',1X,J,1X,'bracketed root intervals!')
STOP

ELSEIF(NB.EQ.0) THEN
  PRINT 16

16 FORMAT(1X,'There are NO ROOTS in the selected interval!')
ELSE
  I=1
ENDIF

TOL=(1.D-6)*(FIB1(I)+FIB2(I))/2.0
DFI=ZBRENT(PFUNC,J,Y,FIB1(I),FIB2(I),TOL)

IF (J.EQ.1) THEN
  DFIP=DFI
ELSEIF (J.EQ.2) THEN
  DFII=DFI
ENDIF

20 CONTINUE

ENDIF
RETURN
END

* MEMBRANE POTENTIAL

DOUBLE PRECISION FUNCTION PFUNC(X,Y,J)

IMPLICIT REAL*8 (A-H,O-Z)

INCLUDE 'C:\FORTRAN\CRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:\FORTRAN\CRISTINA\CELPARAM.FOR'

345
DIMENSION Y(NMAX)

IF (J.EQ.1.D0) THEN
  PFUNC=(DLOG(PNAP*Y(10)/Y(7)+PKP*Y(11)/Y(7)+PCLP*Y(5)/Y(6)+RFP*(Y(3)+Y(6))*((1-1/ROP)/X)/(PNAP*Y(3)/Y(6)+PKP*Y(4)/Y(6)+PCLP*Y(12)/Y(7)+RFP*(Y(3)/Y(6))+(1-1/ROP)/X))-X
ELSEIF (J.EQ.2.D0) THEN
  PFUNC=(DLOG((PNAP*Y(19)/Y(18)+PKI*Y(20)/Y(18)+PCLI*Y(16)/Y(17)+RPI*Y(14)/Y(17))*((1-1/ROI)/X))/
  + (PNAP*Y(14)/Y(17)+PKI*Y(15)/Y(17)+PCLI*Y(21)+Y(18)+RPI*(Y(14)/Y(17))*((1-1/ROI)/X))-X
ENDIF
RETURN
END

* BREAT'S method to solve the non-linear equation (membrane potential)

DOUBLE PRECISION FUNCTION ZBRENT(FUNC,J,Y,X1,X2,TOL)
IMPLICIT REAL*8 (A-H,O-Z)
INTEGER ITMAX,ITER
PARAMETER (ITMAX=100,EPS=1.D-6,NMAX=50)
DIMENSION Y(NMAX)
EXTERNAL FUNC
A=X1

B=X2
FA=FUNC(A,Y,J)
FB=FUNC(B,Y,J)

IF ((FA.GT.0.0.AND.FB.GT.0.0).OR.(FA.LT.0.0.AND.FB.LT.0.0)) THEN
  PRINT 10
  10 FORMAT(1X,'THE ROOT MUST BE BRACKETED IN')
  STOP
  RETURN
ENDIF

C=B
FC=FB
DO 11 ITER=1,ITMAX
  IF ((FB.GT.0.0.AND.FC.GT.0.0).OR.(FB.LT.0.0.AND.FC.LT.0.0)) THEN
    *....adjust the interval
    C=A
    FC=FA
    D=B-A
    E=D
  ENDIF
  IF (DABS(FC).LT.DABS(FB)) THEN
    A=B
    B=C
    C=A
FA=FB
FB=FC
FC=FA

ENDIF

* Check the convergence

TOL1=2.0*EPS*DABS(B)+0.5*TOL
XM=0.5*(C-B)

IF (DABS(XM).LE.TOL1.OR.FB.EQ.0.0) THEN
    ZBRENT=B
    RETURN
ENDIF

IF (DABS(E).GE.TOL1.AND.DABS(FA).GT.DABS(FB)) THEN

    S=FB/FA

    IF (A.EQ.C) THEN
        P=2.0*X*Q
        Q=1.0-S
    ELSE
        Q=FA/FC
        R=FB/FC
        P=S*(2.0*X*(Q producción de 2.0)*R)*(B-A)*(R-1.0)
        Q=(Q-1.0)*(R-1.0)*(S-1.0)
    ENDIF

ENDIF

IF (P.GT.0.0) Q=Q
P=DABS(FA)

IF (2.0*P.LT.MIN(3.0*XQ-1.0,DABS(TOL1*Q),DABS(E*Q))) THEN
    E=D
    D=P/Q
ELSE
    D=XM
    E=D

ENDIF

ELSE
    D=XM
    E=D

ENDIF

A=B
FA=FB

IF (DABS(D).GT.TOL1) THEN
    B=B+D
ELSE
    B=B+DISGN(TOL1,XM)
ENDIF

FB=FUNC(F,Y,J)

11 CONTINUE
PRINT 15
15 FORMAT (1X,'ZBRENT EXCEEDING MAXIMUM ITERATIONS')

ZBRENT=B
RETURN
END

* Subroutine for bracketing the root interval

SUBROUTINE ZBRAK(FX,J,Y,X1,X2,N,XB1,XB2,NB)
IMPLICIT REAL*8 (A-H,O-Z)
PARAMETER (NMAX=50)
INTEGER N,NB,NBB,J
DIMENSION Y(NMAX),XB1(NB),XB2(NB)
EXTERNAL FX
NBB=0
X=X1
DX=(X2-X1)/N
FP=FX(X,Y,I)

DO 11 I=1,N
   X=X+DX
   FC=FX(X,Y,I)
   IF (FC*FP.LT.0.0) THEN
      NBB=NBB+1
      XB1(NBB)=X-DX
      XB2(NBB)=X
   IF (NBB.EQ.NB) GO TO 1
ENDIF
   FP=FC
11 CONTINUE
1 CONTINUE
   NB=NBB
   RETURN
END

* *
*Output Program
* *
SUBROUTINE ASSIGN(IP,YMODEL,SOLNON)
IMPLICIT REAL*8 (A-H,O-Z)

INCLUDE 'C:\FORTRANCRISTINA\MVKIDGLU.FOR'
INCLUDE 'C:\FORTRANCRISTINA\CELPARAM.FOR'

DIMENSION SOLNON(1:109,0:9),YMODEL(NMAX)

*.....Assign the solutions for compartmental values

SOLNON(1,IP) = YMODEL(1)/YMODEL(6)
SOLNON(2,IP) = YMODEL(2)/YMODEL(6)
SOLNON(3,IP) = YMODEL(3)/YMODEL(6)
SOLNON(4,IP) = YMODEL(4)/YMODEL(6)
SOLNON(5,IP) = YMODEL(5)/YMODEL(6)
SOLNON(6,IP) = YMODEL(6)
SOLNON(7,IP) = YMODEL(7)
SOLNON(8,IP) = YMODEL(8)/YMODEL(7)
SOLNON(9,IP) = YMODEL(9)/YMODEL(7)
SOLNON(10,IP) = YMODEL(10)/YMODEL(7)
SOLNON(11,IP) = YMODEL(11)/YMODEL(7)
SOLNON(12,IP) = YMODEL(12)/YMODEL(7)
SOLNON(13,IP) = YMODEL(13)
SOLNON(14,IP) = YMODEL(14)/YMODEL(17)
SOLNON(15,IP) = YMODEL(15)/YMODEL(17)
SOLNON(16,IP) = YMODEL(16)/YMODEL(17)
SOLNON(17,IP) = YMODEL(17)
SOLNON(18,IP) = YMODEL(18)
SOLNON(19,IP) = YMODEL(19)/YMODEL(18)
SOLNON(20,IP) = YMODEL(20)/YMODEL(18)
SOLNON(21,IP) = YMODEL(21)/YMODEL(18)
SOLNON(22,IP) = YMODEL(22)
SOLNON(23,IP) = YMODEL(23)/YMODEL(18)
SOLNON(24,IP) = YMODEL(24)/YMODEL(18)
SOLNON(25,IP) = YMODEL(25)
SOLNON(26,IP) = YMODEL(26)
SOLNON(27,IP) = CDEXPL
SOLNON(28,IP) = CDEXIT
SOLNON(29,IP) = CDXAVIT
SOLNON(30,IP) = PDEXPL
SOLNON(31,IP)=PIDEXIT
SOLNON(32,IP)=SOUTP
SOLNON(33,IP)=SOUTI
SOLNON(34,IP)=DFIB
SOLNON(35,IP)=DFIP
SOLNON(36,IP)=DFII
SOLNON(37,IP)=DFIB
SOLNON(38,IP)=XJFIT
SOLNON(39,IP)=XJLIT
SOLNON(40,IP)=QSIT
SOLNON(41,IP)=QLIT
SOLNON(42,IP)=XJFBT
SOLNON(43,IP)=XJLBT
SOLNON(44,IP)=QSBT
SOLNON(45,IP)=QLBT
SOLNON(46,IP)=PBT
SOLNON(47,IP)=PIIT
SOLNON(48,IP)=PBT
SOLNON(49,IP)=PIBT
SOLNON(50,IP)=PC
SOLNON(51,IP)=PIPL
SOLNON(52,IP)=CIT
SOLNON(53,IP)=CITAV
SOLNON(54,IP)=CBT
SOLNON(55,IP)=CBTAV
SOLNON(56,IP)=CPL
SOLNON(57,IP)=XNAIT
SOLNON(58,IP)=XKIT
SOLNON(59,IP)=XCLIT
SOLNON(60,IP)=XNALIT
SOLNON(61,IP)=XKLIT
SOLNON(62,IP)=XCLLT
SOLNON(63,IP)=CFAITC
SOLNON(64,IP)=CFClTC
SOLNON(65,IP)=CFAIT
SOLNON(66,IP)=CFClTC
SOLNON(67,IP)=SIGIT
SOLNON(68,IP)=SIGION
SOLNON(69,IP)=PSIT
SOLNON(70,IP)=PSION
SOLNON(71,IP)=XKFIT
SOLNON(72,IP)=(SOUTP*1.D+3-297.96D0)*100.D0/297.96D0
SOLNON(73,IP)=(YMODEL(7)-VPOLO)*100.D0/VPOLO
SOLNON(74,IP)=(YMODEL(18)-VITO)*100.D0/VITO
SOLNON(75,IP)=(YMODEL(6)-CVPOLO)*100.D0/CVPOLO
SOLNON(76,IP)=(YMODEL(17)-CVITO)*100.D0/CVITO
SOLNON(77,IP)=1.D3*DNAD
SOLNON(78,IP)=1.D3*DKD
SOLNON(79,IP)=1.D3*DFCD
SOLNON(80,IP)=1.D3*DCAT
SOLNON(81,IP)=1.D3*DALB
SOLNON(82,IP)=1.D3*DCLD
SOLNON(83,IP)=1.D3*DAND
SOLNON(84,IP)=ALBK0
SOLNON(85,IP)=XFCIT
SOLNON(86,IP)=XCLIT
SOLNON(87,IP)=XFAIT
SOLNON(88,IP)=XFALIT
SOLNON(89,IP)=PIROTPL
SOLNON(90,IP)=PIPOTT
SOLNON(91,IP)=CGLUPLM*1.D3
SOLNON(92,IP)=CGLUIM*1.D3
SOLNON(93,IP)=YMODEL(27)
SOLNON(94,IP)=YMODEL(28)
SUBROUTINE OUTPUT(IP,TIME,HCT,SOLNON,BV)
IMPLICIT REAL*8 (A-H,O-Z)

DIMENSION SOLNON(1:109,0:9)

WRITE (10, 90)
TIME, HCT, SOLNON(35,IP)*26.7, SOLNON(6,IP), SOLNON(3,IP)
+ SOLNON(10,IP)*1.D3, SOLNON(11,IP)*1.D3, SOLNON(12,IP)*
+ 1.D3, BV, SOLNON(32,IP)*1.D3, SOLNON(50,IP), SOLNON(51,IP),
+ SOLNON(13,IP), SOLNON(56,IP), SOLNON(93,IP), TIME, SOLNON

RETURN
END