

72-14,921

LEONARD, Joel I., 1939-
DYNAMICS OF PLASMA-INTERSTITIAL FLUID DISTRIBUTION
FOLLOWING INTRAVENOUS INFUSIONS IN
NEPHRECTOMIZED DOGS.

The University of Michigan, Ph.D., 1971
Physiology

University Microfilms, A XEROX Company, Ann Arbor, Michigan

DYNAMICS OF PLASMA-INTERSTITIAL FLUID DISTRIBUTION FOLLOWING
INTRAVENOUS INFUSIONS IN NEPHRECTOMIZED DOGS

by
Joel I. Leonard

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Bioengineering)
in The University of Michigan
1971

Doctoral Committee:

Associate Professor Peter H. Abbrecht, Chairman
Associate Professor David R. Rovner
Associate Professor Jerome S. Schultz
Associate Professor Harvey V. Sparks

PLEASE NOTE:

**Some pages have indistinct
print. Filmed as received.**

University Microfilms, A Xerox Education Company

ACKNOWLEDGEMENTS

I would like to express my appreciation for the encouragement, advice and assistance I received during these stimulating years of interdisciplinary study. Dr. Peter Abbrecht originally directed me towards the interesting problems of body fluid regulation and I am grateful for his personal interest and the advice he provided throughout all stages of this research effort. Dr. Harvey Sparks helped clarify many of the physiological problems that arose and, from him, I discovered the excitement of doing research. Drs. Jerome Schultz, David Rovner and James Conway gave generously of their time in guiding the development of this dissertation. It is a pleasure to thank Miklos Gellai whose surgical skills, technical expertise and patient understanding was invaluable during the experimental phase of this study. I wish to express my appreciation to the staff of the Physiology Department for their support and for the use of the departmental facilities. Finally, I wish to acknowledge the Bioengineering Program and the National Institute of Health for the financial assistance they provided for my academic studies and this investigation.

TABLE OF CONTENTS

	Page
I. INTRODUCTION.....	1
II. REVIEW OF PRIOR WORK.....	5
A. Elements of Plasma-Interstitial Fluid Exchange.....	5
1. Introduction.....	5
2. Transcapillary Filtration.....	5
a. The Starling Hypothesis.....	5
b. Modifications of the Starling Hypothesis.....	7
3. Effect of Peripheral Vascular Regulation on Transcapillary Filtration.....	9
a. Anatomical-Functional Description of the Peripheral Vascular Bed.....	9
b. Vasomotor Adjustments for Controlling Filtration.....	9
c. Vasomotor Mechanisms.....	11
d. Overall Peripheral Regulation.....	12
e. Vasomotor Control of Plasma Volume.....	13
4. The Interstitial Fluid Compartment.....	15
a. Physical State of the Interstitial Space.....	15
b. Tissue Pressure and Compliance.....	16
c. Regulation of Interstitial Homeostasis.....	18
d. Interstitial Fluid Volume.....	20
5. Dynamics of Lymph Formation and Lymph Flow.....	22
6. Protein Concentrations of the Plasma, Interstitial Fluid and Lymph.....	27
7. Transcapillary Protein Transport Mechanisms.....	32
B. Quantitative Models of Extracellular Fluid Regulation and Distribution.....	35
C. Effects of Infusion on Plasma-Interstitial Fluid Distribution.....	40
1. Introduction.....	40
2. Effects of Infusion on Blood Volume Expansion.....	41

	Page
3. Effects of Infusion on Circulatory Function.....	43
4. Effect of Infusion on Lymph Flow and Protein Transport.....	45
III. EXPERIMENTAL METHODS AND MATERIALS.....	47
A. Requirements of the Experimental Design.....	47
B. Experimental Design.....	48
C. Experimental Protocol.....	49
D. Special Methods.....	52
1. Extra-Corporeal Shunt and Counting Chamber.....	52
2. CR-51 Labelling Technique.....	53
3. Blood Volume Calculations.....	53
4. Preparation of Infusion Solutions.....	55
5. Blood, Plasma, and Lymph Analysis.....	55
IV. EXPERIMENTAL RESULTS.....	59
A. Time Course of Observed Variables in Typical Experiments.....	59
B. Variables for Individual Experiments.....	62
1. The Plasma Volume Response.....	62
2. Statistical Analysis of Plasma Volume Response.....	68
3. The Blood Pressure.....	71
4. Variability in Total Red Blood Cell Volume.....	71
C. Averaged Results of Infusate Retention, Blood Pressure and Protein Concentrations.....	73
D. Cardiac Output and Total Peripheral Resistance Responses.....	77
E. Lymph Flow and Lymph Protein Concentration.....	79

	Page
V. DISCUSSION OF EXPERIMENTAL RESULTS.....	81
A. Accuracy of Blood Volume Measurement Techniques.....	81
1. Variability in Red Cell Volume.....	81
2. Accuracy of F_{cell} Ratio.....	82
3. Accuracy of the Coil Technique.....	82
B. Plasma Volume Response.....	83
C. Blood Pressure Response.....	85
D. Cardiac Output and Total Peripheral Resistance Responses.....	87
E. Effects of Innovar.....	88
F. Lymph Flow and Lymph Protein Concentration.....	88
G. Summary.....	90
VI. THE MATHEMATICAL MODEL AND SIMULATION PROCEDURE.....	92
A. The Physiological Model.....	92
B. The Compartmental Model.....	93
C. The Mathematical Model.....	96
D. Variables and Parameters of the Model.....	103
1. Output Variables.....	103
2. Parameter Optimization.....	103
3. Input Data.....	104
4. Describing Functions for Tissue and Lymph Parameters.....	105
a. Tissue Pressure Function.....	105
b. Lymph Flow Function.....	106
5. Assumed Values for Initializing Constants.....	108
a. Capillary Surface Area.....	109
b. Capillary Filtration Coefficient.....	109

	Page
c. Capillary Protein Permeability.....	110
d. Pre-/Post Capillary Resistance Ratio.....	110
6. Calculation of Initial Conditions.....	111
E. Methods of Dynamic Simulation.....	113
1. Purpose of Simulation.....	113
2. Design of Simulation Experiments.....	113
3. The Simulation Procedure.....	114
4. The Computer and Simulation Language.....	119
5. Least Squares Comparator and Optimization Subroutine.....	119
VII. RESULTS OF COMPUTER ANALYSIS.....	122
A. Parametric Studies.....	122
B. Piecewise Optimization Studies.....	129
1. Method of Analysis.....	129
2. Comparison of Responses Between the Four Groups.....	133
a. Response of Optimizing Parameters.....	133
b. Capillary Pressure Response.....	134
c. Response of Plasma Volume.....	135
d. Response of Protein Concentration and Mass....	135
C. Constant Parameter Optimization Studies.....	135
VIII. PHYSIOLOGICAL IMPLICATIONS OF THE SIMULATION STUDY.....	139
A. Simulated Behavior of R, A, and P_c	139
B. Effect of Tissue Compliance on the Simulated Behavior of the Pre-/Postcapillary Resistance Ratio...	141
1. Tyrode's Infusions.....	141
2. Dextran Infusions.....	143
3. Analysis of Tissue Compliance.....	149

	Page
C. Role of Pre-/Post Capillary Resistance Ratio and Sphincter Tone in the Regulation of Plasma-Interstitial Fluid Balance.....	150
D. Increase in Plasma Proteins Following Infusions....	152
E. Evaluation of the Model.....	154
1. Validity of Model.....	154
2. Implications of the Lumped-Parameter Concept...	155
3. Deficiencies of the Model.....	156
F. Significance and Applications.....	157
IX. SUMMARY.....	160
Appendix.....	164
References.....	168

LIST OF TABLES

Table	Page
I. Typical Values of Protein Concentration in Plasma, Tissue and Lymph Fluid of the Dog.....	30
II. Statistical Analysis of Plasma Volume Responses.....	70
III. Mean Values of Red Cell Volumes During Infusion.....	73
IV. Values of Tissue Compliance Used in Simulation.....	106
V. Representative Values of Capillary Parameters.....	108
VI. Assumed Values for Initializing Constants.....	111
VII. Experimental Input Constants for Simulation Studies.....	118
VIII. Variability of Microcirculatory Parameters.....	139

LIST OF FIGURES

Figure	Page
1. Extracorporeal Shunt Used for Monitoring Blood Radioactivity Continuously	51
2. Colloid Osmotic Pressures of Protein and Dextran.....	56
3. Results of a Typical Tyrode's Infusion Experiment.....	60
4. Results of a Typical Dextran Infusion Experiment.....	61
5. Slow Tyrode's Infusion: Individual Experimental Results.....	64
6. Rapid Tyrode's Infusion: Individual Experimental Results....	65
7. Slow Dextran Infusion: Individual Experimental Results.....	66
8. Rapid Dextran Infusion: Individual Experimental Results....	67
9. Variation of Total Red Cell Volume During Slow and Rapid Infusion Experiments.....	72
10. Tyrode's Infusion: Averaged Results of Slow and Rapid Infusion Experiments.....	75
11. Dextran Infusion: Averaged Results of Slow and Rapid Infusion Experiments.....	76
12. Cardiac Output and Total Peripheral Resistance Responses During Tyrode's Infusion.....	78
13. Lymph Flow and Lymph Protein Concentration Responses.....	80
14. Schematic of Plasma-Interstitial Lymph Fluid Circulation.....	92
15. Compartmental Model of the Extracellular System.....	94
16. Flow Diagram Showing Operational Analysis of Plasma-Interstitial Fluid Exchange Model.....	97
17. Describing Functions for Model: Tissue Pressure and Lymph Flow as Function of Interstitial Fluid Volume.....	107
18. Normalized Plasma Volume Responses for the Four Experimental Groups.....	115

Figure	Page
19. Blood Pressure Responses for the Four Experimental Groups.....	116
20. Flow Chart of Simulation Procedure.....	117
21. Effect of R_a/R_v on Simulated Plasma Volume Response.....	123
22. Effect of A on Simulated Plasma Volume Response.....	124
23. Effect of $K_w A$ and $K_p A$ on Simulated Plasma Volume Response.....	125
24. Effect of Tissue Compliance on Simulated Plasma Volume Response.....	126
25. Effect of Lymph Flow on Simulated Plasma Volume Response....	127
26. Piecewise Optimization Results of Simulated Tyrode's Infusions.....	131
27. Piecewise Optimization Results of Simulated Dextran Infusions.....	132
28. Constant Parameter Optimization of Rapid Tyrode's and Dextran Infusions.....	137
29. Simulated Effect of Tissue Compliance on Microcirculatory Variables During Tyrode's Infusion.....	142
30. Comparison of Experimental TPR and Simulated R_a/R_v During Rapid Tyrode's Infusions.....	144
31. Simulated Effect of Tissue Compliance on Microcirculatory Variables During Dextran Infusion.....	145
32. Postulated Changes in Pre- and Postcapillary Resistances During Dextran Infusion.....	148

LIST OF APPENDICES

Appendix	Page
A. Symbols, Notations and Units.....	165
B. Table A-1: Control Values for Each Experimental Animal.....	167
C. Table A-2: Control Values Associated with Individual Experiments.....	168

I. INTRODUCTION

"In future studies of material transfer through biological barriers, of living systems not in a true steady state, it is safe to predict that not only will more effort be expended in individual experiments to obtain information about a sufficient number of independent variables, but also that the student of material transfer will become a most avid user of modern computers". (Leslie F. Nims, 1966)

Rapid changes in blood volume are observed to occur during a variety of pathological or therapeutic situations such as hemorrhage, fluid replacement therapy or artificial hemo-dialysis therapy. If the amount of fluid added or removed is moderate the blood volume usually readjusts to normal within a few minutes to a few hours (Milhorn, 1966). These rapid circulatory adjustments involve essentially two major physiological pathways: excretion through the kidneys and transcapillary filtration to or from the interstitium.

It is becoming increasingly apparent that the latter of these pathways, the "Starling" fluid shift mechanism, plays an important role in both the short-term control of plasma volume (Mellander, 1968), and, by establishing a tight mechanical coupling between plasma and interstitial fluid compartments, in the control of the entire extracellular fluid space (Gauer et al, 1970). In patients suffering from complete or partial renal failure the role of the renal pathway in controlling circulatory fluid dynamics is, of course, minimized. Furthermore, under a hemorrhagic stress in normal individuals, there is an immediate reflex shutdown of urine excretion. Thus in these situations, and in others such as exercise and standing, transcapillary filtration and reabsorption between plasma and interstitial compartments become the major source of plasma volume control.

The general objective of the present research was to obtain a better understanding of the distribution of fluids between these two extracellular spaces. Experimentally this objective can best be achieved by inducing and measuring large changes in transcapillary filtration and compartmental volumes. Most of the classical studies elucidating the fluid shift mechanism have accomplished this by occluding individual capillaries (Landis, 1927) or by perfusing, at various pressures, small capillary beds of single organs (Pappenheimer & Soto-Rivera, 1948). Inasmuch as we were interested in the total extracellular system of the whole animal, we could use

a variety of other stimuli: hemorrhage, upright tilting, direct alteration of blood pressure, or plasma removal via extracorporeal dialysis. However, none of these is as easy to facilitate and control as infusion of fluids directly into the bloodstream.

Compared to the amount of work performed where hemorrhage (and the accompanying absorption of interstitial fluid) was the primary stress, there is relatively little information available on the reverse problem concerning fluid transport into the tissues during moderate transfusions (Gauer & Henry, 1963). Many of the early infusion studies were designed to induce secondary changes in renal function, lymph flow, cardiac performance, etc., and were not concerned with obtaining the dynamic blood volume and blood pressure response data that we desired. In fact, inasmuch as the disappearance of any infused solution can be greatly influenced by the rise in capillary filtration pressure, it is surprising that there has been virtually no effort to quantitatively relate the retention of plasma expanders to the simultaneous increases in circulatory pressures.

More recently, modern isotope tracer techniques and electronic pressure transducers have made it possible to study certain dynamic infusion responses, usually however, in animals with intact renal function (Prather, et al, 1969; Moore, et al, 1966; Wasserman & Mayerson, 1952a). The renal diuretic mechanism is known to reflexly respond very rapidly to even moderate blood volume changes (Gauer & Henry, 1963) thus obscuring the full effects of the infused fluids on plasma-interstitial fluid partitioning. In addition, the complexity of renal function makes it less amenable to include this factor in simple models of body fluid distribution. Thus, we have chosen to study nephrectomized dogs and their short-term blood volume and pressure responses to intravenous fluid infusions.

The theoretical factors governing plasma-interstitial fluid exchange have, to a large extent, been defined ever since Starling (1896) first proposed his theory of transcapillary migration of fluids, salts and proteins. But while verification of many of the controlling mechanisms has been experimentally confirmed in careful investigations of small portions of the vascular bed, there has been correspondingly little attention paid to whole animal studies. Recently, two theoretical papers have laid the groundwork for such an understanding of plasma-interstitial fluid exchange

in the entire animal (Guyton & Coleman, 1967; Wiederhielm, 1968). These authors and others have suggested that the Starling fluid shift mechanism is only one of several important factors in the long term regulation of extracellular fluid distribution. Using computer simulation techniques they were able to show that tissue compliance, lymph flow, protein exchange, and peripheral vessel resistance are equally important factors to be considered for a more complete understanding of this problem. However, there was only a perfunctory attempt to relate their analysis to actual experimental data. In addition, although their models were suitable for obtaining transient solutions they did not feel it necessary to examine their system for other than the steady state case. Finally, while they were able to simulate such clinical situations as inflammation, lymph insufficiency, and hypoproteinaemia, the problem of plasma expansion by infusion of fluids was not studied. A major focus of the current study was to use these models as a basis for simulating the disappearance of infused fluids from the circulation and to compare these responses with experimental results.

If the infused solutions are isotonic and have similar extracellular ion concentrations, net fluid shifts across the cell membrane are not significant (Moore, et al, 1966), and we need only consider distribution within the extracellular compartment. Two different types of fluids were used in this study: iso-oncotic dextran and Tyrode's solution--both isotonic but one colloidal-free. These solutions would be expected to disappear from the circulation at widely different rates. We chose to eliminate the large intracellular fluid compartment from our sphere of examination by infusing such isotonic solutions, since the experimental techniques dealing with changes in cellular volume and composition are relatively difficult to perform, inaccurate and often involve destructive biopsy methods. While total body fluid regulation is certainly important and not well understood, there is still much to be learned from the more restricted system that we have chosen, particularly with respect to dynamic behavior. Additionally, a more quantitative description of plasma-interstitial exchange is needed by the clinician interested in extracellular fluid balance.

To summarize the objectives of this investigation: we have been concerned in general with those factors that affect and control the exchange of fluid between plasma and interstitial compartments. Our objective was to study, both experimentally and theoretically, the dynamic distribution

of intravenously infused solutions across the blood-tissue interface in nephrectomized animals over a period of several hours. We desired to ascertain if certain well-known physiological relationships which are well accepted for the steady state can also account for short-term dynamic behavior. The experimental protocol required accurate determination of the changes in plasma volume as well as any major blood pressure or protein concentration data as a result of the infusion. There is much to be said for examining non-steady state (i.e. dynamic) behavior, especially for complex regulatory systems, as it invariably leads to a better understanding of the interrelationships between variables. Theoretically, we wished to perform an analysis of the most important systems involved in extracellular fluid distribution and by using dynamic computer simulation techniques predict the results achieved experimentally. In particular, we have asked if the observed blood pressure response together with a model of plasma-interstitial fluid exchange can adequately account for the observed blood volume response. This process of testing a model with experimental data often leads to ways in which the underlying theory can be refined and extended.

II. REVIEW OF PRIOR WORK

"It might be imagined that it was desirable to know everything that had been learned about a subject. In practice this is quite impossible, and attempts in this direction are usually a sterile waste of time." (E. Bright Wilson, Jr., 1952)

A. ELEMENTS OF PLASMA-INTERSTITIAL FLUID EXCHANGE

1. INTRODUCTION

Exchange between interstitial and plasma fluids occurs at only two general sites: through the capillary walls and at the sites in the venous vessels where lymph ducts join the circulation. Thus the factors that control transcapillary fluid exchange and lymph flow shall be our primary concern in this review. Inasmuch as large colloidal molecules, mainly the albumin fraction of the plasma proteins, are directly responsible for developing the colloidal osmotic pressure gradients across the capillary walls, we shall also be concerned with the movement of proteins between the two major extracellular compartments. The renal system, which is primarily responsible for the long-term control of plasma volume, especially following infusions, will not be discussed here because this study, both experimentally and theoretically, has eliminated this system from consideration. A recent review, however, deals extensively with the effect of the renal system on the control of extracellular fluid volume (Gauer, et al., 1970).

2. TRANSCAPILLARY FILTRATION

a. The Starling Hypothesis

The first accurate description of the exchange of fluid between blood capillaries and interstitial space was given by Starling (1896). The essential element of the Starling hypothesis is a capillary membrane almost impermeable to plasma proteins which results in a large colloidal osmotic pressure gradient tending to draw fluid from the tissues into the circulation. This force is normally counterbalanced by an equal tendency for the capillary hydrostatic pressure gradient to filter fluid in the opposite direction, out of the circulation. Under the influence of these physical

forces the transcapillary fluid shift system was seen as a self-regulating mechanism for aiding in the homeostatic control of blood and tissue fluid volume. Although reliable techniques were unavailable to Starling to quantitatively support his theory, it is in the main still valid and serves as a basis of our present knowledge.

Quantitatively, the Starling hypothesis defines the net filtration rate of fluid across any small capillary segment as a linear combination of the hydrostatic pressure differences between the capillary (P_c) and tissues (P_t) and the colloidal osmotic pressure differences between the plasma (π_{pl}) and tissue fluid (π_t), all multiplied by a membrane permeability factor, $K_w A$:¹

$$Q_{filt} = K_w A (P_c - P_t - \pi_{pl} + \pi_t) \quad (II-1)$$

A positive value of Q_{filt} indicates net movement into the tissues and a negative one, net absorption into the capillary lumen. The term A represents the effective capillary surface area.

The review of Landis and Pappenheimer (1963) summarizes the attempts to measure each of the factors in the above relationship. The difficulty of studying the micro-circulation can be appreciated when one realizes that at the present time no one has even attempted to measure all of the variables of Equation (II-1) during a single experiment. Nevertheless, the essential accuracy of this relationship has been validated over the last 40 years, starting with the classical studies of Landis (1927, 1934). He was able to measure capillary pressure directly in single capillaries and found that when this pressure was equal to the plasma colloid osmotic pressure, transcapillary fluid movement nearly ceased. These studies have been recently repeated, using refined techniques, by Zweifach and Intaglietta (1968), who concluded that the tissue forces play a more important role in fluid exchange than was originally thought. Verification of the Starling relationship has also been obtained by studying entire capillary networks in isolated organs (Pappenheimer & Soto-Rivera, 1948; Johnson & Hanson, 1966;

¹ $K_w A$ as used here is similar to k_f as used by Landis and Pappenheimer (1963) and CFC, the capillary filtration coefficient, as used by Mellander & Johansson (1968). If K_w is expressed as ml/min-mmHg-cm² surface area, then A is expressed as cm² of capillary surface area in the tissue under consideration.

Mellander, 1960; Folkow, et al, 1963; Levine, et al, 1967).

b. Modifications of the Starling Hypothesis

While these types of studies provided the necessary proof to support the Starling hypothesis, they inevitably led to modifications of the original theory and elucidation of many other factors that Starling did not appreciate or emphasize. Traditionally it had been the view that, due to the decreasing hydrostatic pressure along the entire length of the capillary, fluid was filtered into the tissues near the arterial end of the capillary; and an almost equal amount of fluid was reabsorbed at the venous end. A more recent view suggests that rhythmic contractions and relaxations of the smooth muscles at the entrance of the capillary bed (a muscular response to minute pressure changes known as "vasomotion") permits blood flow in large microcirculatory networks to be controlled in a phasic on-off fashion (Zweifach, 1961; Zweifach & Intaglietta, 1966). During the periods of flow, fluid filters from the capillaries along their entire length and conversely during the periods of no-flow, tissue fluid is reabsorbed. The mechanisms controlling vasomotion frequency and duration are not precisely understood, but they allow neural, humoral and pressure influences to take part in feedback regulation of local blood flow distribution and, thus, plasma-interstitial fluid balance. This view does not invalidate the basic Starling hypothesis as expressed by Equation (II-1), but suggests that the phenomenon of vasomotion is the physical means by which the Starling fluid exchange mechanism is more perfectly regulated. Additionally, it emphasizes that capillaries probably do not act in isolation but as parts of large capillary networks. The entire body of literature relating to the chemical and neural regulation of transcapillary filtration has been summarized in a major review by Mellander & Johansson (1968).

There is evidence that capillary networks are not symmetrical in that changes in arterial and venous pressures effect capillary filtration rates unequally, in part due to unequal upstream and downstream capillary resistances (Pappenheimer & Soto-Rivera, 1948; Haddy, et al, 1957; Mellander, 1960; Johnson & Hanson, 1962). In addition, permeability to water and other substances varies along the length of the capillary endothelium with the highest values of permeability found in the venous capillaries (Wiederhielm, 1966; Zweifach & Intaglietta, 1966, 1968). These changes in permeability are thought to be due to both variation in pore size, and total surface area available for

filtration along the length of the capillary. Some of these same studies have shown that the exchange process involves not only the capillaries, but the smallest venules as well. While Starling proposed a capillary membrane almost totally impermeable to proteins, it was recognized long ago that a certain fraction of plasma proteins are able to cross the capillary barrier and show up eventually in lymph fluid (Drinker & Field, 1931). Grotte (1956) delineated the quantitative relationship between the molecular weight of large molecules and their ability to permeate the capillary membrane. However, even today, the transport mechanism of large molecules is not clearly defined, some authors favoring a restricted diffusion process (e.g. Guyton & Coleman, 1967) and others a bulk flow mechanism through large pores in the venular side of the capillary (Renkin, e.g., Wiederhielm, 1968). A recent report by Garlick and Renkin (1970) suggests that both mechanisms are normally present and the preferential pathway of a particular molecule depends on its molecular size. Molecules smaller than 5000 MW, mostly electrolytes, are relatively unhindered by the capillary membrane in comparison with the larger plasma proteins and therefore do not contribute, under most circumstances, to the net osmotic forces that regulate fluid movement. Hence, the transport mechanisms regarding these smaller substances will not be considered here.

The importance of tissue pressure and tissue colloidal osmotic pressure in affecting transcapillary fluid shifts does not appear to have been appreciated by the earlier investigators. It has usually been assumed that "the tissue component ($P_t - \Pi_t$) is comparatively small; viz., the actual tissue pressure was low and the concentration of protein in the extravascular space was negligible. Both of these assumptions are open to question, in particular the physical pressure outside the capillary wall", (Zweifach & Intaglietta, 1968). In fact, by coincidence or not, it was not until Guyton (1963) succeeded in measuring negative tissue pressures using subcutaneously implanted capsules that the rightful role of tissue pressure began to be recognized (e.g. Wallentin, 1966). Landis and Pappenheimer (1963) present a good summary of the work done up to that time using micropipette methods to measure tissue pressure, while Gauer, Henry and Behn (1970) review the subsequent controversy surrounding the concept of a negative interstitial pressure.

Similarly, there is little general agreement on the normal value of the effective colloidal osmotic pressure in the interstitial fluid. While this value was originally thought to be so low as to be negligible, it is now believed by some that colloidal osmotic pressures in the tissue space may be from 5 to 10 mm Hg (Wiederhielm, 1968). It is probable that a large source of the disagreement comes from a lack of suitable techniques for sampling the extremely thin layer of interstitial fluid as well as the fact that the permeability of the capillary membrane and the nature of the interstitial gel varies from one organ to the next (Mayerson, et al, 1960).

Some of these concepts will be taken up in greater detail in subsequent sections.

3. EFFECT OF PERIPHERAL VASCULAR REGULATION ON TRANSCAPILLARY FILTRATION

a. Anatomical-Functional Description of the Peripheral Vascular Bed

The various vascular circuits may be described by their functionally different consecutive sections: the Windkessel vessels (large arteries), the precapillary resistance vessels (small arteries and arterioles), the precapillary sphincters at the entrance of each capillary loop, the exchange vessels (the capillaries), the post-capillary resistance vessels (venules and small veins) and the capacitance vessels (mainly the distensible veins) (Mellander & Johansson, 1968). Of these segments, the pre- and post-capillary resistance vessels, the pre-capillary sphincters and the capillary exchange vessels have a key position in the regulation of transcapillary exchange and hence of plasma volume. The blood flow to an organ is controlled by the overall flow resistance in the tissues while the distribution of blood within the exchange vessel networks is controlled by the precapillary sphincters (Renkin, 1964). Small vessel resistance and sphincter tone depend on contraction and relaxation of smooth muscle in the vessel walls, and this in turn is subject to control by nervous, hormonal, physical and local chemical factors.

b. Vasomotor Adjustments for Controlling Filtration

Of the factors in Equation (II-1) that determine transcapillary filtration, two of them, the mean hydrostatic capillary pressure, P_c ,

and the effective surface area available for exchange, A , can be controlled directly by powerful vasomotor reactions.² The factors determining capillary pressure have been related by the expression,

$$P_c = \frac{P_a + P_v \cdot (R_a/R_v)}{1 + R_a/R_v} \quad (\text{II-2})$$

where P_a and P_v denote the central arterial and venous pressures respectively, and R_a and R_v , the precapillary and postcapillary flow resistances respectively (Landis & Pappenheimer, 1963). Because it is usually found that R_a changes substantially more than R_v , vasodilation usually leads to a decrease in the pre/post capillary resistance ratio and thus to net filtration, while vasoconstriction leads to an increase in this ratio and net absorption of extravascular fluid into the circulation.

The surface area available for filtration is dependent on the number of capillaries open to blood flow. Precapillary sphincters, situated at the entrance of each of the capillaries, can theoretically control the blood flow to individual capillaries. Thus the control of sphinctor tone will in turn control capillary surface area and net filtration rate.

Hence there are two distinct local adjustments that can be made to control transcapillary filtration: first, regulation of capillary pressure by alterations of the precapillary/postcapillary resistance ratio and

²There is some disagreement whether the permeability coefficient, K_w , can be time-varying. K_w reflects the characteristics of the individual pores (such as pore diameter and length) through which filtration takes place. It is only possible to directly measure the overall permeability, $K_w A$, rather than each of the two terms of which it is composed. Although it has been shown that $K_w A$ can increase during exercise (Kjellmer, 1964) and decrease upon standing (Mellander, 1968), it has not been possible to show conclusively that A rather than K_w is the more significant time-varying quantity. Most of the conclusions regarding this have been based on measuring the permeability of protein or dextran rather than fluid. Thus Mayerson and Wasserman (1952a) show that the disappearance rate constant of albumin during infusions can increase up to a factor of three and Shirley, et al (1957) conclude that this is due to a stretching of the pores (presumably a change in K_w). On the other hand Arturson and Kjellmer (1964) show that $K_w A$ can also increase by a factor of three during exercise but, since dextran permeability does not simultaneously change, they believe the area, A , rather than K_w is changing. Lundren and Mellander (1967) came to similar conclusions.

second, regulation of the size of the capillary surface area available for exchange by means of changes in precapillary sphincter tone. This is the basis of the concept of "autoregulation of capillary fluid filtration" originally proposed by Lundvall, et al (1967).

c. Vasomotor Mechanisms

The changes in resistance or tone referred to above are typically brought about by an active change in the radius of the resistance vessels or sphincter sections. The walls of these vessels or the sphincters themselves are composed of smooth muscle cells which may contract (increasing resistance) or relax (decreasing resistance) in response to specific types of stimuli. Vessel resistances may also be changed passively by variations in transmural pressure, organic changes in the wall, or plugging of the lumen. Passive distention will occur at the onset of a stress such as standing, exercise, or administration of i.v. infusions, but this action is always quickly modified by subsequent active reflexes. We are concerned here primarily with the active component.

In general it is possible to identify three different types of stimuli that bring about this active smooth muscle response or vasomotor reaction: neurogenic, physical (or myogenic), and chemical. The neurogenic stimuli can be thought of as being the important link in a central, extrinsic level of control while the myogenic and chemical stimuli are mediators in a local, intrinsic control of the peripheral circulation.

Central Control.

A most important example of the central control system and the neurogenic stimuli is, of course, the sympathetic adrenergic constrictor fibres which are activated via baro- and chemo-receptors. These fibers, capable of graded discharge, innervate the resistance and capacitance vessels and the precapillary sphincters as well (Ross, 1971). (There does not appear to be any good evidence to suggest that the capillary endothelial walls themselves are affected by any of these stimuli). When stimulated, these fibers consistently increase precapillary resistance more than postcapillary resistance, which results in a lowered mean capillary pressure and a reabsorption of fluid across the capillary membranes. This would be a typical

response of the skeletal muscle vascular beds as a result of a lowered arterial blood pressure caused by hemorrhage (Oberg, 1964).

Local Control.

Local control of the peripheral circulation exists under normal conditions and even if the innervation of the periphery is experimentally blocked. There are two principal mechanisms: a) chemical factors related to released metabolites, and b) mechanical or myogenic factors related to changes in transmural pressure. While the exact chemical factors are still unknown, in general they are released upon increased tissue activity and have the effect of dilating the pre-capillary resistance vessels and capillary sphincters but not necessarily the postcapillary resistance vessels. The overall effect is to increase blood flow to the tissue, capillary pressure, capillary surface area and hence outward filtration of fluid.

Local myogenic control refers to the mechanoreceptor properties of vascular smooth muscle in which distension leads to contraction. Hence, increased transmural pressure leads to relative vasoconstriction, and decreased pressure to vasodilation. Myogenic activity is believed to occur primarily in the precapillary resistance vessels and sphincters and is probably responsible, at least in part, for "auto-regulation of blood flow" (Folkow, 1964) and the phenomena of vasomotion (Zweifach, 1961), both concepts attempting to provide a basis for the maintenance of tissue homeostasis. It has also been suggested that because of this mechanism significant edema formation is prevented during the high hydrostatic loading that occurs upon standing (Mellander, 1968). Increased transmural pressure causes a significant number of precapillary sphincters to close, and the rate of filtration is decreased in proportion to the decreased capillary surface area.

d. Overall Peripheral Regulation

We have described several distinct vascular adjustments that are capable of modulating such factors as surface area, pressure, blood flow and distribution, vessel radius and distensibility, and transcapillary fluid exchange. However, as yet, no clear cut theory has evolved describing how the various intrinsic and extrinsic feedback mechanisms behave to achieve the proper balance of this complex of physical factors and thus serve the needs of each organ and the animal as a whole.

Part of the problem may be that there are a wide variety of competing demands placed upon an equally diverse number of circulatory control mechanisms and that it is possible to single out only a few of these during any one study. Thus, "metabolic autoregulation of blood flow" explains why in certain tissues local blood flow remains relatively constant at a level appropriate to metabolism even when arterial pressure varies widely above and below normal (Renkin, 1964). The phenomenon of "vasomotion" has already been described as the physical means by which a Starling fluid equilibrium may be maintained at the local organ level. The concept of autoregulation has recently been extended to include "autoregulation of capillary fluid filtration", which attempts to account for the regulation of total plasma volume as well as the maintenance of local fluid homeostasis (Mellander, 1968).

There are no basic conflicts between these various approaches to explain tissue homeostasis, and they may really be manifestations of the same phenomena. Renkin (1966) has suggested that "we may be observing bits and pieces of a complex regulatory mechanism for controlling fluid balance." There is general agreement that the balance between intrinsic and extrinsic control of circulation varies from one vascular bed to another. There is also general acceptance of an intrinsic myogenic activity of the resistance and sphincter sections which provides a basic degree of basal tone and homeostatic control (Bohr, et al, 1971). During periods of circulatory stress, however, local chemical agents, centrally induced vasomotor fiber discharge, or even increased myogenic activity can be potentially powerful modulators of this inherent control.

e. Vasomotor Control of Plasma Volume

The most complete discussion relating these peripheral vascular adjustments to the control of plasma-interstitial fluid balance was given in two recent review articles (Mellander & Johansson, 1968; Mellander, 1968). It is suggested that there are two basically different types of active control:

- 1) Normal fluid partition between intra- and extra-vascular compartments is maintained within each separate organ by local intrinsic adjustments. The mechanism of control varies with the demands of the tissue and is not always well defined, but functionally it consists of

readjustment of the pre/post capillary resistance ratio or of precapillary sphincter tone. This type of control has been given the designation "autoregulation of capillary filtration" and has been previously described.

2) Total plasma volume can be regulated for the entire organism by extrinsic neurogenic vasomotor reactions which induce active redistribution of fluid between the plasma and interstitial compartments. In particular, hypovolemic or hypervolemic states can be corrected by altering the mean hydrostatic pressure in certain tissues. This is accomplished by changes in the pre/post capillary resistance ratio via graded discharge of the vasoconstrictor fibers.

While most if not all tissues maintain local fluid homeostasis by local autoregulation of capillary filtration, not all tissues are capable of incurring large changes of interstitial fluid and hence taking part in the second type of control. In some tissues (intestines, myocardium, brain) large transcapillary fluid movement may impair organ function. These organs appear to have a particularly powerful autoregulatory protective mechanism that tends to maintain the pre/post capillary resistance ratio and hence normal capillary pressure even in the face of high extrinsic vasoconstrictor discharge that might otherwise cause tissue fluid depletion (Oberg, 1964).

Other tissues, in particular skeletal muscle and to some extent cutaneous tissue, appear to be more suited to serve as an extra-vascular fluid reservoir in the regulation of total plasma volume. For example, these tissues are capable of absorbing a certain fraction of plasma filtrate during the periods of high hydrostatic loads that accompany standing. In this case, however, gross edema is prevented by local myogenic autoregulation that primarily reduces filtration area rather than capillary pressure. In other circumstances, such as acute hemorrhage or infusions, the needs of the entire organism require a rapid compensatory reaction to either increase or reduce the blood volume respectively. In these cases, the large variations in vasoconstrictor discharge that often accompany these volemic stresses induce a clear-cut and well maintained change in the pre/post capillary resistance ratio which effects capillary pressure and fluid filtration in the desired direction. Thus the central mechanisms may override or compliment local regulation in muscle and skin in attempting to control changes in total plasma volume.

Skeletal muscle in particular functions well as a target organ for restoring normal plasma volume because due to its large total mass (65% of skeleton and fat-free body weight) it contains a large interstitial fluid volume. In addition, it is not enclosed by rigid structures, as are the brain, myocardium, and kidney, which could otherwise limit filtration because of a rapid rise in tissue pressure. Finally, it does not appear that muscle function is greatly impaired by extensive extravascular fluid changes.

In contrast to the great effort given to studying the effects of blood loss on the peripheral vascular regulatory mechanisms no significant attention has been shown toward investigating blood expansion by infusions, the main subject of interest here.

4. THE INTERSTITIAL FLUID COMPARTMENT

a. Physical State of the Interstitial Space

Histological and chemical studies of the interstitial space indicate that it is normally filled with a gelatinous mass of interstitial fluid entrapped in a fine meshwork of mucopolysaccharides and glucoproteins (i.e. the ground substance) and various types of tissue fibers (Rusznayak, et al, 1967). Tissue fluid, entrapped within the macromolecular ground substance, is generally considered to have extremely low mobility (Guyton, et al, 1966a). Rusznayak agrees, stating that "there exists no free interstitial fluid under normal conditions....The condensed ground substance presumably forms a layer over the surface of the connective tissue fibers". Landis and Pappenheimer (1963) have estimated that the average thickness of the layer of interstitial fluid spread over this extra-vascular surface can be no more than 0.5 microns, making it extremely difficult to obtain tissue fluid samples for analysis. However, this extremely fine layer, combined with the pervasiveness of the microvasculature, makes it extremely likely that equilibration between plasma and tissue fluid can take place rapidly by diffusion alone. Thus there is a fair degree of homogeneity between fluids in these two compartments with regard to the more easily diffusible crystalloid molecules.

Under normal conditions the interstitial fluid is thus "non-motile", that is, it is not free to move by bulk flow under the small hydrostatic

gradients that exist within the tissues. The fluid behaves rather like a film between two glass plates which cannot move freely, being held by tremendous surface tensile forces. On the other hand, this fluid cannot be considered "bound water" in the usual sense, inasmuch as it appears to be readily available to diffusible ions. In other words "the resistance that is offered by the physical structure of the gel is essentially no barrier to diffusion of fluid, even though it is a terrific barrier to (bulk) movement of fluid under the influence of the relatively weak hydrodynamic pressures in the tissue spaces" (Guyton & Coleman, 1968).

This is not to imply that there is a complete absence of bulk movement of fluid within the interstices of the tissues. Inasmuch as the lymphatics are able to carry away the net amount of fluid filtered across the capillaries (approximately 20% of fluid originally filtered is not reabsorbed at the venule capillaries, Landis & Pappenheimer, 1963) implies a small degree of motile fluid. Minute channels of motile fluid have actually been observed in normal tissues (Guyton, et al, 1966a). Furthermore, a recent study by Garlick and Renkin (1970) concludes that both convection and diffusion contribute significantly to the plasma-lymph transport of macromolecules. Bulk movement is especially prevalent in instances when edema is created, where "tremendous quantities of fluid can be moved from one part of the tissue spaces to another with a very slight pressure gradient....essentially all of the increase in fluid is in the motile portion of fluid rather than in the non-motile portion" (Guyton & Coleman, 1968).

b. Tissue Pressure and Compliance

It is a fact that the tissue spaces are not so rigid as to fail to accept a large increase in interstitial fluid, nor so compliant as to accept all fluid entering its interstices without concomittantly experiencing a rise in tissue pressure. An increase in interstitial fluid volume can arise from either of two sources; an increased net capillary filtration or a decreased lymph flow. The accompanying increase in tissue pressure acts in a way that tends to counteract both of these initiating actions, i.e., a passive type of control system. The compliance of the interstitial space (change of volume per unit change of pressure) determines the final extent and rate of interstitial volume change when the balance between inflow and outflow has been upset. These facts are little appreciated, and this

may account for the relatively little attention given toward measuring tissue pressure and, especially, compliance. Gauer, et al (1970) summarize the few studies that have recently emerged and the arguments surrounding them.

Tissue Pressure

Landis and Pappenheimer (1963) have reviewed the various methods employed to measure tissue pressure. They show that using micropipette-tipped manometers, resting values of tissue pressure were obtained in the range of +0.5 to 9.0 mm Hg in a variety of human tissues. Since that time a number of investigators, starting with Guyton (1963), have found tissue pressure to be normally subatmospheric, i.e., negative (e.g., Scholander, 1968; Meyer, et al, 1968, Ladegaard-Pedersen, 1970). These investigators have used several distinctly different techniques including wicks made out of synthetic or natural fibers and chronically implanted perforated hollow capsules. The devices are placed within a given tissue and connected to a pressure transducer via a liquid filled catheter. In a series of papers, Guyton, et al, (1965a, 1965b, 1966a, 1966b), the leading proponents of implanted capsules, explored the effect of a negative interstitial pressure on tissue fluid mobility, fluid movement through the capillary wall, and its ability to control interstitial fluid volume. They found that in many parts of the body the pressure averaged between -5 and -8 mm Hg in the subcutaneous and retroperitoneal tissues and their dependent areas and about -4 mm Hg in muscle. A controversy ensued, continuing up to the present, concerning the existence of a normally pervasive negative tissue pressure and the validity of either the implanted capsule method (Stromberg & Wiederhielm, 1970) or the micropipette manometer methods (Landis & Pappenheimer, 1963). The various arguments are summarized by Gauer, et al (1970), who feel that the matter is far from settled.

Tissue Compliance

From a theoretical viewpoint, the fact that the tissue pressure is negative or positive is not as important as the actual compliance of the interstitial space. If one changes the interstitial fluid volume by any arbitrary method, simultaneously measuring tissue pressure and volume, the resulting pressure-volume graph depicts the equation of state for the

tissue space. The slope of this curve, $\Delta V/\Delta P$, represents the compliance. Wiederhielm (1968) suggests using pressure measurements obtained from micro-needle punctures, which correspond to a much more distensible reservoir than that advocated by Guyton (1965). Guyton's measurements with implanted capsules yielded the information that at rest, normal compliance is 4 ml/kg-mm Hg, about 1/24 that suggested by needle puncture techniques, but after the interstitial fluid volume increases by approximately 15% the compliance quite suddenly increases to the value assumed by Wiederhielm (96 ml/kg-mm Hg). Furthermore, Guyton related this change in compliance to the absolute value of tissue pressure. He demonstrated a very low compliance of the interstitial spaces so long as the tissue pressure was negative, but once the pressure rose above the surrounding atmospheric pressure, the compliance increased many fold. This behavior was said to be analogous to blowing up a previously deflated balloon. Wiederhielm rejects the notion of compliance changes.

Admittedly, Guyton's dual concept of negative tissue pressure and of a volume-dependent compliance is extremely attractive. First, the negative tissue pressure becomes a mechanism to explain how the successive layers of tissue can be normally held so tightly together (muscles, muscle sheaths, tendons, fasciae) especially where there are no fibrous attachments, while in edematous conditions they are found to be separated. Secondly, the low compliance can account for the great margin of safety in the prevention of edema. Unless the capillary filtration pressure increases considerably, it is possible for most increases in filtration to be successfully attenuated by relatively large rises in tissue fluid pressure. Thirdly, it provides a basis for a general theory of the regulation of interstitial fluid volume and an important element in the regulation of extracellular fluid volume. This will be discussed in detail in the following section.

c. Regulation of Interstitial Homeostasis

According to Guyton & Coleman (1968) there are three specific findings that can form the basis of a schema for understanding the regulation of both interstitial fluid volume and pressure. These are: a) the normal interstitial fluid pressure is negative rather than positive, b) there is a very non-linear relationship between interstitial fluid pressure and interstitial fluid volume and c) essentially all of the fluid normally present in the tissue spaces is nonmotile.

If negative tissue pressure is a reality, where is the energy source required to maintain this pressure? If the interstitial fluid is mainly non-motile and bound into the ground substance, what are the forces that keep free fluid from accumulating and maintain this "dry" state of the tissues? The answers to these questions are probably related.

Guyton suggests two factors that could cause a negative tissue pressure. First, lymph flow occurs in all regions of the body, removing free fluid from the tissues and returning it to the circulation. Lymph flow not only removes most of the free fluid but also proteins, and it is solely because of a low capillary permeability to proteins and the existence of lymph flow that a gradient of protein concentration exists across the capillary wall. The resulting trans-capillary osmotic gradient can cause further decreases in the volume of fluid in the tissue spaces. Both of these actions, lymph flow and osmotic gradients, act to "suck the (interstitial) bag dry" of almost all except that amount of fluid that is "held in combination with the tissue gel or held by capillarity in other minute interstices of the tissue spaces".

In other words, the interstitial fluid volume can be said to be passively regulated so that the interstitial spaces are "dry" of motile fluid. And further, "the removal of the motile fluid creates a slight negative pressure in the tissue spaces". Conversely, "as long as there is a negative pressure in the tissue spaces essentially no motile fluid will be present".

On the other hand, any increase in tissue fluid, as occurs during infusion, serves to increase essentially only the motile portion of fluid rather than the nonmotile portion. An approximately 30% increase in interstitial fluid volume will cause the relatively low compliant tissue space to increase its pressure dramatically from about -7 mm Hg to 0 mm Hg (atmospheric pressure). When this point is reached several important events occur:

- 1) the conductance of free tissue fluid increases over 100,000 times that in the resting dry state (Guyton, et al., 1966b);
- 2) there is a dramatic increase in lymph flow of about 15 times normal as measured in the thoracic duct and 30 times normal as measured in hind leg lymph ducts (Gibson & Gaar, 1970).

3) the compliance of the tissue increases about 20 fold as the contacting tissue surfaces are pushed further apart (Guyton, 1965).

Gauer, et al (1970) rightly point out, (and this cannot be emphasized enough), that the important factor is not the absolute tissue pressure but the distensibility or compliance of the extravascular space. For it is this parameter that will determine to a large extent the changes in tissue pressure and lymph flow and finally, the ultimate fluid distribution between plasma and interstitial compartments. These authors seem to intuitively favor the concept of a low tissue compliance. They point out that if Guyton's data is correct, then "for all practical purposes, the compliances of the (venous) vascular bed and the interstitial space are identical"....and that "such an unexpectedly tight coupling of the mechanics of the interstitial space and blood volume comes as a welcome surprise". It appears reasonable that the short-term control of blood volume would be facilitated if the intra- and extra-vascular compartment had a matching compliance. This would allow the blood compartment to transmit its needs regarding fluid excess or deficit directly to the interstitial fluid reservoir by pressure mechanisms alone. Furthermore, it suggests that "the (long-term) regulation of the total extracellular fluid volume is primarily handled through mechanisms located in the circulation (i.e. renal control) regardless of their nature."

d. Interstitial Fluid Volume

The purpose of this section is not to completely review the various methods of measuring interstitial fluid volume (IFV) but to consider the importance of this variable as it pertains to plasma/interstitial fluid exchange. It is important to emphasize that any tracer injected into the blood will distribute itself in the plasma in addition to any other compartment. Hence there is no direct method of measuring interstitial fluid volume. Rather, this value is obtained from the difference between extracellular fluid volume (EFV) and plasma volume.

Much has been written concerning the difficulties in obtaining a meaningful measurement of extracellular volume. Two short but well documented reviews are contained in Pitts(1968) and Elkinton and Danowski (1955). Upon a casual glance at this literature, one is struck with the fact that many tracers have been employed to measure EFV and that each one has a

different apparent volume of distribution. They range from inulin, with a distribution volume of 16% of body weight (BW) to thiocyanate and radiosodium, with a volume as high as 30% BW. This wide disparity is due to the ability of extracellular tracers to diffuse into various extravascular fluid phases in varying degrees depending to a large extent on their electrical charges and molecular weight.

Several of these fluid phases have been identified: 1) the ultrafiltrate of plasma and fluid immediately surrounding the capillary including lymph (12% BW); 2) dense connective tissue and cartilage water (4.5%); 3) bone water (4.5%) and 4) transcellular water (1.5%) (Edelman & Leibman, 1959). All these compartments have been associated with total interstitial water at one time or another. In addition, many tracers that are said to measure extracellular fluid volume also enter the intracellular compartment to a limited but unknown extent (including the thiocyanate, sodium, chloride and bromide ions).

Wiederhielm (1968) has suggested that the protein molecules that diffuse across the capillary wall "are restricted to the free fluid phase in the interstitial space, whereas water (but not protein) freely permeates the meshwork of the ground substance", i.e., the immotile fluid phase. This bound fluid is "not available to the protein; i.e., the plasma protein space is not identical with the total interstitial space". The importance of this suggestion is that since the protein molecules are restricted to a relatively small volume, they will exert a higher osmotic pressure than if the bound fluid were also available for dilution. In practice it is impossible to obtain samples of interstitial fluid, especially of the free fluid phase alone, in order to verify this hypothesis.

Inasmuch as tissue fluid oncotic pressure is an important factor controlling capillary filtration, it appears reasonable, in light of the above, to assume that we are interested in that part of the interstitial fluid phase that is available to plasma proteins, the free fluid phase. In all probability this closely corresponds to the first item in the above list of interstitial phases and also to the volume of distribution of the larger extracellular tracer molecules such as inulin (16% BW), sucrose (20% BW) or mannitol (18%) (which upon subtraction of 4.5% for the plasma volume give values for interstitial volume of 11.5%, 15.5%, and 13.5% of BW respectively. Elkinton & Danowski, 1955). Wiederhielm (1967) suggests

a value of 15% as the IFV value found in most mammals, while Pitts (1963) gives a working figure of 16% BW.

In the present study, the absolute value of IFV is important insofar as it helps determine changes in tissue protein concentration. However, incremental changes in IFV seem to be more critical than absolute values for the determination of changes in lymph flow and tissue pressure.

5. DYNAMICS OF LYMPH FORMATION AND LYMPH FLOW

Lymphatic ducts are found in all parts of the body. While their existence has been known since the discovery of the blood circulatory system, what little knowledge has been established in the field of lymphatic dynamics is mainly due to studies performed in the last decade. Several extensive reviews or monographs have been published (Mayerson, 1963; Yoffey & Courtice, 1956; Rusnyak, Foldi & Szabo, 1967) and in the last several years especially, there have been major symposia in the field of lymphology (Collete, et al, 1967; Mayerson, 1968; Ruttiman, 1967). The importance of the lymph system is not usually appreciated. In addition to its special and vital anti-bacterial functions, the flow of lymph is largely responsible for maintaining the physical integrity of the interstitial fluid space and normal fluid balance between the plasma and interstitium. Nevertheless, still lacking is a clearly defined description of the factors responsible for regulating lymph formation and lymph flow. In particular, we need to understand the effects of infusion on lymph flow. For over a century it has been known that an increase in transcapillary filtration due to an increase in capillary pressure is followed by an increase in lymph flow. But little was known about the intermediate steps or mechanisms that caused this behavior. The discussion below summarizes what is presently known about this important system.

The lymphatic vessels start in the tissue spaces as a system of numerous dead-ended thin walled capillaries (termed "initial lymphatics" by Casley-Smith, 1968) which drain into larger and thicker-walled collecting trunks and ultimately empty into the great veins at the base of the neck (Yoffrey & Courtice, 1956). From the physiological point of view, the lymphatic system is primarily a transport system designed to maintain homeostasis of the interstitial environment by draining tissue fluid back to the blood

stream. In this context it acts like a secondary extravascular circulation, although considerably slower than intravascular movement. Lymph, very similar to interstitial fluid, contains macromolecules (mainly plasma proteins) that are able to permeate even healthy capillary walls, as well as many substances derived from cellular metabolism that are found in tissue fluid. Lymph flow also removes excess fluid which accumulates during periods of high net capillary filtration.

We shall be concerned with two of the major problems in understanding how this system functions: a) lymph formation -- how material enters and remains in the initial lymphatics; and b) lymph flow -- how lymph, once formed, is propelled through the lymphatic system. We will try to review the arguments surrounding these questions only insofar as it concerns bulk movement of fluid. In a later section we will consider the movement and concentration of macromolecules.

Lymph Formation

How does interstitial fluid enter the lymphatic system in the first place? We may liken the lymphatic capillary to a flabby bag with holes in its sides large enough to accommodate the passage of both water and solutes, and with a one-way valve at its opening to the larger lymph ducts. The questions become: a) how does one expand the bag so that it can fill, and b) how does one compress the bag so that its contents are forced through the valve without allowing most of them to regurgitate back into the surrounding fluid? There is general agreement on the forces which control filling and emptying of the initial lymphatics (Yoffey & Courtice, 1956; Casley-Smith, 1967; Rusnyak, et al., 1967).

Under normal conditions, protein leakage across the blood capillary wall and release of large molecules by active tissues tends to increase the osmotic accumulation of interstitial fluid. This additional fluid swells the tissues causing the connective tissue fibers attached to the lymphatic capillaries to pull on their walls and thus distend the vessels. The resulting pressure differential between the connective tissues and the lymphatics is absolutely essential to ensure the bulk flow of fluid into the initial lymphatics. Thus, as the formation of tissue fluid increases, the tissue pressure rises, the lymphatic vessels dilate, and the pressure differential causes them to fill. Just as filling is accomplished by external tensile forces, so the initial lymphatics are emptied by external

compressive forces which squeeze their contents past the first lymphatic valve. This action can be accomplished by muscular activity, although that is not really necessary, as slight increases in the connective tissue pressures, e.g., from arterial pulsations, respiration, etc., may be sufficient to cause this sequence of events (Casley-Smith, 1967). The pores in the initial lymphatic wall (actually intercellular junctions) that allow fluid to pass into the vessels do not ever close completely during the compressive emptying phase, and so there is a certain amount of regurgitation, especially of small molecules, back into the tissues (Casley-Smith, 1968).

Lymph Flow

If the lymph system were simply a system of rigid tubes, we could easily describe lymph flow in terms of the mean pressure drop across the system and the resistance to flow. However, the lymphatic system is much more complicated. It is composed of a network of elastic tubes of ever widening diameters, containing numerous valve flaps along its length that allow fluid to flow in one direction only. Although an adequate quantitative description of the fluid dynamics in this system has not yet been formulated, we might presume a number of factors that would influence lymph flow: the absolute pressure and pressure variations at either end of the lymph system (i.e. tissue pressure and central venous pressure); elasticity of vessel walls; and the resistance of lymph vessels and valves. Since the lymph ducts are very thin walled, they are affected by external pressures from such mechanical sources as respiratory movement, arterial pulsing, cardiac activity, peristaltic movement and skeletal muscle contraction. All of these factors have been shown to directly effect the flow of lymph (Rusnyak, et al, 1967). In addition much evidence has been accumulating, strongly suggesting an active component to lymph flow; that is, the walls of the larger lymphatic tubes contain contractile elements which, by independent contraction and relaxation, act to propel lymph fluid. Any rhythmic change in tone in the lymphatic wall taking place between two one-way valves would in reality cause a pump-like action; and if such tonal changes were synchronous along the length of the duct the resulting peristaltic wave would be a powerful force for lymph flow. There have been relatively few well documented cases of this type of rhythmic pulsation in man and animals (Hall, 1969; Ruttiman, 1967; Rusnyak, et al, 1967), but its existence is fairly well

accepted. There is less agreement as to the relative contribution of these active and mechanically passive modes to total lymph flow. Moreover, the stimulus and controlling mechanisms for active propulsion are yet to be uncovered.

Effect of Pressure on Lymph Flow

There is little quantitative data available relating the flow of lymph to any of the factors, active or passive, which have been shown to affect it. One approach which has been reported by several authors is to assume that the flow of lymph, as is true of any fluid in a tube, is possible only from a region of higher pressure to a region of lower pressure. Thus factors such as cardiovascular pulsations, muscular or peristaltic movements, or increased capillary pressure increase lymph flow only to the extent that they increase distal lymphatic pressure. The fluid which has gained access to the lymph capillaries produces a pressure which may well ensure a uniform lymph flow and the drainage of the lymphatics (Rusnyak, et al, 1967). It would appear logical, therefore, to attempt to correlate lymph flow with a pressure close to the point of origin of lymph, for example, the pressure within lymphatic capillaries or, more practically, tissue pressure.

Guyton & Coleman (1967, 1968) have presented a model of lymph flow based partly on experimental and partly on indirect evidence. They suggest that lymph flow is primarily a function of interstitial tissue pressure. However, their model requires that normal tissue pressure is negative. Since it is inconceivable that a negative pressure can maintain lymph flow against a positive venous pressure (without an external source of energy), their model includes a "lymphatic pump", which refers to the movement or pulsations in the tissue (resulting from such factors as arterial pulsation). Thus, the tissue pulse pressure is added to interstitial fluid pressure, the net result being periodic pulses of positive tissue pressure which push fluid into the lymphatics. The presence of valves prevents fluid from flowing backwards and maintains distal lymphatic pressure at atmospheric or above. Using an electrical analogy, this description of lymph flow past valves and driven by a pulsating pressure is similar to removing the negative portion of an a-c signal using a half-wave rectifier. This view is shared by several other workers (Szabo, 1967, DeLangen, 1961).

The concept of pulsating flow is theoretically attractive, especially when dealing with one-way valved flow in a low pressure system. Flow through

valved tubes would be enhanced by pulsations especially if the mean peripheral tissue pressure were below the pressure necessary to open the valves. For this reason it should be possible to correlate lymph flow with those factors that regulate the magnitude of the pulse: respiration rate and depth, heart rate, pulse pressure, skeletal muscle contraction, etc. There is little quantitative data of this nature available.

As attractive as the lymphatic pump hypothesis is, it is not easily amenable to quantification, inasmuch as transducers are not yet available that can actually record these fine and rapid tissue movements. To circumvent this difficulty several investigators have attempted to correlate mean tissue pressure with lymph flow (Guyton & Coleman, 1968; Gibson & Gaar, 1970). Using data obtained from saline infusion experiments, they determined that a sigmoidal relationship exists between these variables. That is, lymph flow increases almost linearly with tissue pressure for moderate infusions, but as tissue pressure (which is normally negative in their experiments) approaches atmospheric, lymph flow increases dramatically. For massive infusions, when tissue pressure rises above atmospheric, lymph flow reaches a maximum value. The upper limit is thought to be due to the high tissue pressure exerting itself on the walls of the lymphatics and restricting its flow. For flow from the thoracic duct, the maximum lymph flow rate is said to be 15 times normal flow while lymph flow from the hind limbs can achieve values as high as 30 times resting flow.

It is not easy to visualize how a chronic rise in systemic venous pressure, such as would occur during venous congestion or rapid infusions, would effect lymph flow. On the one hand, this would lead to a higher capillary filtration rate, increased tissue pressure and hence greater lymph formation. On the other hand, a greater resistance to lymph flow due to higher outflow pressure near the region of the thoracic duct. A study by Szabo (1967) indicates that increased venous pressure is indeed accompanied by an increase in lymph flow in spite of the added resistance. However, he also showed that during venous congestion the increases in flow observed in an open cannulated thoracic duct is several times as high as would occur in the intact animal whose thoracic duct remains connected with the cervical veins. Hence, the results of almost all previous investigations in which lymph flow was measured from open lymph ducts during high central venous pressures may be questioned (Foldi, 1967). Observed increases in flow in such situations are probably overestimated

compared to their values in the intact animal.

There has been much data collected regarding lymph flow in the normal animal. Mayerson (1963) states that in different mammals thoracic duct lymph flow is relatively constant at 2 ml/hr per kg body weight. Rusnyak, et al (1967) show that about half of the total lymph flow originates from the kidneys and most of the remaining half is from the liver, intestinal tract, and hind legs. Their data suggest that the thoracic duct does not carry all the lymph introduced into the peripheral lymphatics. They ascribe the difference to a) additional pathways for lymph entry into the venous system other than the thoracic duct, and b) more importantly, to lymph fluid absorption from the lymph nodes and ducts into either the tissue spaces or directly into blood capillaries. However, it is not known for certain how much fluid, if any, is really lost from the lymph ducts themselves once the fluid has entered the lymphatic system (Mayerson, 1963).

To summarize: lymph formation is determined not only by the presence of mobile tissue fluid, but by such external forces as muscular contraction, respiratory or peristaltic movements, and cardiovascular pulsations which create a periodic pressure differential between tissue and lymph spaces. Lymph flow is maintained by these same extrinsic forces as well as intrinsic contractile properties of the larger lymph vessel walls, intermittantly raising the pressure in the more distal parts of the lymphatic system. Valves are so arranged as to prevent a backflow of lymph. Little is quantitatively known about the effects of infusion on the mechanisms affecting lymph flow, although there is some empirical data relating infusion volume and tissue pressure to lymph flow rate.

6. PROTEIN CONCENTRATIONS IN THE PLASMA, INTERSTITIAL FLUID AND LYMPH

The term plasma proteins refers not only to the abundance of proteins dissolved in the circulating plasma (viz., albumin, γ -globulin, iron-binding globulin, β -lipoproteins and fibrinogen) but to the entire pool of proteins dissolved in all the extracellular fluids; i.e., the plasma, interstitial and lymph fluids. In fact, the primary distinguishing factor between these different extracellular fluids is their protein concentration. The chief osmotically active protein in normal mammalian plasma is albumin representing about 50% of total protein, but because of its relatively

small size (MW = 70,000) compared to other proteins (as high as 1,000,000 MW) albumin exerts a proportionately larger percentage of the total oncotic pressure (up to 75%). Roughly half of the total body pool of the plasma proteins is normally present in the plasma and the remainder is located extra-vascularly. (It is now known how this extravascular pool is distributed between lymph and interstitial fluid). As emphasized by Gitlin (1957) the large quantity of plasma protein is not a stagnant pool but is continually being exchanged between the three fluid pools via lymph flow and transcapillary exchange. Thus there is a dynamic equilibrium of proteins, from the vascular system to the interstitium, thru the lymph system, and back to the vascular system.

The relative concentrations of protein in plasma, tissue fluid, and lymph at steady state depend primarily upon two factors: 1) the effective permeability of the blood capillary and lymph capillary membranes to proteins, and 2) the mean rate of lymph flow. (Rates of protein synthesis, release and catabolism would affect the equilibrium but are thought to be relatively slow and thus will not be considered). The capillary filtrate contains proteins in lower concentration than is found in the plasma since the capillary membrane restricts the passage of protein to a much greater extent than water. While water and salts are reabsorbed by bulk flow and by diffusion back into the circulation, proteins are not reabsorbed to any significant extent. Instead proteins passing through the capillary mix with existing tissue fluid and pass into the lymph capillaries.

One theory postulates that proteins are absorbed by lymphatics in much the same concentration as in the tissue fluid while another hypothesis states that there is some concentrating mechanism within the initial lymphatic capillaries and/or the remainder of the lymph system.

Disagreement centers basically on the permeability properties of the lymphatic membrane walls. If, as Yoffey and Courtice (1956) suggest, the walls of the initial lymph capillaries contain pores that are freely permeable to both large and small molecules, this would imply that: a) tissue fluid enters the lymph capillaries by bulk flow without change in composition, b) when extra-lymphatic pressure compresses the vessel there is some flow of lymph past the first valve but a considerable leakage of fluid back into the tissue space and c) if there is no further modification of lymph fluid on its journey through the larger lymph trunks, than lymph emerging from the thoracic duct has the identical composition as the tissue

fluid from which it was derived. Among the proponents of this theory are Drinker and Field (1931), Gitlin (1957), and Courtice (1968). This description is theoretically capable of accounting for lymph flow, but clearly the behavior of the initial lymphatics is that of a "relatively inept...inefficient...leaky pump system" (Mayerson, 1963).

A more recent theory based on electron micrographic evidence leads to quite different conclusions. Casley-Smith (1967) proposed that intercellular junctions in the initial lymphatic walls are capable of allowing relatively free passage of all molecules during the filling phase just as in the previous theory. However, during lymphatic compression, intralymphatic pressure forces fluid past the first lymph valve, and simultaneously the tissues press on the lymphatic capillary walls, pulling the endothelial cells over each other and causing the junctions to seal. This minimizes leakage back into the tissue fluid and hence, the terminal lymphatics may be said to function as "efficient force pumps", with both intercellular junction inlet valves and intralymphatic outlet-valves. However, although it appears that there is little regurgitation of the contents of the vessels, Casley-Smith (1968) does propose that the junctions do not ever seal completely during the emptying phase, and that smaller molecules (water, salts) can leak out to some extent, thus concentrating the lymph fluid with regard to large protein molecules. This model, suggesting that lymph fluid has a higher protein concentration than the tissue fluid from which it was derived is compatible with the conclusions of Rusnyak, et al (1967) and Landis and Pappenheimer (1963), who hold the view that lymph and tissue fluid can no longer be considered identical. The hypothesis is difficult to test directly because it is nearly impossible to collect true tissue fluid from mammals under normal conditions.

It appears, then, that present evidence does not allow us to make quantitative conclusions regarding the relationship between lymph and tissue fluid, or, concerning the protein concentration mechanism, if any, within the lymph system. Even without admitting to the identity of the two fluids, many authors agree that changes in protein concentration of extracellular fluid and lymph show a parallel trend. In every study in which some kind of relationship between the two fluids is required, the investigators invariably assume that the protein concentration of lymph fluid and tissue fluid is identical (Johnson, 1966; Guyton & Coleman, 1967; Boyd, et al, 1968; Wiederhielm, 1968; and Garlick & Renkin, 1971).

Representative values for the protein concentration in plasma, interstitial and lymph fluids of the dog are shown in Table I. Values for the plasma and lymph are the most reliable inasmuch as these fluids are well defined and easily obtainable. But as already explained, samples of tissue fluid are extremely difficult to obtain and the values shown are the only ones known to this author representing direct measurements in the dog. In this case the tissue fluid was obtained by allowing it to collect in subcutaneously implanted perforated capsules over a period of weeks and removing small samples with a hypodermic syringe. It should be noted that Prather's data for the muscle and skin tissue fluid is slightly higher than that reported for the leg lymph, which is opposite to what might be expected if we assumed there was a protein concentrating mechanism in the lymph system. Note also that the lymph from the thoracic duct is more representative of liver and intestinal lymph than that from the leg.

TABLE I

Typical Values of Protein Concentration in Plasma, Tissue,
and Lymph Fluid of the Dog

	No. of Animals	Conc. (gm%)	COP (mm Hg)*	Reference
Plasma	20	5.9	19.7	Prather (1968)
	88	6.3	21.5	Rusnyak (1967)
Tissue Fluid (Muscle & Skin)	20	2.5	6.5	Prather (1968)
Lymph:				
thoracic duct	65	4.1	11.5	Rusnyak (1967)
liver	30	5.0	15.5	"
intestine	32	2.8	7.5	"
leg	20	1.8	4.4	"

*COP values determined from protein concentration and Equation (3.5)
as given by Landis & Pappenheimer (1963).

It appears that lymph originating from different areas may contain different amounts of protein depending on the protein permeability of the capillary membrane in that area. The splanchnic organs, for example, are known to have a much higher permeability than skin or muscle (Mayerson,

Wolfram & Shirley, 1960). Lymph from the thoracic duct represents an average concentration of lymph from all parts of the body -- the actual concentration depends on both the concentration of lymph from each tissue and the flow rate of lymph from that tissue. Inasmuch as resting thoracic lymph is derived mainly from the splanchnic organs in the nephrectomized dog, it would be expected that the protein concentration of thoracic lymph would be more representative of the lymph of these organs.

It is interesting to compare some of these values with those obtained either by indirect methods or with those of other species. For example, human plasma is usually found to have a higher protein concentration, about 7 gm% (Landis & Pappenheimer, 1963), and rabbit plasma lower, about 5.3 gm% (Haljame & Freden, 1970; Zweifach & Intaglietta, 1968) than that for the dog. This would mean that if everything else were equal, the capillary pressure in man would have to be 5-7 mm Hg higher than in the dog to maintain homeostatic control of plasma volume. Although almost all mammalian species have plasma COP values in the range 15 - 25 mm Hg, a difference of a few mm Hg in determining the true value is crucial in calculating filtration rates when one considers that the net Starling driving force for net filtration is normally less than 1 mm Hg. Another of the few studies reporting direct tissue fluid sampling concludes that the protein concentration of rabbit subcutaneous tissue fluid has a value of 2.0 - 2.5 gm% (5 - 6.5 mm Hg) or about 1/3 that of rabbit plasma. Using this ratio for the dog, the comparable value of tissue fluid in that animal should be about 2.0 gm% or slightly below that reported by Prather (Table I). If one accepts the identity of lymph and tissue fluid, then these results are in agreement with those of Garlick and Renkin (1970) who observed the ratio of lymph/plasma albumin concentration to be approximately 0.31 in the hind limbs of dogs. Landis and Pappenheimer (1963) summarized the data for indirect measurements of tissue fluid protein concentration based on lymph studies during normal and pathogenic situations and a variety of assumptions. They conclude that the concentration of interstitial fluid may be considered to vary from 0.7 to 2.1 gm% (1.5 - 5 mm Hg) for the hind limb, while lymph in the same resting extremity has values of 1.3 - 3.3 gm% (3.0 - 9.0 mm Hg).

More important to the present study are average values of interstitial fluid for the whole body, which have been estimated by both Wiederhielm (1967) and Landis and Pappenheimer (1963). Using their assumptions that

total plasma proteins are about evenly distributed between plasma and interstitial compartments and that interstitial fluid volume averages from 3.0 - 3.6 times the plasma volume, it is possible to show that the total average protein concentration for the tissue fluid of the dog is between 1.7 and 2.0 gm% (4.2 - 5.0 mm Hg), which is close to that reported for hind limbs. However, in later reports Wiederhielm (1968, 1971) suggested that the actual effective tissue oncotic pressure is much higher than this, perhaps between 8 - 10 mm Hg (which means a protein concentration just outside the capillary of 3.0 - 3.5%). These values were obtained by assuming a) that tissue fluid available for protein dilution is much lower than the total interstitial fluid, and b) that other osmotically active species besides proteins are present in the interstitium, viz., mucopolysaccharides. It appears that accurate values of tissue fluid colloidal osmotic pressure must await further study.

7. TRANSCAPILLARY PROTEIN TRANSPORT MECHANISMS

At the time that Starling (1896) proposed his concept of transcapillary filtration it was known that the capillary wall must be somewhat permeable to proteins and that the permeability properties were different in various regions of the vascular bed. Three-quarters of a century later we still do not know the precise mechanisms responsible for plasma/interstitial protein transport.

The proteins dissolved in the extracellular fluids can be grouped into three broad categories according to molecular size: 1) albumin, MW = 69,000, $a = 110 \text{ \AA}$; 2) γ -globulins, MW = 150,000, $a = 55 \text{ \AA}$; 3) lipoproteins, macroglobulins and fibrinogens, MW = 350,000 to 10^6 , $a = 110 \text{ \AA}$ (where MW refers to molecular weight and a refers to molecular radius, Landis & Pappenheimer, 1963; Boyd, et al, 1969). Albumin, which contributes the greatest number of particles per unit weight, usually is found to exert between 50 - 80% of the total plasma COP, depending on the species. However, since all protein fractions are found in both capillary and pericapillary fluid, it is important that any theory must account for the transcapillary exchange of both low and high molecular weight fractions.

Three fundamentally distinct anatomical pathways have been suggested through which molecules as large or larger than albumin might cross the capillary wall: 1) pores smaller than $40 \text{ \AA}$ radius, 2) larger pores or

leaks between 250 - 800 Å radius, and 3) pinocytotic vesicles that move inward and outward across the capillary endothelial cells (Renkin, 1966; Garlick & Renkin, 1970; Landis & Pappenheimer, 1963). Macromolecular transport through pores can be accounted for by several mechanisms depending on the relative size of pores and protein molecules, and the presence or lack of a significant fluid filtration gradient. These mechanisms can be listed as: unrestricted diffusion, restricted diffusion, hydrodynamic filtration (bulk flow movement of solutes and solvent down a pressure gradient through pores large enough so as not to selectively restrict any solutes), and molecular sieving (a combination of restricted diffusion and restricted filtration, i.e., filtration through pores small enough so that some solute molecules are partially restricted). Of these, it appears that restricted diffusion is the simplest theory that can adequately account for the transport through small pores of molecules no larger than albumin (Renkin, 1966, 1968; Garlick & Renkin, 1970). For molecules larger than albumin, there is general but not universal agreement that transport occurs by bulk flow filtration or unrestricted diffusion through large pores, presumably located in the venous end of the capillaries (Mayerson, et al, 1960; Grotte, 1956; Landis & Pappenheimer, 1963; Renkin, 1966, 1968; Garlick & Renkin, 1970; Boyd, et al, 1969; and Wiederhielm, 1968). An alternative hypothesis to the large pore theory is vesicular transport, first proposed by Mayerson, et al (1960) and considered more seriously by Renkin (1964) and Garlick and Renkin (1970). In vesicular transport material is transported by an active process from one surface of an endothelial cell to the other in minute vesicles. These vesicles have been identified in electron micrographs, but their role in protein transport remains to be established.

Irregardless of which mechanisms are at work, certain experimental data regarding macromolecular transport have been obtained and must be explained. Renkin (1966, 1968) has analyzed results of several studies in which the steady state lymph/plasma concentration ratios of dextran were measured subsequent to injecting graded sizes of this macromolecule into the blood (Grotte, 1956; Mayerson, et al, 1960). He established that capillary permeability to dextran falls sharply between 1,000 and 20,000 MW ($\alpha = 21\text{Å}$ to 32Å) for capillaries of the leg and cervical regions, these values being somewhat higher for the capillaries of the

intestine and liver. Above this range of sharp restriction, molecular transport still takes place, but at a greatly diminished rate, and little or no further restriction is encountered at least up to 400,000 MW. More recent studies confirm these observations (Boyd, et al, 1969; Garlick & Renkin, 1970).

Renkin interprets this data to mean that the lower molecular weight dextran passes through a small pore system (25 - 40 Å radius) under a diffusion mechanism that restricts transport tremendously as the test molecules approach the size of the pores. However, the larger molecules (greater than 50 Å radius), whose passage does not appear to be restricted with increasing molecular weight, are hypothesized to cross the capillary membrane either: 1) through a large pore system by unrestricted diffusion or bulk flow, or 2) by vesicular transport. The more recent study of Garlick and Renkin (1970), while extending the theoretical treatment of vesicular transport, fails to reach any new conclusions regarding the nature of the large molecule transport system.

One unfortunate aspect of Renkin's earlier analysis was that he correlated permeabilities of dextran based on molecular weight rather than molecular size. It appeared to be implicitly assumed that dextran of 70,000 MW would have a similar permeability as albumin (MW = 69,000). This is untrue, for Garlick and Renkin clearly show that the capillary permeability of albumin is more closely related to dextran of 20,000 MW rather than 70,000 MW. The reason for this seems to be that dextran, lacking the electrical charges of protein, is a less compact, looser and therefore larger molecule. For example, the molecular radius' of albumin and dextran (70,000 MW) are 35.5 Å and 60 Å respectively, while dextran of 20,000 MW has a radius of 32 Å.

Thus, the available evidence seems to suggest that in order to account for the entire range of molecular sizes known to cross the capillary wall, several different mechanisms or pathways are probably operating simultaneously: small pores, large pores, and vesicles. For example, a macromolecule much larger than 40 Å can probably cross the capillary membrane only through the large pores or vesicles, while a small molecule such as albumin is able to utilize all three pathways. Clearly, if the large pores were very numerous, albumin could be transported almost exclusively and very rapidly through that route, the path of least resistance. But this would cause albumin concentrations in tissue fluid and plasma to be nearly identical, contrary to experimental evidence. Because of

considerations such as these, it was recognized rather early that if large pores did exist they would have to comprise less than 1% of the total small pore area (Grotte, 1956; Landis & Pappenheimer, 1963; Renkin, 1964). Using these assumptions, Garlick and Renkin concluded that albumin transport is approximately evenly divided between the large and small molecular pathways. They also interpreted their results as showing that large pores alone are incapable of accounting for all observed transport of the larger molecules (between 40 and 130 Å radius). They suggest vesicular transport as an attractive alternative mechanism.

To summarize: separate transport systems appear to exist for small and large molecules. The small system consists of pores approximately 25 - 40 Å radius, permitting molecules not much larger than albumin (or dextran of less than 20,000 MW) to pass by a mechanism that can be described as restricted diffusion. The large molecular transport system is not well understood. It can be accounted for either by 1) a small number of very large pores (perhaps as high as 800 Å radius) through which most molecules pass unrestricted in bulk flow or diffusion, or 2) an active transport system of cytoplasmic vesicles.

B. QUANTITATIVE MODELS OF EXTRACELLULAR FLUID REGULATION AND DISTRIBUTION

The fluids of the body are often thought of as being divided into distinct compartments or phases, each with different compositions separated by membranes that may or may not form a contiguous surface. Starling's description (1909) of the extracellular fluid is still valid. He described this large fluid compartment as being comprised of three smaller fluid phases, namely:

- "1) The circulating blood, contained in a closed system of tubes and everywhere separated from the tissues by a layer of (capillary) endothelium,
- 2) The lymph, also contained in a closed system of endothelial tubes connected at one or more points with the blood vascular system,
- 3) The tissue fluid, filling all the spaces of the body and in immediate contact with the tissue cells."

If one were to compare the compositions of these three fluids with regard to organic and inorganic substances, it would be found that, except

for the protein concentration, all three fluids are nearly identical (Selkert, 1963; Rusnyak, et al, 1967). Thus a model of the extracellular fluid could be represented by three circularly connected compartments containing the plasma, tissue and lymph fluids respectively and separated by membranes whose effective permeability to all substances but protein is the same.

A model such as this has been the basis of interpreting most of the observations concerned with body fluids and their regulation. Although the body of pertinent literature is large, the thrust of the conclusions reached is descriptive rather than predictive. It is rare when we are provided with an analytical working tool, that is a model, capable of simulating observed results accurately and predicting new events, particularly for the complete system. We would like to review here those few studies that deal with the quantitative aspects of the entire extracellular system. We are primarily interested in "whole-body" models that deal with the distribution of fluid between the interstitial fluid space and plasma volume.

One important category of models describes the movement of protein through the extracellular fluids, both in the steady and non-steady state (Areskog, et al, 1964; Grotte, 1956; Landis & Pappenheimer, 1963; Wasserman & Mayerson, 1956; Johnson, 1966; Boyd, et al, 1969; Renkin, 1966; Garlick & Renkin, 1970). Typically these are two compartment models of the plasma and tissue fluid in which lymph is assumed to be identical in composition with interstitial fluid. They have been helpful in explaining the disappearance of tracer macromolecules that were injected into the circulation, and in providing values for the effective permeability of protein across the capillary wall. Inasmuch as protein osmotic forces play a crucial role in the movement of fluids between compartments, these studies were invaluable in providing a description of how proteins are distributed in extracellular fluids. However, in all cases the volume of fluid comprising each compartment is assumed to be constant.

Theoretically, the treatment of a time-varying fluid volume due to osmotic forces leads to non-linear differential equations whose analytical solutions are difficult to obtain. The quantitative studies of Jacobs (1934), who dealt effectively with non-steady state osmotic fluid fluxes across erythrocyte membranes, demonstrate that the appropriate mathematical tools have been available for a long time. However, it was only with the

advent of analog and digital computers and their use by biologists that non-steady state behavior of compartmental volumes was studied seriously, and it has been only in the last five years that these techniques were applied to problems of extracellular fluid distribution.

One of the first attempts to deal quantitatively with overall body fluid regulation and composition was reported in the companion papers by Bradham, et al, (1964) and DeLand and Bradham (1966). They were able to compute fluid and electrolyte distributions in four well defined body compartments (red cell, plasma, interstitial and cellular fluids) from a model which accounted for the chemical mass action laws and electrochemical forces. Hundreds of chemical equations had to be solved simultaneously. In particular, they were considering a thermodynamic concept of the body rather than a kinetic or mechanistic model; one which could only deal with equilibrium conditions rather than being capable of simulating time transients as well. Other limitations of this model were that all membranes were considered to be impermeable to proteins, lymph flow was not considered explicitly, pressures were presumed constant, there was no compensatory chemical or neural regulation and renal function was not considered. They attempted to validate their model with experimental data obtained from infusion experiments in nephrectomized dogs, the same procedure used in the present study. But as already implied, they were interested only in steady state conditions.

The next two major contributions, chronologically speaking, were reported under a single cover (Reeve & Guyton, 1967). In the first of these, Beneken and DeWit were concerned primarily with a mathematical description of the human circulatory system in order to determine pressure, volume and flow characteristics as they affect circulatory regulation. The scope of their study was confined to the plasma compartment only. However, they did consider several important control elements that influence the dynamic response to infusions: the transcapillary fluid shift mechanism, stress relaxation and central control of peripheral resistance. While they do not consider body fluid distribution, their estimation of time constants for certain cardiovascular controlling systems will be valuable in interpreting our own experimental results.

The second and most important of the above mentioned papers dealt more explicitly with the details of plasma/interstitial fluid dynamics (Guyton & Coleman, 1967). It was the first time that a systems analysis

was made that included most of the elements concerned with both fluid and protein exchange: the regulation of interstitial volume and pressure, the dynamics of lymphatic flow, the regulation of tissue colloidal osmotic pressure and the regulation of capillary pressure. The authors were able to show that when the mechanical aspects of circulatory function (i.e. pressure-flow-volume relationships) are combined with factors influencing extracellular fluid partitioning and regulation (i.e. fluid and protein transport mechanisms, renal function), the result is 1) a "far more powerful feedback regulatory system than the combined effects of the autonomic reflexes, the hormonal regulatory systems and other factors usually invoked to explain circulatory regulation", 2) an extremely stable total system, and 3) an extremely difficult system to investigate. The study is most valuable in providing an appreciation of how simple physiological processes can interact to produce unsuspected powerful regulatory systems. Analog computer solutions were obtained to show the effects of changing capillary pressure, plasma colloid osmotic pressure, lymphatic flow and cardiac output. Emphasis was placed, however, on long term (days and weeks) and steady state effects and there was only one serious attempt to relate their simulated results to experimental observations, those made during the onset of experimental hypertension. Furthermore, although there is an attempt to account for the action of autonomic reflexes and autoregulatory effects, this portion of the model is really unsuitable for studies of short term behavior. Gauer, et al, (1970) have reviewed parts of this study, tending to agree with the concepts of interstitial fluid regulation but taking issue with the assumptions concerning renal function. Nevertheless, this report along with several more recent papers (Guyton & Coleman, 1968, 1969) remains as the best overall quantitative description of long-term regulation of the circulation that is available.

Another important systems analysis of the dynamics of transcapillary fluid exchange was given by Wiederhielm (1968), who was not concerned with the mechanics of circulatory function. In fact, his simulation may even be considered to be a one-compartment model, that of the interstitial fluid volume, in which there is exchange with the plasma volume via lymphatics and capillary exchange. But the circulatory parameters such as blood pressure, plasma colloidal osmotic pressure and plasma volume that are assumed to be dependent variables in Guyton & Coleman's model are

considered by Wiederhielm as independent variables, i.e. forcing functions. The plasma volume, in fact, never appears explicitly in his formulation. Nevertheless, by obtaining steady state analog computer solutions he was able to simulate the qualitative responses to perturbations that cause lymphedema, inflammation, and hypoproteinemia. Like Guyton's model he successfully demonstrated the self-regulating factors that protect the body from edema. A unique feature of Wiederhielm's model, however, is the major effort made to deal with a more realistic model of the capillary fluid shift mechanism. Guyton implicitly assumed a uniform cylindrical capillary with homogeneous permeability characteristics and an average capillary pressure, constant throughout the length of the tube. Wiederhielm's model, however, was more in keeping with the Starling-Landis concept of a capillary that filters fluid at the arterial end and reabsorbs at the venous end. Using recent experimental observations he was able to update the traditional model by allowing for a higher fluid permeability and higher effective surface area at the downstream end of the capillaries. By dividing the capillary into two consecutive sections, one for filtration and one for absorption, he was able to demonstrate mathematically that capillary filtration rates are effected more by changes in venous pressure than by changes in arterial pressure, a finding observed in the laboratory (e.g. Pappenheimer & Soto-Rivera, 1948). In addition, there were two other fundamental differences between this and previous models. First, Wiederhielm assumed that proteins leave the vascular circulation not by diffusion as is commonly assumed but by bulk flow leakage of plasma through a large pore system in venous capillaries. This means that instead of exchange down a protein concentration gradient, the driving force is a hydrostatic pressure gradient between venous and interstitial pressures. Second, Wiederhielm assumed a tissue compliance about 20 times greater than that of Guyton, rejected the later author's assumption of a non-linear compliance, and furthermore regarded Guyton's findings of a normal negative tissue pressure as being questionable. Although he was unable to disprove Guyton's assumptions, he was able to show that the body's protective mechanisms against edema are not dependent on a non-linear tissue compliance but could also be explained by a combination of other factors. Still unresolved by these studies are the questions of 1) whether a two segmented capillary model, although more realistic, produces significantly more accurate simulation results than the uniform capillary model, and 2) whether a protein diffusion model is more accurate than a bulk flow model.

To make this review complete we should mention a very recent study that purports to describe "the dynamic exchanges of water and solute between the body fluids" and has been used to "predict the changes in water distribution due to rapid infusion of hyperosmotic, diffusable solutes in neprectomized dogs" (Wolf & McNamee, 1971). Unfortunately, only a brief abstract is currently available.

In summary, of the four quantitative studies reviewed here regarding distribution of plasma/interstitial fluid, only one considers the problem of disappearance of infused fluids, but the model is only suitable for the steady state (DeLand & Bradham). A second did not actually consider events within the tissue compartment, being concerned basically with circulatory function (Beneken & DeWit). Two most important reports (Guyton & Coleman; Wiederhielm) present a clear mathematical interpretation and summary of certain well accepted physiological principles, although in certain cases the underlying mechanisms are not precisely understood, particularly with regard to protein transport, tissue compliance and lymph dynamics. While neither of them were concerned with short term transient behavior, nor with studying distribution of infused fluids, their models are presented as a closed system of differential equations which can be appropriately modified for this use. These studies can be considered first generation models with emphasis on trends and qualitative comparison with experimental results. There was no attempt to utilize simulation-optimization techniques that are capable of estimating parameters and offer the potential of distinguishing between alternate models.

C. EFFECTS OF INFUSION ON PLASMA-INTERSTITIAL FLUID DISTRIBUTION

1. INTRODUCTION

The object of this section is to review a cross-section of the many studies performed on man and other animals in which intravenous infusion was the primary experimental stress. Most of the studies cited have been found useful in designing our experiments and interpreting the results.

Relatively few infusion studies were designed to study plasma-interstitial fluid distribution. The vast majority of them were primarily aimed at examining other physiological phenomena such as renal function,

(Zuidema, 1956; Strauss, 1957), treatment of hemorrhagic shock (Moore, 1966; Smith, et al, 1965), plasma protein transport (Wasserman & Mayerson, 1952; Mayerson, et al, 1960), lymph dynamics (Starling, 1909; Gibson & Gaar, 1970), tissue compliance (Guyton, 1965), circulatory pressure-flow regulation (Conway, 1966; Prather, 1969; Guyton, et al, 1958), and isotope tracer distribution (Albert, et al, 1957; Reeve, et al, 1953; McNeill, et al, 1963). In addition, there is a large body of literature, clinically oriented, that is concerned with evaluating the ability of certain plasma substitutes to maintain an adequate circulating plasma volume (e.g., Takaori, et al, 1970; Wasserman & Mayerson, 1954; Davies, et al, 1963).

We have limited our search to studies involving physiological saline or dextran infusions reported within the last 20 years because it is only recently that isotope tracers, radioactivity detectors and electronic transducers have become widely available to obtain accurate continuous recordings of fluid volumes and blood pressures. Even so, reports of dynamic data of this type are rare. And in those cases where this data are presented, the conclusions may be found to conflict with other reports, due, for example, to incompatibility of homologous blood and plasma infusions (Remington & Baker, 1959) or to non-uniformity in the dextran fractions and their concentrations (Wasserman & Mayerson, 1954).

The areas most relevant to the present study are a) the direct effects of infusion on blood volume expansion and b) secondary effects of infusion on circulatory function, protein transport and lymph function.

2. EFFECTS OF INFUSION ON BLOOD VOLUME EXPANSION

In general, the persistence of a plasma substitute in the circulation depends primarily upon molecular size, the quantity administered, resistance to enzymatic action, and the presence or absence of hemorrhage (Takaori, et al, 1970). Absence of kidney function will improve the apparent effectiveness of a particular plasma expander as will the presence of macromolecules in the infusion fluid. While the plasma volume may return to normal anywhere between one hour (for saline) to 24 hours (for dextran) after an infusion in a normal, intact animal (Wasserman & Mayerson, 1952; Guyton, 1963), an arenal animal will achieve a quasi-steady state in several hours (DeLand & Bradham, 1966), at which time the plasma volume will be expanded above control levels. Inasmuch as the disappearance of any infused solution

is known to be greatly influenced by the rise in capillary filtration pressure, it is surprising that there has been virtually no attempt to quantitatively correlate these variables.

It has long been recognized that infusion of an isotonic salt solution such as physiological saline or Tyrode's solution expands the extracellular compartment without mobilizing intracellular fluid (Pitts, 1968; Moore, et al, 1966). However, an often quoted corollary is that while the infusion produces a greater absolute increment in interstitial fluid than in plasma volume, both compartments increase proportionately (Bradham, et al, 1964; Strauss, 1957). This is not necessarily true, for as Strauss points out, the actual distribution between plasma and interstitial fluid is determined by a variety of transcapillary (Starling) forces and cannot readily be predicted. In fact, the data of Bradham, et al (1964) and McNeill, et al (1963) both show disproportionate amounts of infused normal saline remaining in the plasma of nephrectomized dogs.

The large diuresis that accompanies saline infusions (Zuidema, 1951) is capable of eliminating almost the entire infused volume within 2 hours and returning the plasma volume to normal, regardless of the quantity administered (Wasserman & Mayerson, 1952a). However, short term behavior is volume dependent, as the percent of the infused volume retained in the plasma, at the end of 30 minutes, varied from 50% for small infusions (250 ml) down to 8% for massive infusions (2000 ml). In general, infusions which exceed 3/4 of the original plasma volume do not serve to increase plasma expansion and may cause edema, since a large portion of the disappearing fluid escapes into the interstitium as well as the urine.

After moderate infusions (250 ml) of isotonic saline into nephrectomized animals a steady state was reached within several hours. At this time the percent retention in the plasma was between 30 and 40% of the infused volume in one study on five dogs (Bradham, et al, 1964) and between 35 and 58% in a second study on two dogs (McNeill, et al, 1963). The former of these investigations revealed that so-called "isotonic" saline solution (0.9% NaCl) is capable of osmotically attracting a significant amount of intracellular fluid, but that this does not occur if the infused fluid is more similar in electrolyte concentration to extracellular fluid (e.g. Hartmann's Ringer or Tyrode's solution).

Theoretically, it should be possible by using iso-oncotic fluids to expand plasma volume without concomitantly affecting interstitial volume

(Strauss, 1957). This is the basis for using plasma or the less expensive plasma substitutes containing large colloids (Dextran, PVP, hydroxyethyl starch) to maintain circulating blood volume after hemorrhage. Of course, the ideal, perfect plasma expander cannot be realized since the expanded blood volume would cause an increase in filtration pressure resulting not only in a dramatic diuresis but in an increase of transcapillary fluid movement into the tissues. Both factors would lead to a decrease in plasma volume (Wasserman & Mayerson, 1954).

Most studies show that, as expected, infused dextran solutions remain in the circulation to a greater extent than colloidal-free solutions. The actual percent retention depends greatly on 1) the molecular size of dextran, with small molecules being more easily excreted and transported into the tissues (Wasserman & Mayerson, 1952b, 1954), and 2) the concentration of dextran, the normal clinical grade (6% by wgt) having almost 3 times the oncotic pressure of dogs' plasma (Prather, 1969; Marty & Zwiefach, 1970). Moderate infusions of hyperoncotic solutions are capable of moving not only tissue fluid into the circulation (Prather, 1969), but perhaps also mobilizing cellular fluid into the interstitium (Takaori, et al, 1970). Thus it is often observed in these cases that the plasma volume, a few minutes after infusion, has increased by more than the volume infused (Davies, et al, 1963).

3. EFFECTS OF INFUSION ON CIRCULATORY FUNCTION

The expansion of blood volume by infusion has been found to be a useful technique for studying such circulatory phenomena as vascular compliance (Gauer, 1956; Guyton, 1951; Gupta, 1966; Kerr & Kirklin, 1970 and Shoukas & Sagawa, 1971), cardiac performance (Prather, 1969; Guyton, et al, 1958) and peripheral resistance (Conway, 1966; Carey, et al, 1965). Many of these investigations have been reviewed by Gauer, et al (1963, 1970), who point out that compared to studies involving depletion of the blood volume by hemorrhage there has been relatively little work done with infusion experiments.

Some of the earlier studies have revealed a general pattern of response to a rapid infusion, irregardless of the type of solution used. For example, it is found that not only is the blood volume initially increased but there may also be dramatic increases in the cardiac output,

blood pressures and decreases in the total peripheral resistance (Guyton, et al, 1951, 1958; Gauer, et al, 1956; Carey, et al, 1965). After the infusion has ceased, these variables tend to return toward normal, but the time course of this return may be quite different for each variable. For example, the cardiac output may return to normal well before either the blood volume or blood pressures. In addition, if the infusion is administered very slowly, these changes may be less dramatic or may not be observed at all.

The initial rise in blood pressures is caused by the resistance of the blood vessel walls to stretch. Inasmuch as the compliance of the venous system is about 200 times that of the arterial system, there may be a more dramatic rise in arterial than in venous pressure and a shift of excess plasma into the venous system (Gauer, et al, 1956, 1963). Simultaneously with the rise in mean circulatory pressure there is a passive dilation of the vessels and decrease in peripheral resistance. Both effects are major factors in increasing venous blood return to the heart and hence increasing cardiac output (Guyton, et al, 1958).

Following the infusion there are several important compensatory mechanisms that aid in returning these variables toward normal. As summarized by Guyton (1963) these are: 1) the rise in arterial pressure causes a pressoreceptor reflex resulting in a decrease of cardiac output and peripheral vascular tone; both these effects would aid in lowering circulatory pressures, 2) the initial rise in capillary pressure would cause immediate outward filtration through the capillary walls into the interstitial spaces and into the urine; these adjustments are capable of returning the blood volume to normal within one to 24 hours, and as a result the mean systemic pressures, peripheral resistance and cardiac output will also return to normal, and 3) the occurrence of stress relaxation, the intrinsic ability of the vascular walls to stretch slowly when the pressure rises, will allow the vasculature to accommodate the increased blood volume and aid in lowering circulatory pressures.

The decrease in peripheral resistance due to the pressoreceptor reflex and the effects of stress relaxation both compensate for the increased blood volume by increasing the compliance of the vasculature. However, there are some fundamental differences between these two phenomena although relatively little is known about stress relaxation. The pressoreceptor reflex is fully effective within a minute, it acts primarily on the

peripheral resistance vessels and the heart, and is abolished by central nervous system blockade. On the other hand, stress relaxation becomes fully effective between 30 minutes and perhaps hours, appears to affect primarily the veins, and is not abolished by central blockade. Stress relaxation may not be observed unless the blood volume remains expanded for longer than a few minutes. The work of Prather, et al, (1969) indicates that the central reflexes offer only partial compensation to the effects of increased blood volume while the stress relaxation mechanism causes a more delayed, though more powerful compensation.

In addition to the three compensatory mechanisms mentioned by Guyton, it is known that there are also local "autoregulatory" mechanisms that are capable of exerting dramatic changes in peripheral resistance in response to changes in blood pressure (Mellander & Johansson, 1968). Relatively little is known about the effects of this "myogenic" response to infusions except that it acts primarily on the peripheral vessels in such a way that an increase in transluminal pressure causes a reflex constriction (or increase in tone). This, it should be noted, is opposite to the action of both the central pressoreceptor reflex and stress relaxation phenomena. Myogenic autoregulation has been invoked to account for a delayed but sustained increase in total peripheral resistance reported in several cases following long term blood expansion (Conway, 1966; Guyton, et al, 1971). Many of these aforementioned effects may never be seen if the infusion is given slowly enough because the regulatory mechanisms described have had sufficient time to fully compensate for the effects of increased blood volume.

4. EFFECT OF INFUSION ON LYMPH FLOW AND PROTEIN TRANSPORT

Infusions have been used for a long time to demonstrate that lymph flow dramatically increases with increasing circulatory pressure (Starling, 1909; Wasserman & Mayerson, 1952a). The most careful studies seen to date have been by Wasserman and Mayerson, who showed a linear relationship between the infused volume of saline and the peak value of the transient rise in thoracic duct lymph flow, and by Gibson and Gaar (1970), who investigated the same phenomena for hind limb lymph. Both of these studies appear to indicate an eventual upper limit of lymph flow if the infusion volume is great enough. While it is generally believed that, at rest, most of the thoracic duct lymph flow originates from the splanchnic organs

(Rusnyak, et al, 1967) no data is available to determine if this is also true during infusion experiments. In general, the role of lymph flow in maintaining homeostasis following either hemorrhage or infusion remains unclear. A possibly related phenomena which is not yet completely understood is the transient rise in lymph flow, often observed, following hemorrhage or hypovolemia (Hidale, et al, 1965).

Similarly, there is seemingly conflicting data concerning the effects of infusion on the plasma proteins. Prather (1969), Wasserman & Mayerson (1952a) and Moore, et al, (1966) review previous studies in which plasma proteins are observed to either increase or decrease following infusions of dextran or physiological saline. It is felt that any change in circulating proteins due to an infusion represents a complex trade-off between "1) the degree of distension of the interstitial space as a result of the infusion, since a greatly distended interstitial space may hold much of the protein which might ordinarily have gone back to the circulation via the lymphatics; 2) the rate of protein leaving the capillaries; 3) the amount of albumin present in interstitial fluid available for mixing with the plasma filtrate; and 4) the rate of lymph flow (Wasserman & Mayerson, 1952a). An additional factor might be the concentration of the lymph which, particularly in the hepato-splanchnic area of the circulation, might be very high, especially in the initial stages of infusion or hemorrhage (Moore, et al, 1966). It is not believed that any changes in protein mass measured during short-term infusion experiments arises from cell metabolism but rather that it comes from pre-formed protein in either the plasma or tissue-lymph fluid.

Shirley, et al, (1957) have proposed that infusion of fluids results in stretching of the capillary endothelial pores through which protein diffuses, and this supposedly can account for the several fold increase in albumin leakage observed by Wasserman & Mayerson (1952) during massive infusions. However, Prather (1968) reports a greater leakage of protein following tyrodes than dextran infusions (33 ml/kg) even though the circulatory pressures and volume were higher in the latter case. Furthermore, the study of Lundgren and Mellander (1967) presents evidence that disputes the stretched pore theory. They observed no change in the capillary permeability of several molecules during incremental increases of venous pressure.

III. EXPERIMENTAL METHODS AND MATERIALS

A. REQUIREMENTS OF THE EXPERIMENTAL DESIGN

The primary experimental task was to monitor the partitioning of fluid between the plasma and interstitial compartments for several hours following an infusion. Important features of the experimental protocol designed to accomplish this were:

- a) Infused fluids were required to be isotonic with respect to the crystalloid concentration of plasma to help ensure that they would remain in the extracellular compartment (Pitts, 1968). That is, it was assumed that these fluids would become distributed between the plasma and interstitial compartments without causing significant fluid shifts into or out of the cellular compartment.
- b) Experimental animals were bilaterally nephrectomized prior to infusion to ensure that there would be no external fluid loss (other than a small sensible and insensible water loss). This is desirable because it would be difficult to make accurate material balances on the infused fluid if an unknown fraction of this fluid were excreted from the body. We were more interested in studying fluid distribution within the body than in examining the more difficult problem of total control of blood volume via both internal and external pathways.
- c) Blood volume changes were measured by making a single injection of ^{51}Cr labelled red cells, after which the radioactivity of the blood was continuously monitored in a specially designed arterio-venous shunt. If it is assumed that there was no loss of tracer and no change in red blood cell volume during the course of the experiment, this procedure provides a continuous record of plasma volume changes.
- d) The spleen was removed from these animals to prevent sequestering or release of red cells in response to anesthetic and cardiovascular effects.

It was assumed that the difference between the volume infused at any time and the measured increase in blood volume gave the amount of fluid which had entered the interstitial space.

The rate with which an infused isotonic fluid disappears from the circulation will depend in large measure on the concentration of osmotically active substances in the injected fluid. Since the capillary membrane is very permeable to all the smaller crystalloidal solutes, it is only the less numerous but larger sized colloidal molecules (whose permeabilities decrease with increasing molecular size) that are capable of inducing a sustained osmotic gradient across the blood-tissue interface.

In this study two extreme cases were considered: a) A colloid-free isotonic solution should disappear most rapidly from the circulation. Tyrode's solution, closely approximating the extracellular crystalloid composition, was used for this case. b) A fluid containing a colloidal substance that is relatively impervious to the capillary membrane should disappear much more slowly. Two such solutions were prepared by dissolving different dextran fractions (average MW = 80,000 and 250,000) in Tyrode's solution.

There is an additional problem when dealing with dextran solutions. Most "clinical grades" of dextran are highly hyperoncotic (Marty & Zweifach, 1970), that is, when they are infused they cause interstitial fluid to be transiently absorbed into the circulation. There is reason to believe that these transient decreases in interstitial fluid volume may induce fluid shifts out of the cellular compartment (Takaori, et al, 1970). To minimize this possibility dextran solutions were prepared that were iso-oncotic as well as isotonic with plasma (i.e., COP = 20 mm Hg). In addition, the volume of solution infused was chosen to be about half of the original plasma volume; large enough to provide a reasonable degree of accuracy in determining blood volume changes but not so large as to induce gross changes in interstitial fluid volume.

B. EXPERIMENTAL DESIGN

Splenectomy, bilateral nephrectomy, and thoracic lymph duct cannulation were done in twelve mongrel dogs of both sexes (average weight = 19.7 ± 1.5 kg).¹ The day after surgery two consecutive intravenous infusions were administered, each followed by a suitable response period.

¹ Deviations shown in the text refer to one standard deviation.

The twelve dogs were divided into three groups according to the type of solution infused:

- Group I: 6 dogs infused with Tyrode's solution.
- Group II: 3 dogs infused with dextran 80 (MW = 60,000-90,000) dissolved in Tyrode's.
- Group III: 3 dogs infused with dextran 250 (MW = 200,000-300,000) dissolved in Tyrode's.

The Tyrode's solution was isotonic and the dextran solutions were prepared to be iso-oncotic with respect to plasma. The volume of each infusion was calculated as 2.5% of body weight and averaged 495 ± 38 ml.

The following variables were measured continuously prior to, during and after the infusions: blood radioactivity, mean arterial blood pressure (MAP) and mean venous pressure (MVP). In addition, frequent blood samples were obtained to measure or calculate hematocrits, plasma protein concentrations, plasma oncotic pressure, total plasma osmolality, sodium thiocyanate distribution volume and Evan's Blue distribution volume. Lymph flow was measured over 10 minute intervals. The lymph was returned to the animal except for samples which were analyzed for plasma protein concentrations and oncotic pressure. In three of the Group I dogs, cardiac output was measured at frequent intervals using dilution of cardio-green.

C. EXPERIMENTAL PROTOCOL

The night prior to surgery the animals were fasted but not deprived of water. Early the next morning, under sodium pentobarbitol anesthesia (30ml/kg), a 15 cm long abdominal incision was made 1 cm to the right of midline. The spleen was removed, care being taken to squeeze as much blood as possible from this organ before ligating the vessels. After removing both kidneys, the bladder was drained and squeezed empty before finally closing the incision. A 7 cm ventral neck incision was made to expose and cannulate the left thoracic lymph duct and left subclavian vein, using PE 50 and PE 140 tubing for the respective vessels. After lymph flow was established, a lympho-venous shunt was made as described by Doemling and Steggerda (1962) and Witte, *et al* (1970). Lymph was allowed to flow through the shunt overnight. In three dogs, the lymph duct was not cannulated but rather these

were implanted with jugular vein and carotid artery catheters in preparation for cardiac output studies.

Following the surgical procedure, 500 ml lactated Ringers solution was administered intravenously in order to make up for evaporative and metabolic losses (O'Connor and Potts, 1969) and ensure adequate hydration during the 24 hour recovery period. No food was given after surgery, but water was available to the animals. Late in the evening a blood sample was obtained for preparing the ^{51}Cr labelled red cells. At this time sodium thiocyanate tracer (25 mg/kg) was injected subsequent to obtaining a control plasma sample. This tracer normally takes 4-5 hours to equilibrate (Eder, 1951).

Early the next morning, Innovar-Vet (McNeill) was administered (0.125 cc/kg initially, later as needed at an average rate of 0.03cc/kg/hr). Under local anesthesia PE 240 catheters were placed in the right femoral artery and vein (for blood pressure recordings), the left femoral artery and vein (for arterio-venous shunt), and the right cephalic vein (for infusing fluid). The animal was placed on its side in a comfortable semi-prone position on top of a 1-inch piece of foam padding and a warm heating pad. Statham transducers (Model No. P23B) were connected to arterial and venous catheters with short lengths of PE tubing, and pressures were recorded continuously on a Grass Polygraph. Zero reference level was taken as 8 cm above table top (Conway, 1966). An arterio-venous shunt, containing a polyethylene coil placed in the well of a gamma counter, was connected between left femoral artery and vein (see Figure 1). The lympho-venous shunt was opened and timed collections of lymph were made in a graduated cylinder, placing the open end of the lymphatic catheter level with the vertical midpoint of the neck. Lymph was returned to the animal via the shunt. In those cases where cardiac output measurements were performed, dye was injected into the jugular catheter and carotid arterial blood was drawn through a densitometer.

One-half hour after catheters were implanted, Lipo-Heparin was administered intravenously (150 units/kg initially, later as needed at an approximate rate of 35 units/kg/hr), and blood flow was started through the extra-corporeal shunt. ^{51}Cr labelled blood was injected into the vein via a rubber injection bulb in the shunt system. A short time later Evan's blue dye was similarly injected to measure the pre-infusion plasma volume. A period of $3/4$ hour to $1\frac{1}{2}$ hours prior to the first infusion was allowed for

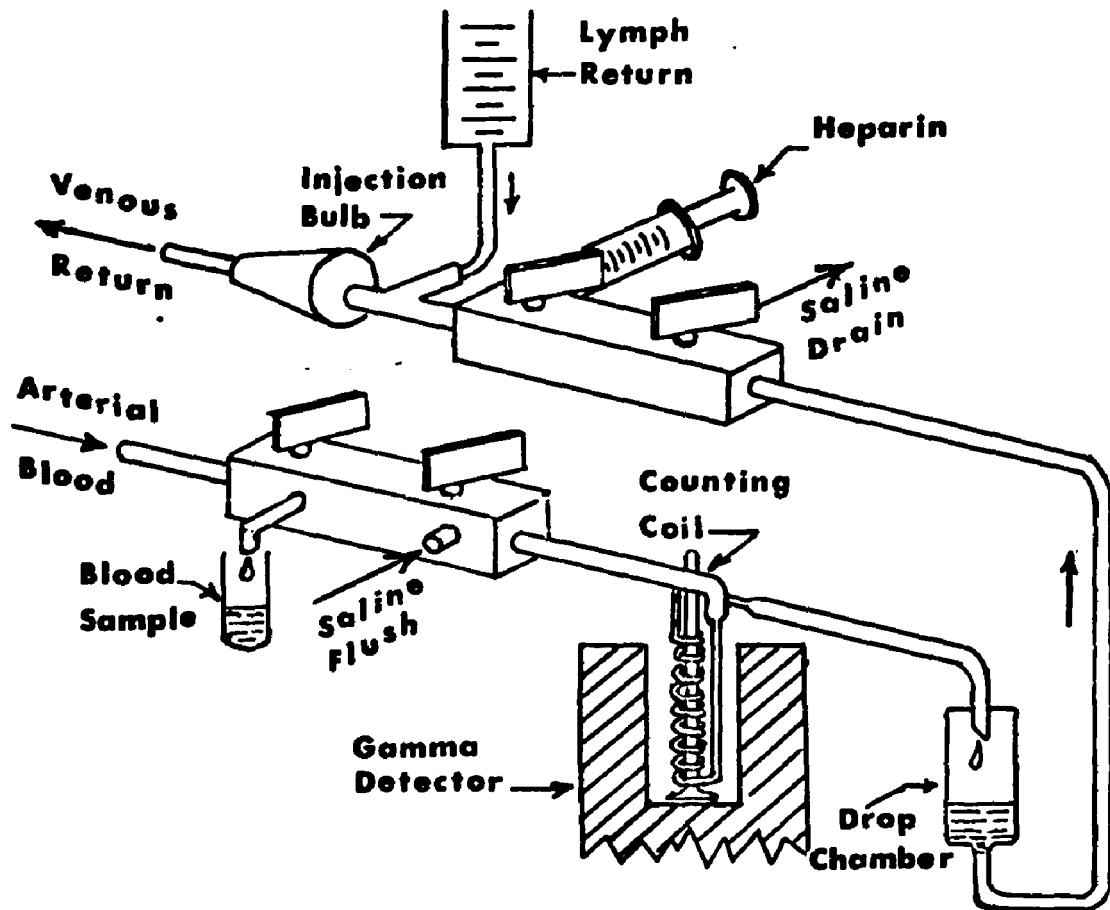


Figure 1. Extracorporeal shunt used for monitoring blood radioactivity continuously, returning lymph to animal, obtaining blood samples and injecting tracers and drugs.

equilibration of the ^{51}Cr tracer and for obtaining control records for MAP, MVP and lymph flow, as well as control blood samples.

The slow infusions were administered continuously at a rate of 15.3 ml/hr through a Harvard Infusion-Withdrawal Pump fitted with 50 cc syringes. The rapid infusions (100 ml/min) were beyond the fastest speed of the pump and were administered manually through 50 cc syringes. Infusion fluids (warmed to 38°C) were introduced thru the cephalic or femoral vein, the slow infusion always preceding the rapid infusion. All experimental variables were monitored either continuously or by taking frequent blood samples (averaging one 5 ml sample every 20 minutes for 2 - 4 hours after each infusion). Thus, each experiment consisted of a control period, an infusion period and a response period. The control period for the second infusion, of course, was merely the last segment of the previous response period.

D. SPECIAL METHODS

1. EXTRA-CORPOREAL SHUNT AND COUNTING CHAMBER

The shunt, illustrated in Figure 1, was constructed with stainless steel stopcock manifolds and fittings and polyethylene and PVC tubing. This shunt, siliconized prior to each run, was equipped with fittings to allow for blood sample removal, tracer injections, heparin administration, lymph return, saline flushing of the shunt, and a drop chamber for measuring flow rate. The primary purpose of the shunt, however, was to measure blood radio-activity. The counting coil, previously described by Albert (1963), was inserted into a 4-inch NaI crystal scintillation well coupled to a scaler/analyzer/printer equipped with a rapid digital printout. Blood was propelled through the shunt by arterial pressure at an average rate of 10 ml/min. The capacity of the entire shunt was less than 15 ml (about 11 ml of which was contained in the coil itself) so that a complete change of blood through the counting coil took place at least every 1 1/2 minutes. Once every hour and a half blood flow was stopped, blood was removed from the shunt into a syringe, and the entire system was flushed with saline to obtain a new background count and remove any coagulated blood from the walls of the system. The original blood was then replaced into the shunt system and blood flow was resumed. A single background value was used in the calculations by averaging several of these measurements.

2. ^{51}Cr LABELLING TECHNIQUE

The method used was modified after Gray & Sterling (1950) and Albert (1963). Ten ml blood were moved from the experimental animal the night prior to the experiment and placed in a tube containing several drops of heparin. Approximately 0.7 mc of $\text{Na}_2^{51}\text{CrO}_4$ solution were then added and the mixture was allowed to incubate in an ice bath for one hour, carefully mixing several times with a Pasteur pipette. The plasma was then separated by centrifugation and the red cells were washed four times by recentrifugation with Tyrode's solution. After the final wash the hematocrit was adjusted to about 0.4 with Tyrode's. The amount of free ^{51}Cr remaining in the supernatant after this procedure was ascertained to be less than 0.5% of the total activity. A calibration standard was prepared by diluting a fraction of the labelled blood mixture (1:500). The actual micro-hematocrit was also determined. Finally the blood was drawn into a syringe, carefully weighed, and stored at 4°C overnight.

As a rule the labelling procedure was about 75% efficient and the activity was such that after dilution of the tracer in the animal's blood the counting rate measured with the coil-counter system was between 10,000 - 20,000 cpm. The total activity injected into the animal (approximately 150 mc) is much higher than is usually administered for single determinations of blood volume. This high dose was necessary, however, to give a high degree of accuracy in obtaining counting rates over a short period of time.

3. BLOOD VOLUME CALCULATIONS

The counting coil was calibrated with a known dilution of labelled blood. Blood volume at time i was calculated from the equation:

$$BV_i = \frac{D^* - \sum_{j=1}^i V_j A_j}{A_i \times F_{\text{cell}}} \quad (\text{III-1})$$

where

D^* = dose injected (cpm)
 = (activity of calibration standardized corrected for free ^{51}Cr) x (dilution factor of standard).

V_j = j th blood sample removed, ml

A_j = coil activity at time of j th sample (cpm)

A_i = coil activity at time i (cpm)

F_{cell} = ratio of whole body hematocrit:venous hematocrit.

The summation term is included to account for radioactivity removed in sampling. All activities in the above equation were corrected for background (less than 1/50 of total activity). The F_{cell} ratio (Reeve, et al, 1953) was determined for each animal during the control period from the Evan's blue space (PV), ^{51}Cr red cell volume (RCV) and venous hematocrit (HCT) according to the equation:

$$F_{\text{cell}} = \frac{\left[\frac{\text{RCV}}{\text{PV} + \text{RCV}} \right]}{\text{HCT} \times 0.97} \quad (\text{III-2})$$

The single injection-continuous monitoring method of determining blood volume can be performed with greater speed, less chance for error and with accuracy and reproducibility comparable to that achieved by the traditional multiple injection-test tube batch method. (Albert, et al 1956).

Total circulating red cell volume was calculated from the expression:

$$\text{RCV}_0 = D^* \times 0.97 \times \frac{\text{HCT}_1}{A_1} \quad (\text{III-3})$$

A value for red cell volume can be calculated only at those time, t_1 , when the hematocrit is measured, in contrast to the continuous measurements of blood volume. Radioactivity removed in sampling need not be included if only the original cell volume is required.

4. PREPARATION OF INFUSION SOLUTIONS

Tyrode's solution was prepared according to the specifications in U.S. Dispensatory.² Dextran was obtained from General Biochemical Corp. in fractions of 60,000 - 90,000 and 200,000 - 300,000 MW. (These will be designated as D₈₀ and D₂₅₀ respectively). Enough dextran was dissolved in Tyrode's solution so that the COP of each solution was 20 mm Hg as measured on the colloid osmometer. (This is the average COP for canine plasma as determined by Prather, 1968). The colloid osmotic pressure of different concentrations of these dextran solutions are shown in Figure 2. Values are also shown for clinical grade (6 wt pct) "Dextran 70" (Marty and Zweifach, 1970) and plasma proteins (Landis and Pappenheimer, 1963). Note that "dextrans with molecular weights similar to those of plasma proteins exert a colloid osmotic pressure that is much higher than that of plasma (although)...the reason...has not been clearly defined" (Marty & Zweifach). This discrepancy may have something to do with the recently reported data that dextran has a much higher molecular radius than proteins of equal molecular weight (Garlick and Renkin, 1970).

The total osmolality for all solutions was 295 ± 5 mosm/kg H₂O. The actual volume infused was 25 ml/kg body weight. This amount would represent, for example, 50% of the control plasma volume assuming an average blood volume of 90 ml/kg and a hematocrit of 0.45.

5. BLOOD, PLASMA, AND LYMPH ANALYSIS

Blood samples were removed via the shunt manifold at approximately 20 minute intervals. Samples were immediately centrifuged and the plasma stored at 0°C. Approximately 100 ml blood, equal to 5 - 10% of the initial blood volume, were removed during the course of the 2-infusion experiment.

Hematocrits were determined in duplicate by the micro-hematocrit technique using an International centrifuge and reader. The value obtained was multiplied by 0.97 to account for trapped plasma (Swan & Nelson, 1968).

Protein concentrations in plasma and lymph were measured by the biuret method with albumin standards (Gornall, et al, 1949; Prather, 1968) and

²U.S. Dispensatory (1937), 22nd Edition, H.C. Wood & C.H. LaWall (Ed.), Philadelphia: J.B. Lippincott Co., p. 637.

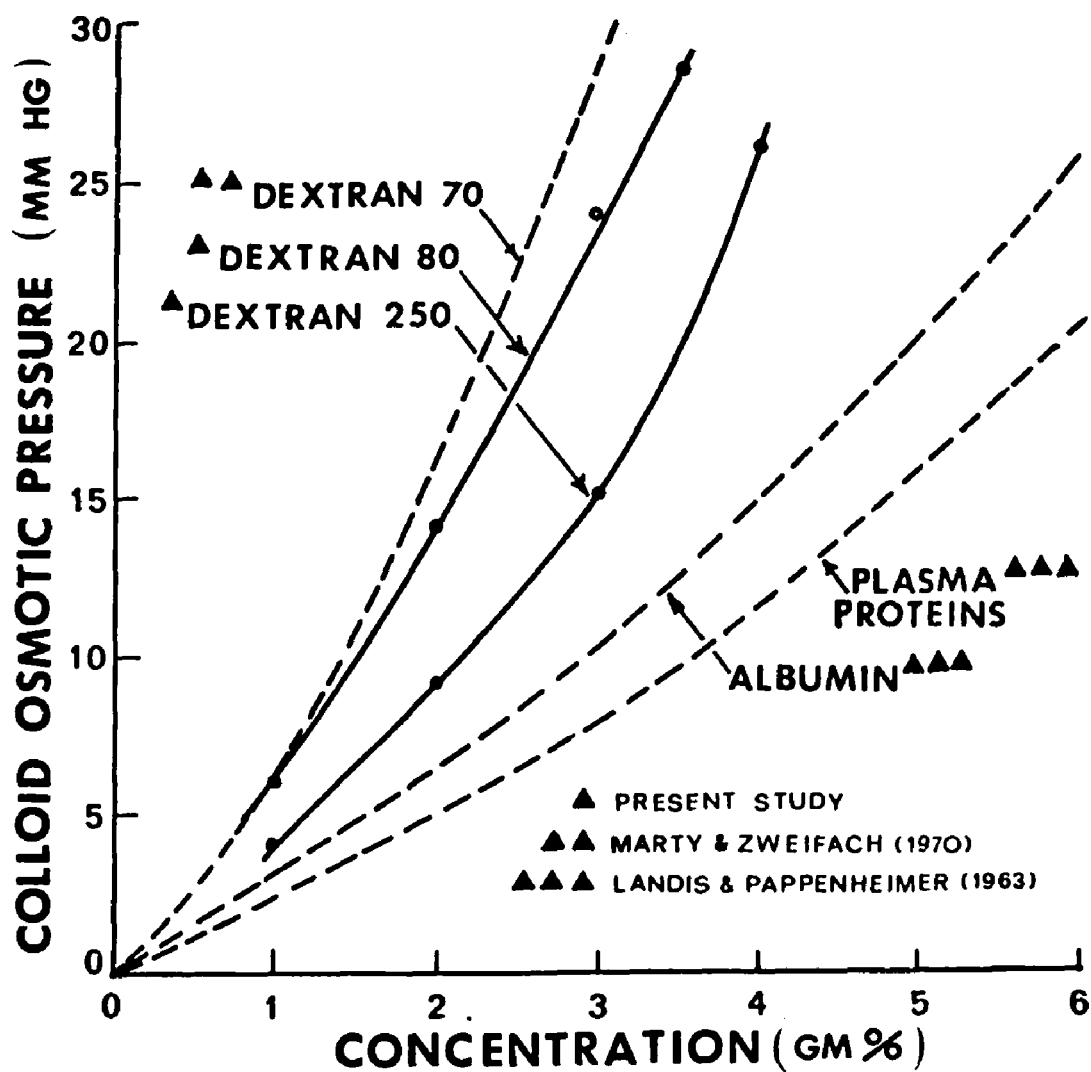


Figure 2. Colloid osmotic pressures of protein and dextran expressed as a function of concentration.

verified with an Hitachi hand-held protein refractometer (Neuhausen, 1923).

Colloidal osmotic pressure (COP) was measured using a specially constructed lucite osmometer attached to a Statham pressure transducer (Model No. 23P). The apparatus, based on the design of Prather, et al (1968), incorporated an Amicon UM-10 membrane which retains particles over 10,000 MW. The osmometer, whose response time was between 1 - 2 minutes, was calibrated with albumin standards. Successive readings on the same sample showed a standard deviation of 0.1 mm Hg. The value of COP can be accurately estimated from the protein concentration if no other colloids are present in the plasma (Landis & Pappenheimer, 1963; Prather, et al, 1968). However, in the presence of dextran, both protein and dextran contribute to the total COP (which must then be measured directly).

Evan's blue space was measured during the control period and taken to be a measure of plasma volume. Approximately one mg Evan's blue dye/kg body weight was injected as a 0.5% solution and four subsequent blood samples were drawn at 10 minute intervals. The spectrophotometric analysis was performed by diluting the plasma samples 1:1 with saline, and the dye-in-saline standard 1:1 with pre-injection plasma. The Evan's blue space was calculated in the usual manner by extrapolation to the time of injection.

Thiocyanate extracellular space was determined colorimetrically, with a modification of the method outlined by Edner (1951). The injected dose (approximately 20 mg/kg) was prepared as a sterilized 30 mg/ml sodium thiocyanate solution and was allowed to equilibrate about 8 hours prior to obtaining five one-half hour plasma samples.

⁵¹Cr Batch Analysis: Four blood samples were obtained during the course of the experiment to determine the blood volume from the conventional batch test-tube method. These were used to validate the continuous blood volume measurement technique. Deviation between the two methods was less than 5%.

Total osmolality was determined from plasma samples obtained immediately before and after each infusion using an Advanced Instrument Co. (freezing point depression) osmometer.

Lymph samples were taken at frequent intervals from the thoracic duct (approximately 20 ml/infusion experiment) and analyzed for protein and/or COP.

Cardiac output was determined by injecting indocyanine green (1.5 mg/ml) and obtaining indicator dilution curves (Conway, 1966). The equipment consisted of a Gilford densitometer, a servo-channel of a Gilson recorder,

and a dye injection apparatus. The concentration of the calibrating standard was 1 μ liter of dye solution/cc blood. Curves were integrated by the trapezoid method.

IV. EXPERIMENTAL RESULTS

SCHOFFENIELS: "...I am wondering whether your observations could not be considered as artifact."

CASLEY-SMITH: "Yes, they are artifacts; everything is an artifact.... However, this is the same everywhere in Science. We only study the artifactual representations of the real world, whether we count things, read meters, weigh substances or look at electronmicrographs. The point is not whether we have artifacts, but whether (these artifacts) bear a constant and predictable relationship to the real world." (From a transcript of a discussion in J.M. Collette, et al (1967), Editors, "New Trends in Basic Lymphology".)

A. TIME COURSE OF OBSERVED VARIABLES IN TYPICAL EXPERIMENTS

Tyrode's or dextran solutions were infused into a total of 12 dogs (average weight = 19.7 ± 1.5 kg). The infusion rate was either "slow" (15.3 ml/min) or "rapid" (100 ml/min) and the volume of each infusion was 2.5% of the pre-surgical body weight (average infusion volume = 495 ± 38 ml). Various control period measurements and statistics associated with each animal are shown in Table A-I (Appendix).

Some of the results of two typical experiments are shown in Figures 3 and 4. In the first experiment (Figure 3) Tyrode's solution was infused and in the second (Figure 4) dextran 80 was infused. A typical experiment consisted of: a) a control period of at least one hour's duration, b) a slow infusion (average infusion time = 0.539 hours) and subsequent recovery or response period of 2-4 hour duration, and lastly, c) a rapid infusion (average infusion time = 0.0826 hours) and a corresponding 2 hour recovery period. In two experiments (Dog #20 and #22) a second rapid infusion was administered in which good repeatability of response was demonstrated.

The Tyrode's solution disappeared from the circulation much more rapidly than the dextran solution, the dotted line indicating the theoretical volume if 100% of the infusate was retained in the blood. Also note the similarity of the hematocrit and protein concentration time profiles as the blood is diluted with each infusion. Protein concentration was determined by two methods, refractometry and biuret chemical analysis, and the close agreement between these methods is shown. In Figure 3 COP was calculated from the protein concentration and Equation (VI-19), while in Figure 4, direct results, using the colloidal osmometer, are presented.

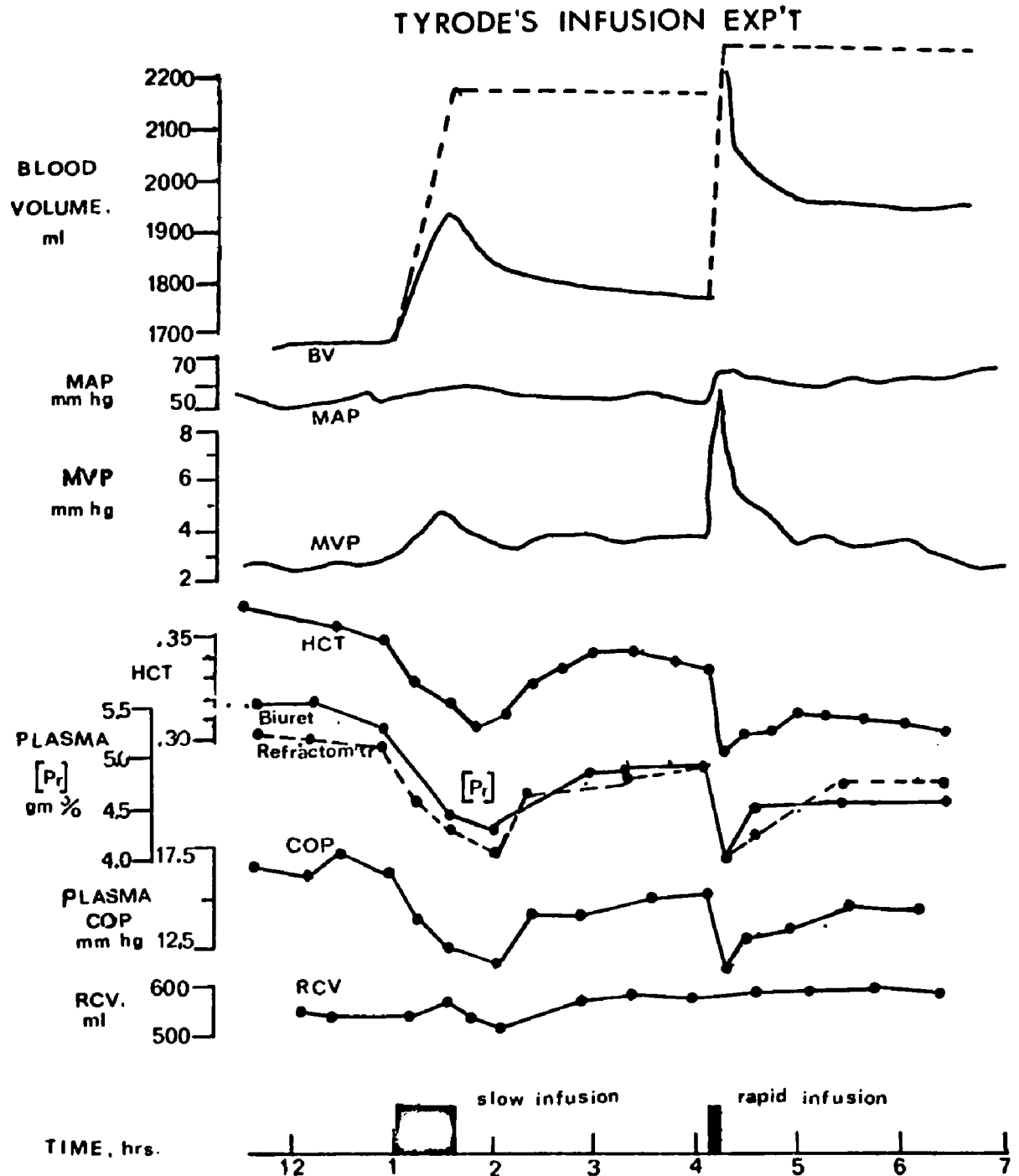


Figure 3. Results of a typical experiment (Expt. #27) showing the effects of administering two consecutive infusions of Tyrode's solution.

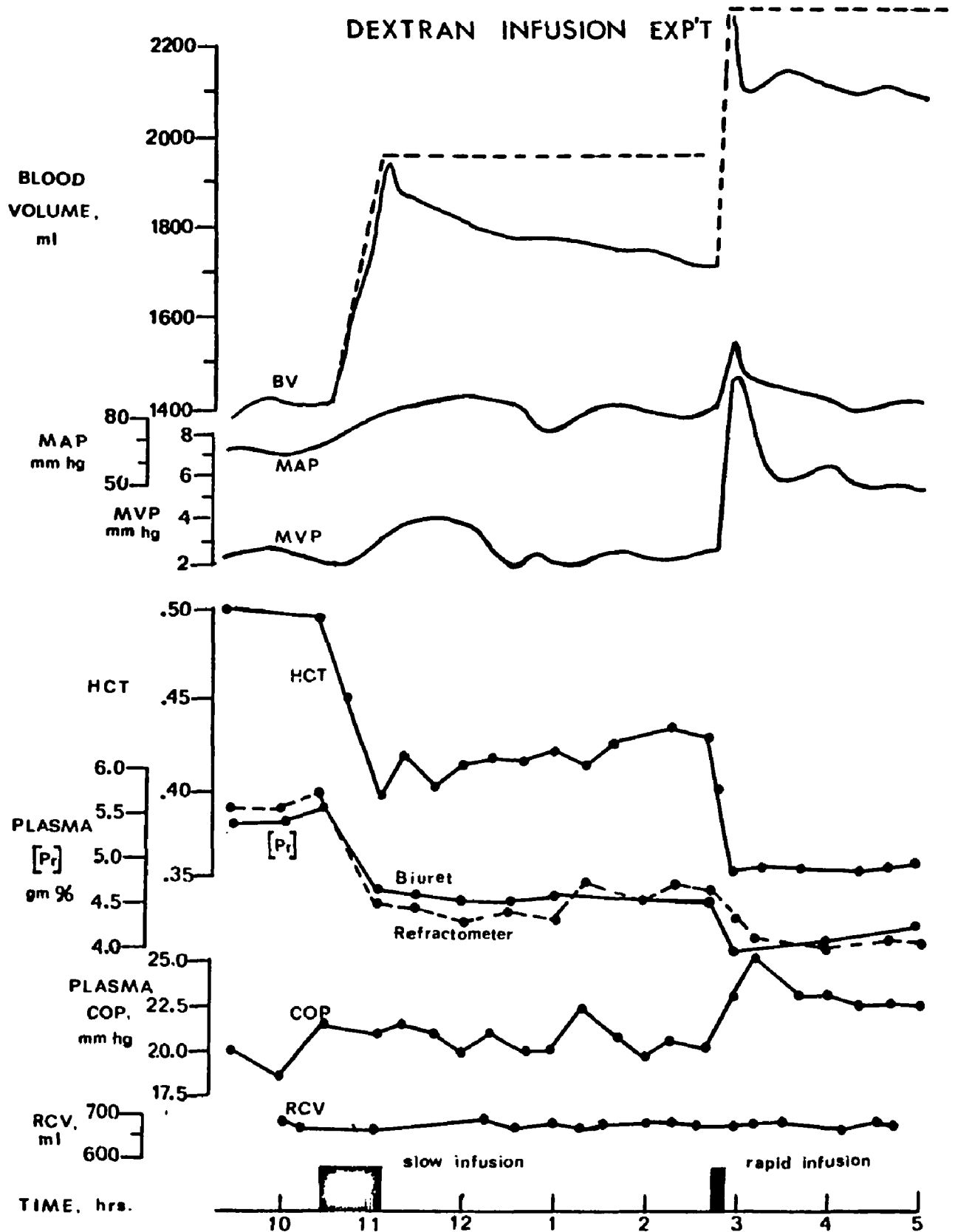


Figure 4. Results of a typical experiment (Expt #24) showing the effects of administering two consecutive infusions of dextran solution.

B. DYNAMIC RESPONSE OF IMPORTANT VARIABLES FOR INDIVIDUAL EXPERIMENTS

The total of 25 separate infusions were divided into four groups: slow Tyrode's infusions (6 animals), rapid Tyrode's infusions (7), slow dextran infusions (6), and rapid dextran infusions (6). Figures 5 to 8 show the results of each of these infusion experiments. The variables illustrated are those that most critically affect the plasma disappearance of an infused solution: the arterial and venous blood pressures and the plasma protein concentration [see Equations (II-1) and (II-2)]. Values for the variables are shown as differences from control levels. Control values for plasma volume, PV_0 ; arterial pressure, $P_{a,0}$; venous pressure, $P_{v,0}$; and plasma protein concentration, $[Pr]_0$, are given in Table A-II (Appendix).

1. THE PLASMA VOLUME RESPONSE

The plasma volume has been further transformed by dividing the difference from control level by the total volume infused. If FR is the fraction of infusate retained in the plasma, then

$$FR = \frac{PV(t) - PV_0}{V_{inf,T}} = \frac{BV(t) - BV_0}{V_{inf,T}}$$

$PV(t)$ and $BV(t)$ are the plasma and blood volumes at any time, t , and $V_{inf,T}$, is the total volume infused during a particular infusion experiment. The subscript "o" refers to control values. Because the actual period of infusion, ΔT_{inf} , differed for all experiments, it was convenient to let $t = 0$ represent the end, rather than the beginning of the infusion period. During the infusion period, $-\Delta T_{inf} < t < 0$, FR is proportional to the increase in plasma volume and is not the fraction retained because the denominator of the above equation is a constant at all times.

Figures 5 to 8. Individual experimental results for the four infusion groups showing the effects of infusion on the plasma volume, PV , mean arterial blood pressure, P_a , mean venous blood pressure, P_v , and plasma protein concentration, $[Pr]_{pl}$. Results are presented as deviations from control. Values for PV_o , $P_{a,o}$, $P_{v,o}$, and $[Pr]_{pl,o}$ are presented in Table A-2 (Appendix). Infusion ends at time = 0.

Figure 5. Responses to slow infusions of Tyrode's solution.

Figure 6. Responses to rapid infusions of Tyrode's solution.

Figure 7. Responses to slow infusions of dextran solution.

Figure 8. Responses to rapid infusions of dextran solutions.

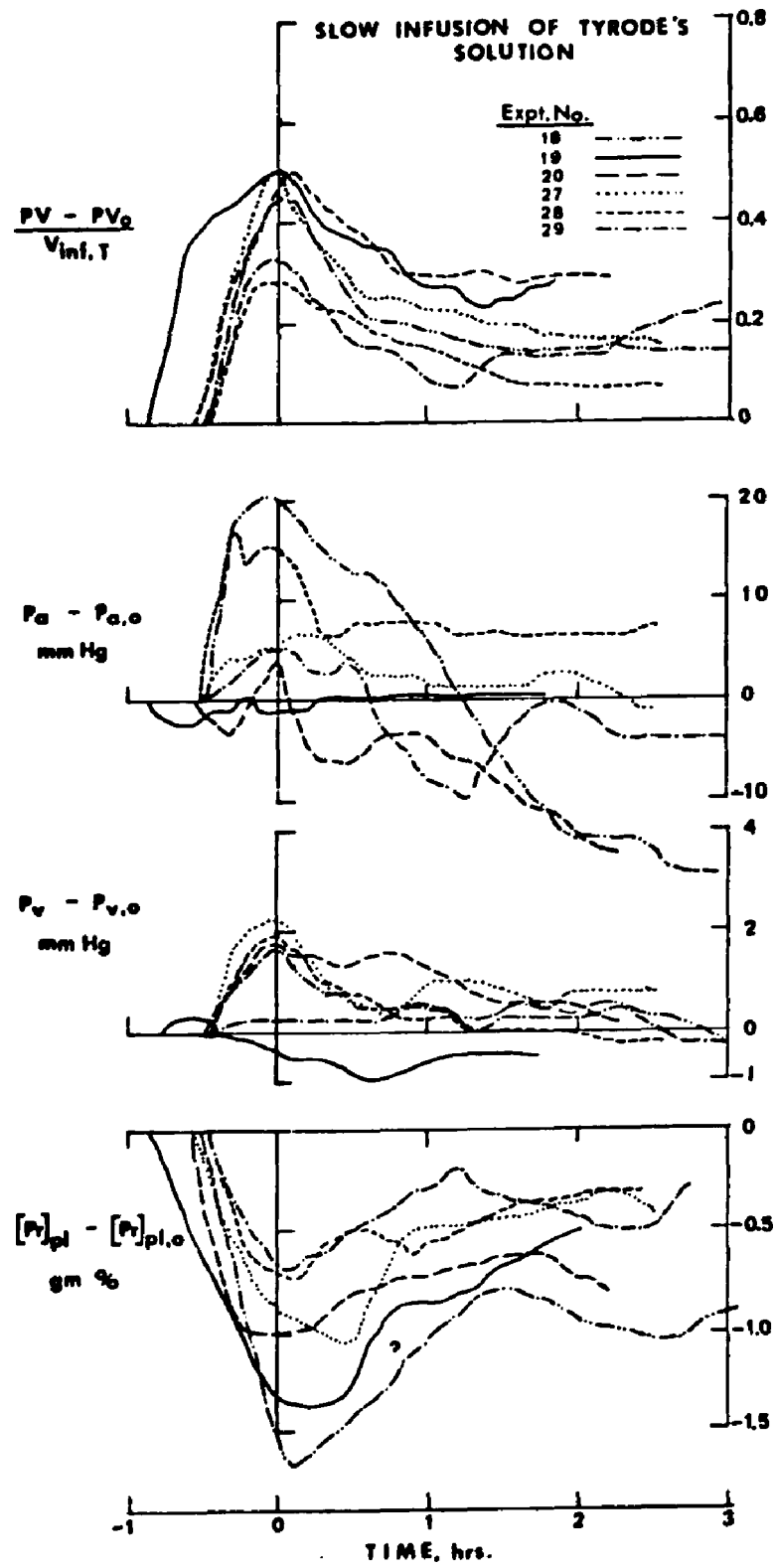


Figure 5.

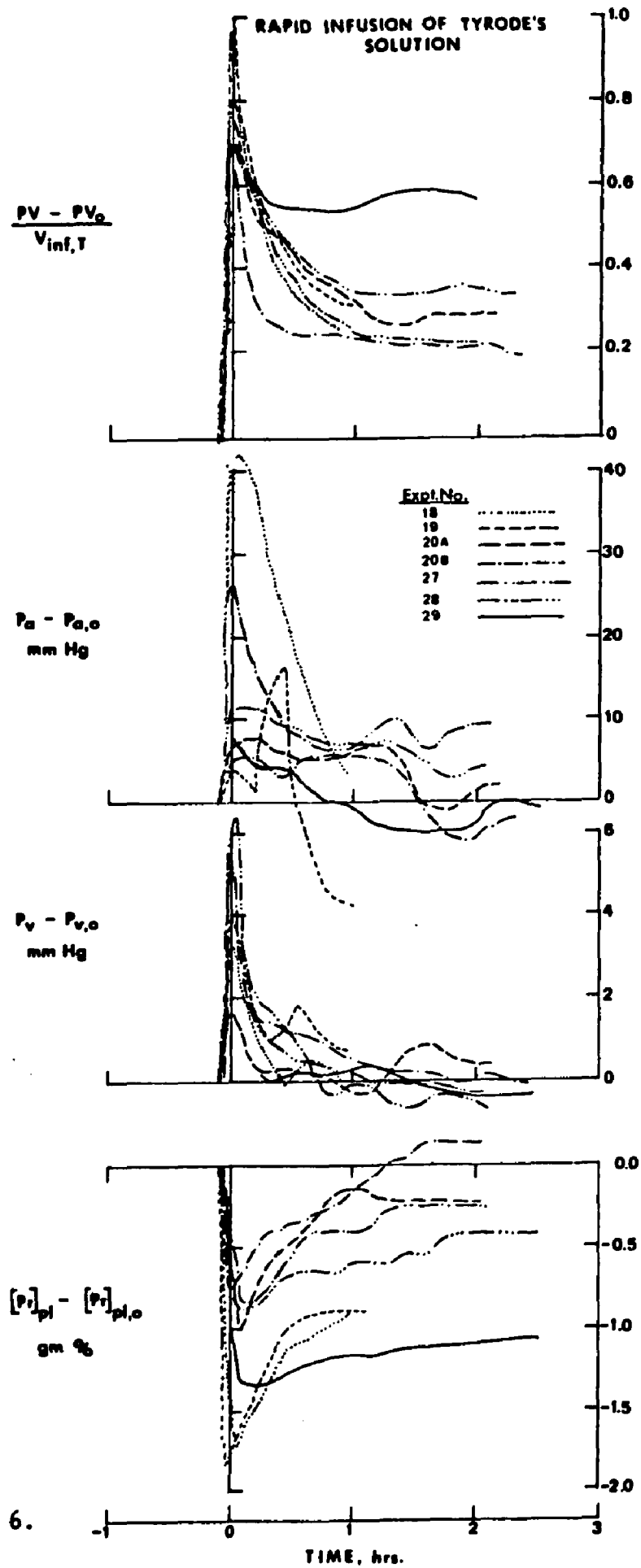


Figure 6.

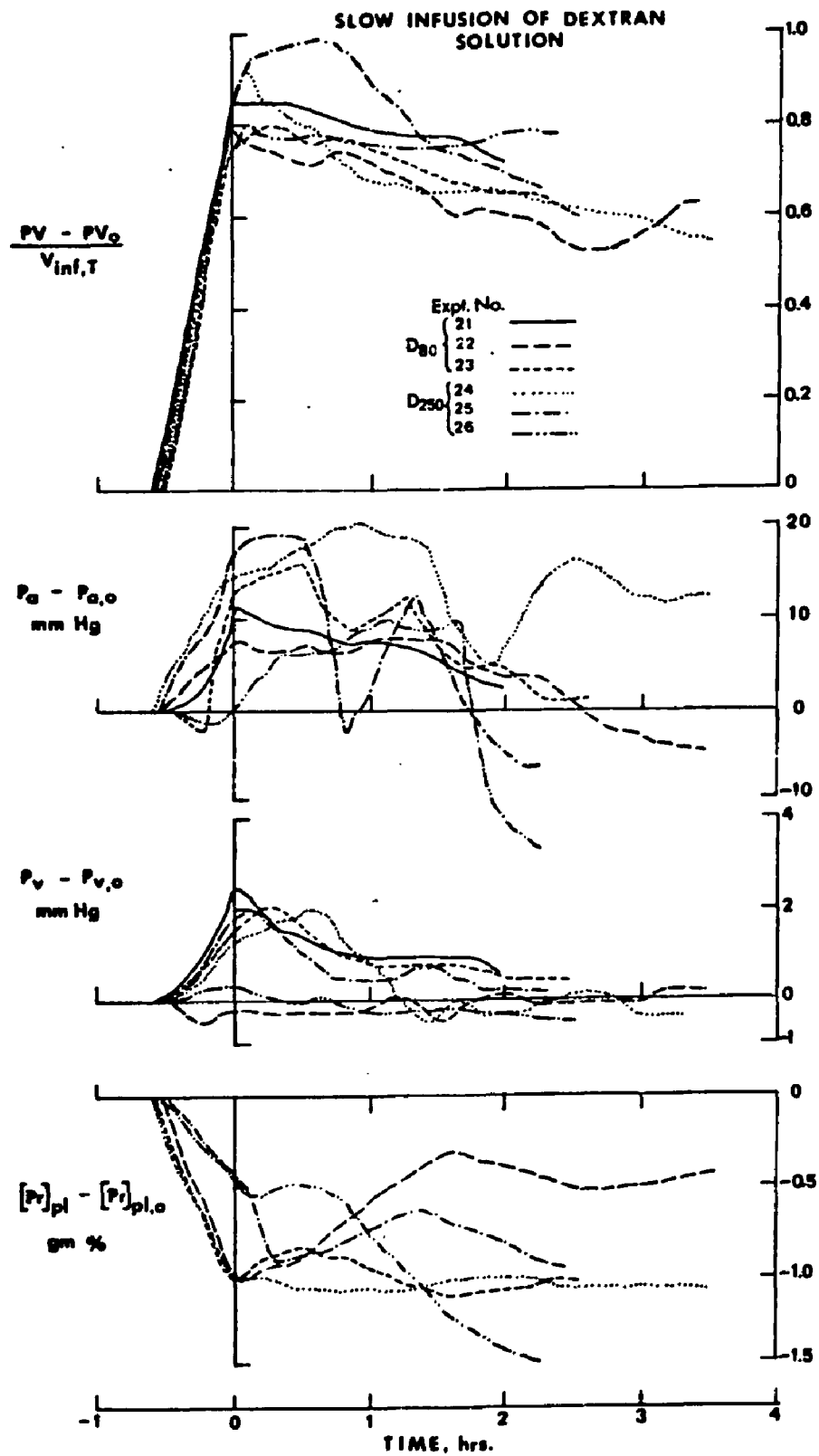


Figure 7.

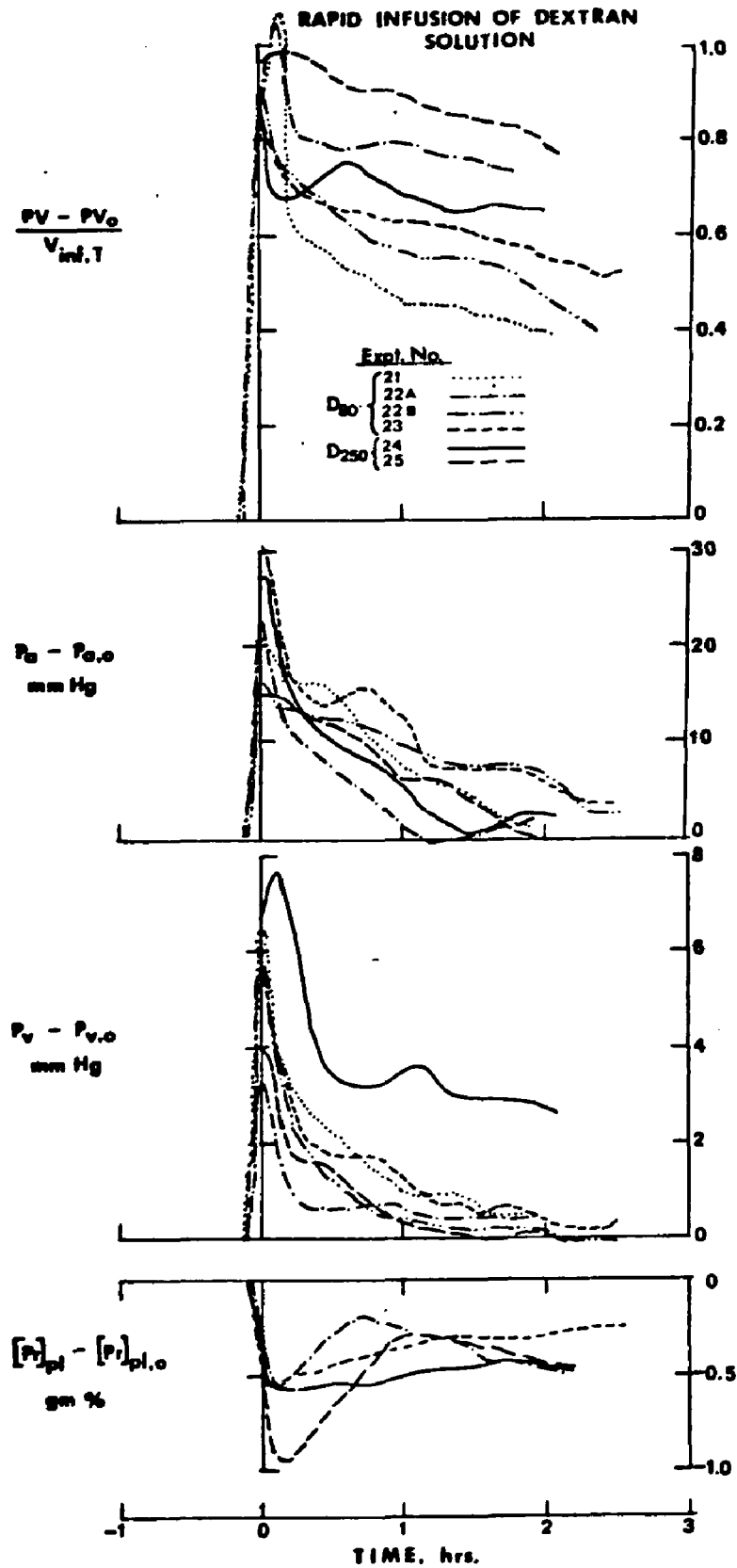


Figure 8.

2. STATISTICAL ANALYSIS OF PLASMA VOLUME RESPONSE

This section presents a detailed statistical analysis of the plasma volume responses. The analysis was performed in order to test the statistical differences between the four groups. In particular we desired to ask the following questions pertaining to the data shown in Figures 5 through 8:

- 1) Are the results obtained with dextran 80 different from those obtained with dextran 250 --
 - i) comparing results from slow infusions only?
 - ii) comparing results from rapid infusions only?

If we combine results from both dextran grades --

- 2) Are the results obtained from the slow infusions different from those obtained with rapid infusions --
 - i) for Tyrodes solution?
 - ii) for dextran solution?
- 3) Are the results obtained with Tyrodes solution different from those obtained with dextran --
 - i) comparing results from slow infusions only?
 - ii) comparing results from rapid infusions only?

If we combine fast and slow infusion experiments for each of the two solutions --

- 4) Are the results obtained with Tyrodes solution different from those obtained with dextran solution?

Statistical Methods

The basic approach in answering these questions was to reduce each of the continuous curves of Figures 5 - 8 into four 1/2 hour segments, starting at $t=0$ and ending at $t=2$ hours. (Beyond 2 hours there was not a sufficient number of experiments in each group to form meaningful statistical conclusions.) The value of the ordinate for each time segment was the average value of

that segment. Two cases of a group (e.g. fast and slow infusion or Tyrode's and dextran solutions) can be compared using the F statistic from a 4-dimensional one-way analysis of variance (time is treated as a 4-dimensional vector variable)(Morrison, 1967). As an example, consider question 2 (i) above. The experimental plan for this analysis would look like:

COMPARISON OF SLOW AND FAST RATES OF
INFUSION FOR TYRODE'S SOLUTION

Infusion Rate	Time Segments			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
	(Fraction Retained)			
Slow	$\bar{X}_{S1}$	$\bar{X}_{S2}$	$\bar{X}_{S3}$	$\bar{X}_{S4}$
Fast	$\bar{X}_{F1}$	$\bar{X}_{F2}$	$\bar{X}_{F3}$	$\bar{X}_{F4}$

where the $\bar{X}$'s represent the mean value of FR at each condition for several duplicate experiments. The comparison between fast and slow infusions is reduced to testing the hypothesis of whether the respective averages of $\bar{X}_S$ and $\bar{X}_F$ are equal at each time segment; i.e. is it true that $\bar{X}_{S1} = \bar{X}_{F1}$, $\bar{X}_{S2} = \bar{X}_{F2}$, $\bar{X}_{S3} = \bar{X}_{F3}$, and $\bar{X}_{S4} = \bar{X}_{F4}$? In the other comparisons the two infusion rates would be replaced by two dextran grades or 2 isotonic solutions.

It is important to note that testing the equality of means' hypothesis at the four time intervals should not be performed by four separate t-tests because the plasma volumes at consecutive intervals are not independent of each other, and in fact are highly correlated. This time dependency is of course expected since the percent retention at one time effects the value at a later time. The effect of time dependency can be accounted for by performing a one-way multivariate analysis of variance -- treating time as a 4-dimensional vector -- hence the F-statistic can be considered to reflect not merely the overall difference between two groups of data but the differences in their time profiles.

The analysis of variance was performed using a computer console oriented library program, CONSTAT, and subprogram MANO (Multivariate Analysis of Variance), prepared by the Statistical Research Laboratory of the University

of Michigan. The results of this analysis are summarized in Table II. The level of significance is the computed type I error. If the attained level of significance is 0.05, for example, we can reject the hypothesis that the two data sets (fast and slow infusion in the above example) are from the same population with only a 5% chance of being wrong.

TABLE II
STATISTICAL ANALYSIS OF PLASMA VOLUME RESPONSE

<u>Comparison</u>	<u>Test Statistic</u>	<u>Degrees of Freedom</u>	<u>Level of Significance</u>
1) D ₈₀ vs D ₂₅₀			
i) Slow infusion	t = 0.885	4	0.43
ii) Fast infusion	t = 1.54	4	0.20
2) Slow vs Fast Infusion			
i) Tyrodes	F = 3.72 **F = 1.42	(4,6) (3,7)	0.075 0.32
ii) Dextran	F = 1.32	(4,7)	0.35
3) Tyrodes vs Dextran			
i) Slow infusion	F = 40.0	(4,7)	0.00007
ii) Fast infusion	F = 19.2	(4,6)	0.0014
4) Tyrodes vs Dextran			
Overall	F = 21.6	(4,18)	0.000001

** Compares last 3 time segments only; omits early portion following infusion.

Note that in Table II, the comparison between the two grades of dextran was performed with a simple t-test rather than the more complicated analysis of variance. This method was deemed advisable because the number of experiments in each of these groups was so small (2 or 3). Instead of dividing the FR curves into 4 time segments, the average value of all 4 periods was used for each experiment. The hypothesis tested was: $\bar{X}_{80} = \bar{X}_{250}$, where $\bar{X}$ represents the fraction retained for each of the two dextran fractions

averaged for each experiment over the 2 hour period following infusion and again averaged for the several duplicate experiments.

Statistical Results

Based on the above analysis it appears that the following conclusions can be drawn with regard to the plasma volume responses:

a) There is no strong evidence that the results obtained from the two dextran fractions are different. (For this reason the results obtained for D_{80} and D_{250} infusions will be pooled in the remainder of the study.)

b) There appear to be significant differences between results obtained with dextran as compared to those obtained with Tyrodes solution.

c) There appears to be no strong evidence to conclude that the rate of infusion of any given isotonic solution effects its overall disappearance from the plasma except perhaps for the period immediately after the infusion ends.

3. THE BLOOD PRESSURE RESPONSE

There appears to be some qualitative difference between the arterial pressure responses of the slow and rapid infusions. During the slow infusion the arterial pressure might be rising during one experiment while it is falling or remaining relatively constant during a comparable time interval of another experiment. By contrast, the arterial pressure responses for the fast infusions as well as most of the venous pressure curves for all four groups exhibit a singular pattern, rising for a brief period during the infusion and then immediately showing a tendency to return to pre-infusion levels.

4. VARIABILITY IN TOTAL RED CELL VOLUME

In Figure 9 are shown the results of all the total circulating red cell volume measurements obtained during each of the infusion experiments.

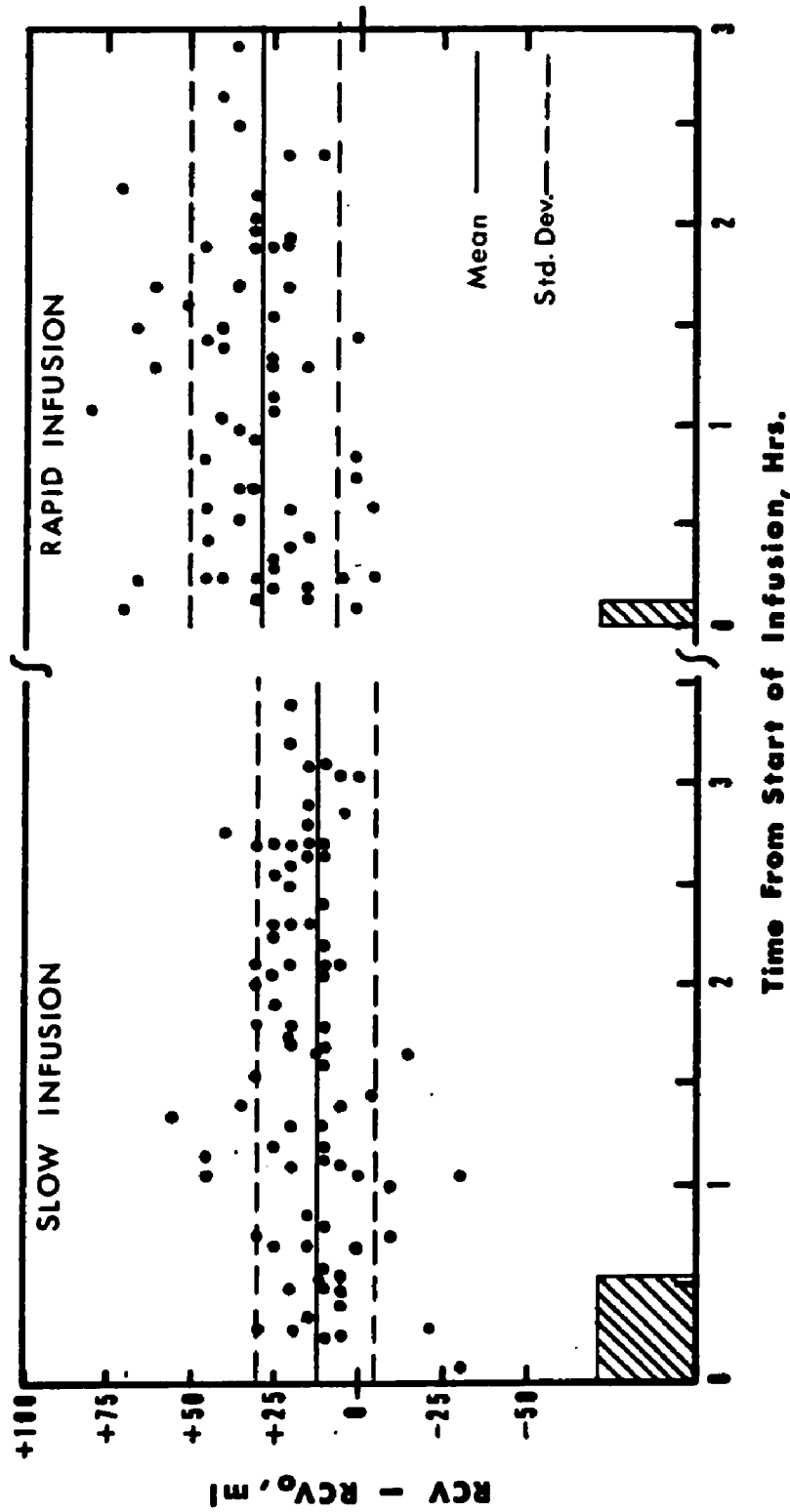


Figure 9. Variation of total red cell volume during all slow and rapid infusion experiments. Results are presented as deviations from control ($RCV_0 = 504$ ml).

Values are given as the difference from the control volume to allow comparison among animals with widely different red cell volumes. Each point on the graphs represents the time at which a blood hematocrit was obtained. The control volume, RCV_0 , represents the mean of several measurements made prior to the first infusion. The horizontal lines are the mean and standard deviations of the dependent variable ($\overline{RCV}$). A linear regression analysis resulted in a line whose upward slope (3m./hr) was not considered statistically significant ($p > 0.10$). Table III summarizes these results.

TABLE III
MEAN VALUES OF RED CELL VOLUMES DURING INFUSION

<u>Variable</u>	<u>Slow Infusion</u>	<u>Rapid Infusion</u>
1. Control Red Cell Volume, RCV_0	504 $\pm$ 110	
2. Avg. Post-Infusion Red Cell Volume, $\overline{RCV}$	515 $\pm$ 112	537 $\pm$ 107
3. Difference, $\overline{RCV} - RCV_0$	12 $\pm$ 16	34 $\pm$ 19
4. % Deviation, $(3/1) \times 100$	2.4%	6.4%

Essentially these results show that the red cell volume, determined by single tracer injection and calculated up to 8 hours apart, shows a variation of less than 7%

C. AVERAGED RESULTS OF INFUSATE RETENTION, BLOOD PRESSURES, AND PROTEIN CONCENTRATIONS

The individual runs of the four groups of data presented in the last section were averaged (by interpolation at 0.1 hour intervals) and the results appear in Figures 10 and 11. Only the first two hours of each run were used because only half of the runs were much longer than this. Dashed lines represent $\pm$ one standard error. Certain trends in the response of the variables become more evident when the results are averaged.

Figures 10 and 11. Averaged results of slow and rapid infusion experiments showing effects on plasma volume, mean arterial pressure, mean venous pressure, and plasma protein concentrations. Mean values are indicated by solid lines. Dashed lines indicate one standard error.

Figure 10. Averaged results during Tyrode's solution infusion experiments.

Figure 11. Averaged results during dextran solution infusion experiments.

INFUSION OF TYRODES SOLUTION

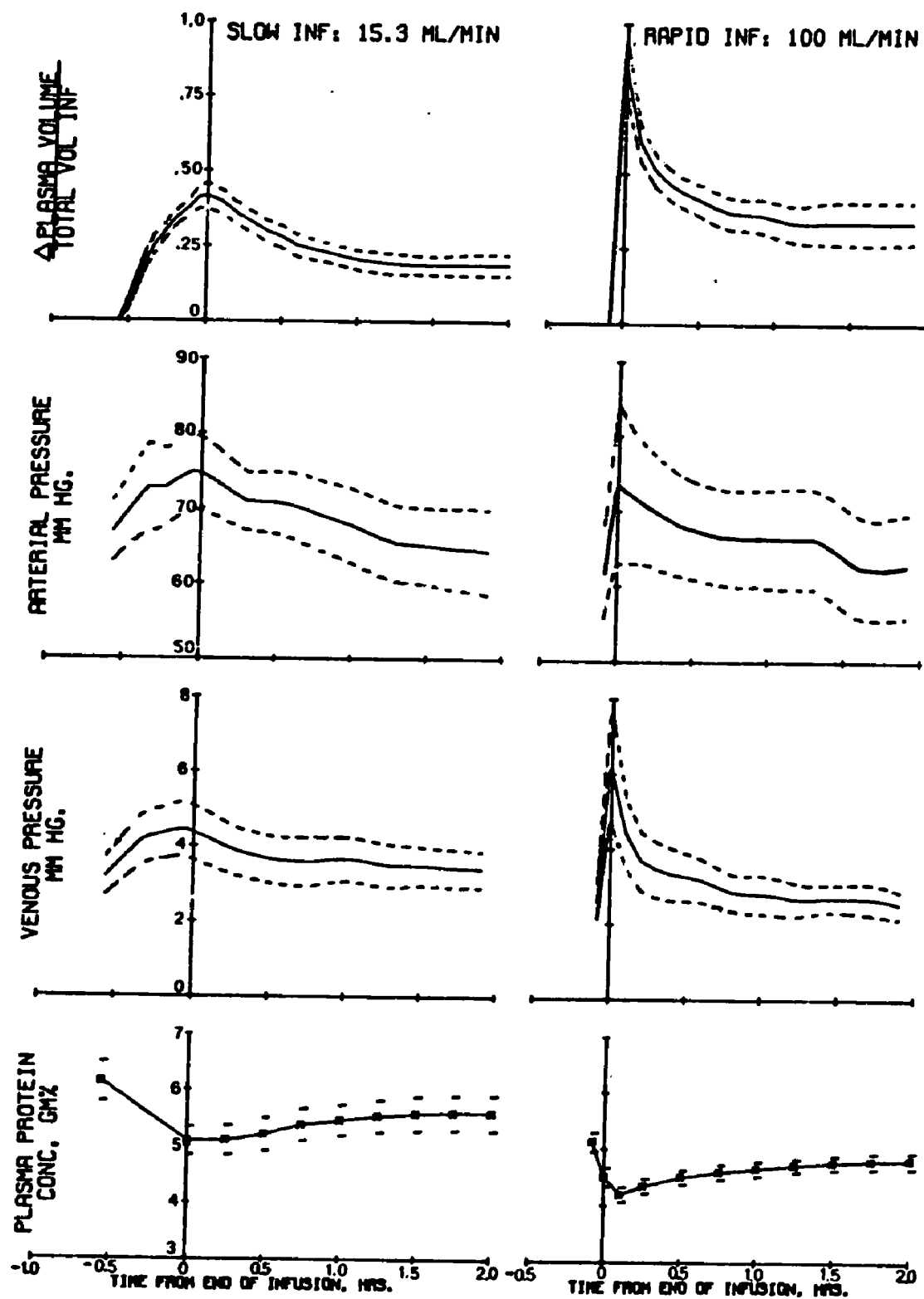


Figure 10.

INFUSION OF DEXTRAN SOLUTION

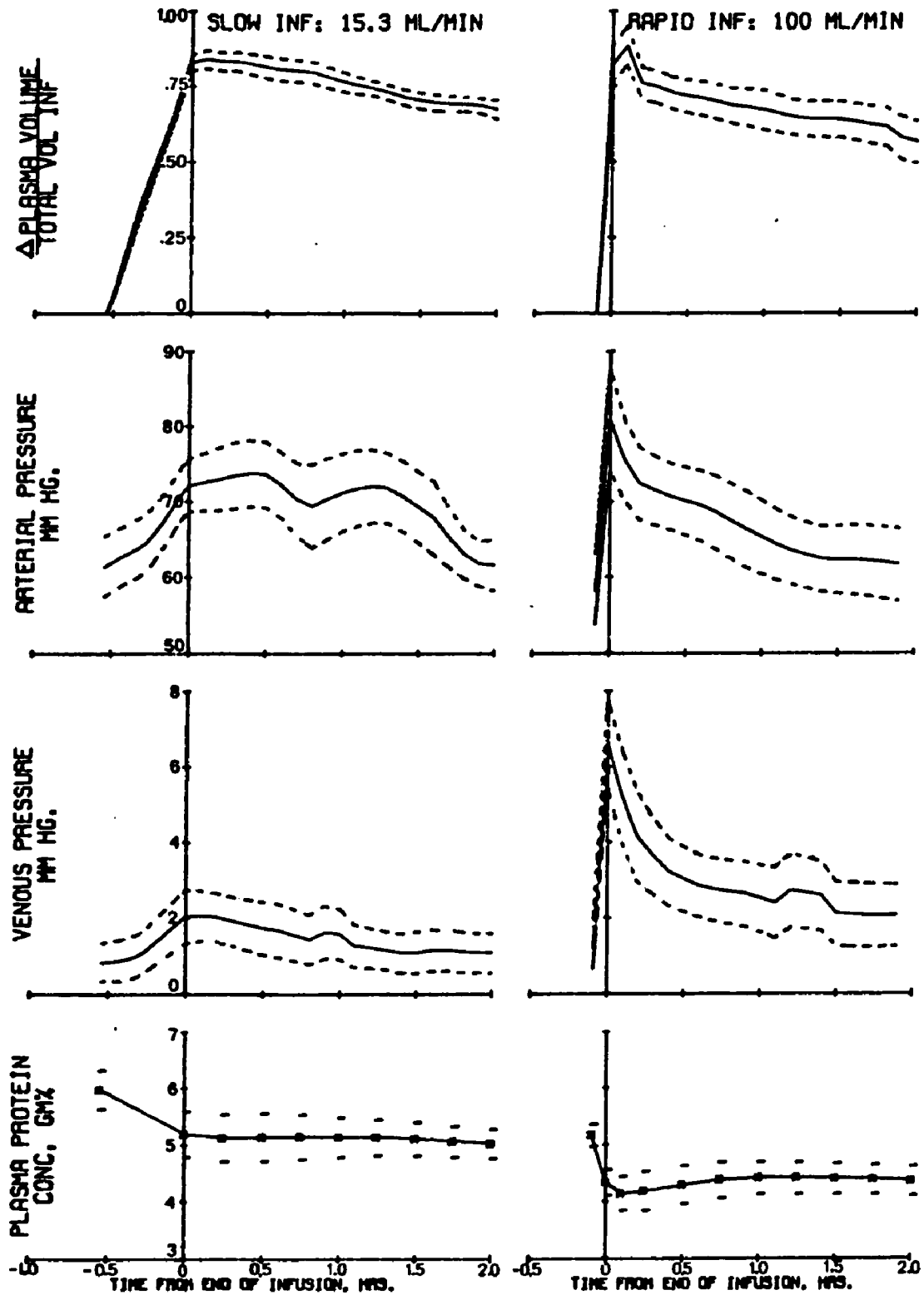


Figure 11.

a) The percent retention for slow and rapid Tyrodes infusions averaged 20% and 35% respectively at 2 hours although there was no statistically significant difference between these two groups (see Table II and Figure 18). On the other hand, the percentage of dextran solutions retained at 2 hours was 70% and 60% respectively, thus exhibiting more than twice the retention in the blood as that shown for Tyrodes.

b) The averaged arterial pressure response seems greater in magnitude and more sustained for dextran than for Tyrodes infusions. While this is true as well for the venous pressure, it is not as dramatic (see Figure 19).

c) The arterial pressure time constant of the return to baseline is approximately the same for both rapid and slow Tyrode's. In contrast, while dextran expands the plasma to essentially the same extent no matter what the infusion rate, the arterial pressure returns toward control much more quickly following the rapid infusion.

d) As expected, the protein concentration rebounds during the response to Tyrode's infusion, but remains depressed after the dextran infusion.

D. CARDIAC OUTPUT AND TOTAL PERIPHERAL RESISTANCE RESPONSES

The cardiac output (CO) responses following Tyrode's infusions in three animals are shown in Figure 12. Total peripheral resistance (TPR) was calculated using the expression:

$$TPR = \frac{P_a - P_v}{CO}, \text{ mm Hg/ml/min.}$$

In all experiments cardiac output increased and peripheral resistance decreased either during or immediately following the infusion. The recorded changes were more dramatic following the rapid, rather than the slow, infusions. The longer term (i.e. 2 - 3 hour) TPR response was inconclusive for slow infusions, the three experiments showing either a decrease, increase or no change, while during the rapid infusions the trend in all three experiments was to rise dramatically after an initial decrease. In two of the three experiments the rise following the rapid infusion was over 35% above control levels. This increase was due to a decrease in cardiac output either back to or below control level, while the systemic pressures remained slightly above control.

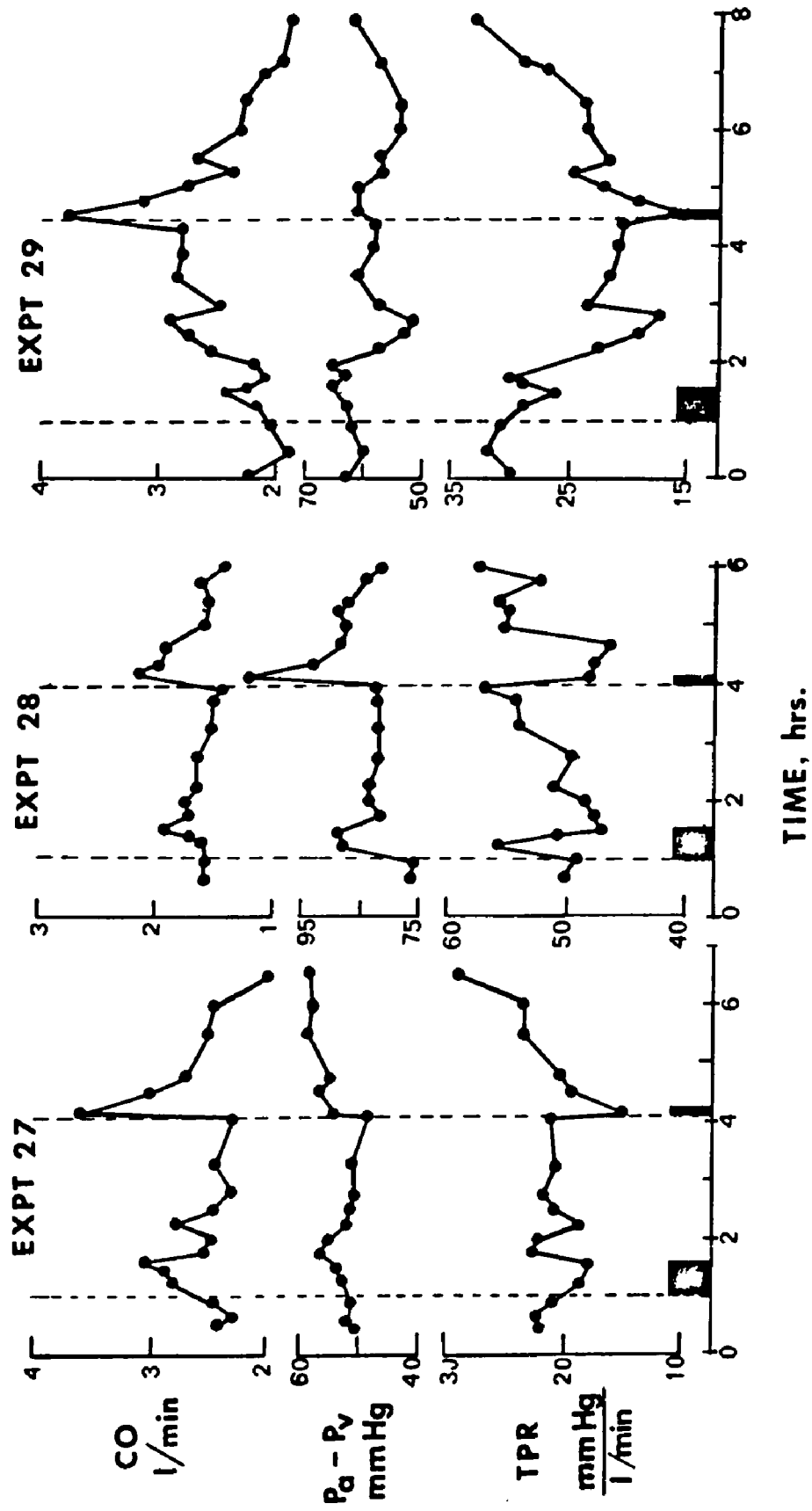


Figure 12. Cardiac output and total peripheral resistance responses following infusion of Tyrode's solution in three animals.

E. LYMPH FLOW AND LYMPH PROTEIN CONCENTRATION

The thoracic duct cannulation was successful in only one of the animals in which Tyrode's was infused and in five of the six animals of the dextran group. The measurements of lymph flow and lymph protein concentration are presented in Figure 13. (Experiment #22 was one of two experiments in which two rapid infusions were made).

Although statistically insignificant, there appears to have been a slight and gradual increase in lymph flow during the slow infusions of both Tyrode's and dextran solutions. In contrast, the rapid infusions induced the greatest lymph flow at the beginning of the response period (regarded as significant for dextran, by the sign test), but it appears to have been only a transient response, reaching as much as three times the control value for the Tyrode's infusion and then rapidly returning to resting levels. (Two individual rapid dextran infusions (not shown) reached peak values of twice the control values, but they also returned to resting values within 2 hours). The lymph protein concentration did not change dramatically from control over the long run, except for the rapid Tyrode's infusions in which the concentration decreased by as much as 50% over 2 hours.

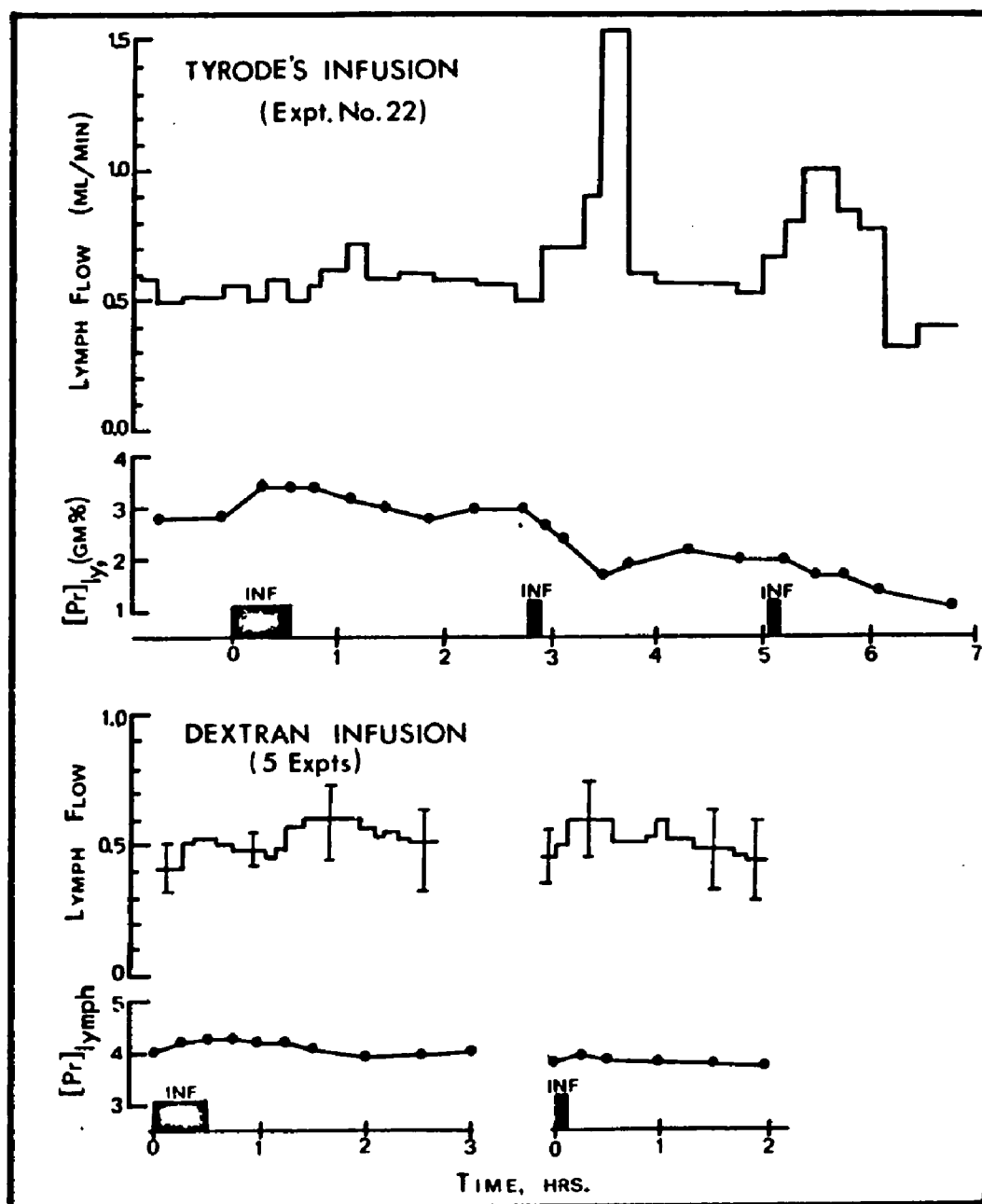


Figure 13. Lymph flow and lymph protein concentration responses following infusions of Tyrode's and dextran solutions.

V. DISCUSSION OF EXPERIMENTAL RESULTS

"The problems of physiology are so complicated that, to put it tersely, one cannot expect to be able to reason correctly from the facts for more than five minutes at a stretch." August Krogh (1959).

A. ACCURACY OF BLOOD VOLUME MEASUREMENT TECHNIQUE

The accuracy of the blood volume response depends on several important factors: a) the ability of labelled red cells to remain intact and evenly mixed in the circulation throughout the experimental period, b) the accuracy and constancy of the F_{cell} ratio, and c) the accuracy and reproducibility of successive blood activity measurements.

1. VARIABILITY IN RED CELL VOLUME

The results of Figure 9 and Table III indicate that the total circulating red cell volume deviates less than 7% from control even 8 hours after the tracer injection. The variation for each infusion experiment is about half of this amount. In addition, the scatter of data near the start of the infusion is no worse than it is elsewhere, in spite of the fact that both hematocrit (HCT) and blood activity (A) are changing dramatically (up to 30%) at these early times.

The red cell volume as calculated from Equation (III-3) should theoretically remain constant during an experiment regardless of the volume of blood removed for sampling or the volume of solution infused. If either cell free fluid were introduced by infusion or taken away by filtration into the tissue spaces, both labelled and unlabelled red cells would be diluted or concentrated to the same extent and the ratio, HCT/A, should remain constant. The data of Figure 9 suggests that there is a small increase in red cell volume during the course of the experiments. This is probably due to an increase in the actual ^{51}Cr red cell distribution volume which occurs as the labelled cells find their way into areas of the circulation, such as the bone marrow, which are slow to equilibrate. It is also possible that the infusion itself is forcibly opening up new areas. Lysing of the red cells is an alternative argument to account for RCV increase. However, most studies dealing with red cell life-span studies do not find this to be a problem even over a period of several months.

2. ACCURACY OF F_{cell} RATIO

The average value for the F_{cell} ratio obtained in these studies, 0.904, agrees well with the value of 0.90 obtained by Reeve, et al (1953a) and 0.88 obtained by Gillett and Halmaggi (1970) using splenectomized dogs. It appears from several previous studies that F_{cell} does not dramatically change during moderate changes of blood volume. Gillett and Halmaggi (1970) showed that the F_{cell} ratio falls after a hemorrhage-reinfusion experiment from 0.94 to 0.88 in intact dogs, but they did not repeat these experiments on splenectomized animals. Reeve, et al (1953b) infused 400-800 ml Dextran in splenectomized animals and showed little or no change in F_{cell} . Although Prather, et al (1969) did not measure F_{cell} itself, they report that the red cell volume remained constant at the beginning and end of two hour infusion-response experiments that were quite similar to the present study with the exception that the spleen and kidneys were left intact. The role of the spleen as a depot for red cell storage and its effects during infusion and anesthesia have been discussed by Reeve (1957) and Gilmore (1965).

3. ACCURACY OF THE COIL TECHNIQUE

Albert (1956) has demonstrated that the coil technique of measuring activity of a liquid is as accurate as the conventional test-tube method. In theory, there should be less variation between measurements of replicate samples because of the constant geometry of the system and lack of need to pipette fluid. There have been no previous reports on the use of the coil to measure blood activity continuously using an extra-corporeal shunt, although its use was suggested by Albert (1963). Used in this fashion, the resolution of continuous blood volume measurements is limited by the residence time of blood as it passes through the coil (1 minute in these experiments) and the total counts (10000 minimum) obtained during the time allotted for counting (1 minute). It is felt that this system could resolve successive samples greater than one minute apart. It is also reasonable to suppose that the relative errors involved in measuring blood volume changes are no greater than those shown for total red cell volume measurements (i.e. less than 4% over the course of a 4-hour infusion period) and perhaps much better since the blood volume, unlike the total red cell volume, does not depend on hematocrit measurements.

The absolute errors however, are impossible to assess from this information. Remington and Baker (1961) have found that successive measurements of blood volume, using separate injections of ^{51}Cr labelled cells, resulted in a standard deviation of less than 3% of the total blood volume. Donovan, et al (1964) found that the agreement between the plasma volume, as simultaneously measured by T-1824 and ^{51}Cr tagged cells, was better than 1%.

B. PLASMA VOLUME RESPONSE

The averaged results (Figures 10, 11 and 18) show that after 2 hours, high molecular weight dextran solutions exhibit about 2 1/2 times the retention in the blood as that shown for Tyrode's solution. This is easily explained by the presence of the large colloidal molecules in the dextran solutions that help maintain the plasma oncotic pressure. In contrast, Tyrode's dilutes the plasma proteins and reduces the oncotic gradient for reabsorption. This, coupled with the probable increase in capillary pressure during infusion, provides a larger driving force for transcapillary filtration than that associated with dextran. In fact, this high filtration gradient for Tyrode's causes half of the infused volume to leave the circulation by the end of the slow infusion and 15 minutes after the rapid infusion, reaching an apparent steady state after 1.5 hours. (This is in agreement with the suggestion of Deland and Bradham (1966). The fact that dextran solutions show a slow but continuous disappearance from the plasma is probably due to both a slow leak of colloids across the capillary membrane and a sustained increase in capillary pressure. It is not likely that metabolism of the dextran molecules affects the response since Wasserman and Mayerson (1952b) have shown that the metabolic half-time of dextran is 27 hours.

At steady state, the percent retention of the infused Tyrode's solution (25 ml/kg) is 20% and 35% for the slow and rapid infusions respectively (not a statistically significant difference). It should be noted that this represents only an average 9% increase in original blood volume. For nephrectomized dogs there are only meager data with which to compare these results. Bradham, et al (1964) infused 5 nephrectomized dogs with isotonic saline (20 ml/kg) and found that the percent retention in the plasma at steady state

was between 30 and 40% (avg = 36%). A similar study using only 2 nephrectomized dogs yielded values of 35% and 58% for saline (9ml/kg) and Hartmann's lactated Ringer's solution (16.5 ml/kg), respectively. These results essentially agree with those of the present study for the rapid infusion, but are higher than were obtained for the slow infusion. Our statistical analysis failed to find any difference in steady state values due to the rate of infusion. In contrast to these studies on nephrectomized dogs, others have shown that the diuretic response of intact animals allows the plasma volume to return to normal within 2 hours after infusion of saline or Tyrode's (Wasserman and Mayerson, 1952a; Prather, et al, 1969). Comparable studies in humans show a slower return to normal resting levels; between 4 and 32 hours (Moore, et al, 1966).

It is even more difficult to find data with which to compare the results from our dextran studies, as there have been virtually no studies reported that have used iso-oncotic dextran (about 2% wt concentration), nor any careful study on the effects of dextran concentration. It is now known that clinical dextran, 6% (MW = 70,000) or 10% (MW = 40,000), the most commonly used forms, are highly hyperoncotic (Prather, et al, 1969; Marty & Zweifach, 1970) and as such they cause an expansion of plasma volume up to several times more than the volume infused by osmotically attracting interstitial fluid. Hence, the disappearance of these solutions from the vascular compartment will be expected to be somewhat different than those obtained in the present study. The only known studies in which the arenal plasma response to infused dextran was reported show that at 2 hours the percent retention was 25% (range 0 - 69%) for average MW = 10,600, and 30% (range 0 - 61%) for average MW = 60,000 (Wasserman & Mayerson, 1952b; 1954). These results are lower than the 65% average retention we report for 2 hours after infusing dextran of MW greater than 80,000. A possible explanation for the discrepancy is the admittedly large variability in the molecular weight spectrum of the solutions used in Wasserman and Mayerson's studies. The fact that our plasma responses to D_{80} and D_{250} were statistically undistinguishable is not surprising in light of the studies of Grotte (1956), Renkin (1964) and Garlick and Renkin (1970). These investigators have observed that the capillary permeability to dextran remains essentially constant for molecular weights above 20,000 to 40,000.

C. BLOOD PRESSURE RESPONSE

Both arterial and venous pressures rise to peak values at or near the end of infusion and then decay to within ± 2 mm Hg of control levels within 2 hours (Figures 10 and 11). The magnitude and time constants of this often observed response are in excellent agreement with previously reported values (Gauer, et al, 1956; 1963; 1970). The peak values of the arterial response are many times greater than the corresponding venous response because of the greater compliance of the low pressure system. That the average pressure response is generally higher in the case of dextran, in contrast to Tyrode's, is undoubtedly due to the greater retention of the former infused volume.

There is also a difference in response behavior that depends upon the rate of infusion, particularly for dextran solutions. It is well known that the baroreceptors of the carotid sinus give a considerably greater response when the pressure is increasing rapidly than after they have adapted to a higher steady state value (Riggs, 1970). This rate sensitivity means that the rapid infusion activates the arterial pressure control system more effectively than the slow infusion. The data for dextran support this by showing a dramatic decrease in arterial pressure immediately after the fast infusion, but essentially no decrease until one hour after the slow infusion, in spite of the fact that dextran expands the plasma to essentially the same extent no matter what the infusion rate. The baroreceptor-sympathetic reflex pathway initiates a decrease in peripheral resistance and cardiac performance in response to an increasing arterial pressure. These events have been observed to occur immediately following Tyrode's infusions (Figure 12). The corresponding responses for dextran, while not measured, would be expected to be similar.

However, the neural reflexes that control arterial pressure via the carotid sinus become fully effective within one minute after the initiation of a pressure change (Prather, et al, 1969). This means that the observed gradual return of the arterial pressure to control values for both slow and fast infusion are probably not due to the action of the baroreceptor reflex pathway except for the first few minutes after infusion. In a similar study, Prather, et al (1969) have presented evidence strongly suggesting that stress relaxation (i.e. a delayed vascular compliance change) is responsible for the observed fall in arterial pressure, especially

during the latter portions of the experiment. Moreover, they show that the degree of stress relaxation is much greater for infused dextran solutions than for Tyrode's.

Other factors which might be responsible for the observed changes in circulatory variables following infusion are: a) increased transcapillary filtration, b) redistribution of blood, and c) local autoregulatory mechanisms. It is obvious that the decreased blood volume due to increased transcapillary filtration has much to do with the fall in blood pressure and cardiac output (Guyton, 1963). There is also a redistribution of blood among the various segments of the systemic and pulmonary circulations following any change in blood volume (Gauer, et al, 1970). This comes about because of the differences in compliance of these various segments, with most of the newly infused volume tending to be shunted to the low pressure, highly distensible venous segments. Little is really known about the nature of this time-varying redistribution, but it undoubtedly contributed to the changes observed in the blood pressure response. Finally, Mellander and Johansson (1968) have summarized the effects of increased pressure on peripheral autoregulatory mechanisms. In particular, the relatively slow myogenic response to increasing pressure will tend to increase peripheral resistance, thus counteracting to a certain extent the action of the rapidly adjusting centrally controlled baroreceptor reflex. It is not perfectly clear how these two opposing mechanisms will actually resolve themselves. Little is known about the long term effects of this local mechanism and nothing has been reported that shows the response to infused fluid. However, there is nothing to suggest that this response is responsible for a fall in circulatory pressure; in fact, just the opposite is indicated.

It has been observed that the arterial pressure following the slow infusions exhibits more or less random behavior as it decays toward control levels (see Figures 5 and 7). The sudden rising and falling of pressure in certain experiments was thought to be somewhat related to the various stages of anesthesia of the animals under the influence of Innovar. There seems to be little correlation between the changes in arterial pressure and the accompanying blood volume changes, as has been observed by other investigators measuring net capillary filtration or capillary pressure (e.g., Tarazi, 1970; Zweifach, 1971; Guyton, 1966b). Guyton suggests two reasons for the lack of correlation between arterial

pressure and filtration that might be applicable here: a) the pre-capillary resistance is carefully autoregulated so as to maintain a constant capillary pressure in the face of large changes in arterial pressure, and b) because of a high pre-/post-capillary resistance ratio the capillary pressure is so close to the venous pressure that the arterial pressure changes are damped before their effects are felt at the capillary level.

D. CARDIAC OUTPUT AND TOTAL PERIPHERAL RESISTANCE RESPONSES

On the average it appeared that there was no change in TPR and CO during the slow infusions although there was a considerable difference among the three experiments. The most consistent changes, however, were observed during the rapid infusion.

The cardiac output results for both slow and fast infusions are in excellent agreement with the observations reported by Guyton, et al (1958) and Prather, et al (1969), who concluded that rapid infusions of blood, saline, or dextran cause an immediate increase in venous return and consequently cardiac output by increasing the mean circulatory pressure and by reducing the resistance to venous return. They state that such effects are not seen to any great extent if infusions are given slowly, or even for more than a few minutes after a rapid infusion because: i) the blood volume returns toward normal due to transcapillary movement and ii) stress relaxation increases the vascular capacity and decreases mean systemic pressure.

Peripheral resistance responses to infusion have not been studied well, and it is unfortunate that these measurements were not obtained during all of our infusion experiments. The initial decrease in TPR that was observed in all experiments is probably the result of: 1) a passive distention of the vessels due to blood volume expansion and 2) active vasodilation via the baroreceptor-sympathetic pathway in response to the sudden increase in blood pressure. It has already been noted that the neural reflexes become fully effective within several minutes of a pressure stimulus and the present results tend to confirm this.

However, the most interesting result emanating from these studies is the observation of the dramatic increase of TPR in all three of the rapid infusions after an initial decline. The only comparable study that this author is aware of was performed by Conway (1966) in which infusions of

a blood-dextran mixture were administered in intact, unanesthetized, normovolemic dogs with and without autonomic blocking agents. His results exhibit trends similar to ours in cardiac output, blood volume and TPR responses. The mechanism responsible for the increased resistance is not perfectly understood, but probably is due to a delayed myogenic constriction in response to the increased blood flow and pressure in the peripheral tissues (Guyton, et al, 1971). This phenomena will be discussed further in Chapter VIII.

E. EFFECTS OF INNOVAR

Innovar, a neuroleptanalgesic, was chosen in this study mainly because of its ease of i.m. administration, its potent tranquilization and the analgesia produced, its ability to maintain a relatively constant blood pressure and the absence of cardiac depression (Corssen, et al, 1965). However, it is recognized that arterial hypotension follows administration of the drug, resulting from a moderate decrease in peripheral vascular resistance and an increase of peripheral vascular capacitance (Dixon, et al, 1970). These changes in vascular tone have been reported to stabilize within minutes of intravenous injection in the dog. Droperidol, one of Innovar's active ingredients, is known to block the alpha effects of epinephrine but not of norepinephrine. The most obvious effect of Innovar on our results was the depression of the mean arterial pressure to levels between 60-70 mm Hg.

F. LYMPH FLOW AND LYMPH PROTEIN CONCENTRATION

It has been previously reported that infusions of normal saline solutions induce rapid and transient increases in lymph flow in the dog (Starling, 1909; Wasserman & Mayerson, 1952a). However, no data were available to determine whether this occurred in nephrectomized dogs as well. The cause of the transient nature of this phenomena has never been satisfactorily explained. The increase in lymph flow has been attributed to increases in interstitial volume, interstitial pressure, circulatory pressure pulses, cardiac, respiratory or intestinal movements, active and passive muscle movements and adrenergic constrictor agents acting directly on the larger lymphatics (Rusnyak, et al, 1967; Guyton & Coleman, 1968;

Leandoer & Lewis, 1970). Certainly some of these stimuli have been present in these experiments.

If, as Guyton and Coleman (1968) and Gibson and Gaar (1970) have suggested, lymph flow can be considered to be a function of tissue pressure (and therefore of tissue fluid volume), one would expect lymph flow to be independent of infusion rate *pre se*, but rather a direct function of the quantity of infused fluid that disappears into the extravascular spaces. The present data show quite the opposite in certain instances. While Tyrode's solution has been shown to enter the interstitial compartment to a greater extent than dextran (for any infusion rate), the increases in lymph flow for the slow infusion are almost identical for both Tyrode's and dextran. On the other hand, the rapid Tyrode's infusions show a greater increase in flow than the rapid dextran, although lymph flow returns to normal in both cases, in spite of the fact that the interstitial space has presumably been expanded more by the Tyrode's solution. These results indicate that other factors than changes in tissue volume or mean tissue pressure have important effects on lymph flow.

Additionally, the changes in lymph flow are in the opposite direction from what would be expected if circulating pressure pulses were its major determinant. The pulse pressures induced by rapid dextran infusions were observed to be greater than those associated with rapid Tyrode's infusions, and yet the flow of lymph increased less for dextran.

The observations we have made, admittedly based on meager evidence, add to the belief that lymph flow changes are due to more than one factor, and that little is really known about the relative significance of these factors. An hypothesis that might account for the data we have obtained is the following: It has been suggested that the bulk of tissue fluid is tightly held to the ground substance by surface forces and cannot be considered free or mobile (Guyton & Coleman, 1968). The transient nature of lymph flow may be postulated to arise from the filtered fluid first drastically increasing the mobile fluid phase and then, as it diffuses through the tissues, becoming part of the immobile phase. An increase in mobile fluid is likely to decrease the resistance of the tissues to convective movement (Guyton, et al, 1966a) and increase the flow of fluid into the lymphatic capillaries. If the rate of filtration is slow, the excess fluid may have time to become "bound" and unavailable to the lymphatics.

The flow of lymph would depend upon both the filtration rate and the amount filtered. This mechanism could account for a greater change in lymph flow with Tyrode's infusion than dextran as well as explaining the difference in lymph flow response between fast and slow infusion rates.

Lymph protein concentration decreased substantially during the rapid Tyrode's infusions. In fact, the decrease was so severe that it brings into question the traditional model of extracellular fluid and protein dynamics. According to this view fluid filters across the capillaries and mixes with the interstitial fluid prior to entering the lymphatics (Landis & Pappenheimer, 1963; Renkin, 1964). Assuming that lymph and tissue fluid are identical, and that the net quantity of interstitial protein is relatively constant during the 2 hour experiment, it can be calculated that the observed change in lymph concentration would entail a tissue fluid volume available for mixing of less than 1000 ml. However, the total interstitial fluid volume in these animals is at least 4 times this amount! This may signify that lymph fluid is not representative of the total tissue fluid but rather lymph represents the smaller mobile, rather than the immobile, fluid phase. This is somewhat similar to Wiederhielm's (1968) suggestion that the protein space is smaller than the total interstitial fluid space.

An alternate explanation for the dramatic decrease in lymph concentration is that during infusion a disproportionate amount of thoracic lymph may emanate from the muscle tissues. It is known that the capillary protein permeability of muscle is very low, leading to a muscle lymph protein concentration which is normally less than 1/2 that of the thoracic duct (see Table I). Gibson and Gaar (1970) have shown that during infusions the lymph flow from muscles can increase up to 30 times resting values, while maximum thoracic lymph flow is only 15 times normal (Mayerson, 1963). However, no comparable studies have been performed with other organs.

G. SUMMARY

The extracellular system's response to infusions may be summarized by considering the temporal sequence of events. The primary effects are: 1) the expansion of blood volume, 2) increase of circulatory pressures, 3) passive distension of the vessels, followed quickly by 4) an increase in cardiac

output. Secondary to this are certain defense mechanisms, both active and passive, which are brought into play and tend to counteract these effects, bringing the circulatory system to a new steady state: 1) there is an increase in vascular capacity caused in part by central reflexes, whose effects last only for several minutes, and in part by stress relaxation, an intrinsic mechanism, which is more delayed in onset and more lasting in its effects; 2) a redistribution of blood into the more distensible regions of the vasculature aids in reducing arterial pressure and the load on the heart; and 3) an increase in transcapillary filtration, which reduces the excess circulatory volume. Other factors which may be considered to be more delayed tertiary homeostatic mechanisms to protect the integrity of the interstitium include: 4) myogenic autoregulation at the local organ level with readjustment of the pre-/post-capillary resistance ratio and capillary surface area ostensibly to limit the rapid filtration into the tissues, and 5) a rise in tissue pressure which also aids in limiting filtration in addition to increasing lymph flow, thus minimizing fluid loading of the interstitium.

VI. THE MATHEMATICAL MODEL AND SIMULATION PROCEDURE

"The art of model building and model working is to put only into the models those things you can understand and see how far you can go with them. When those are reasonably understood, or when their inadequacies are understood, then the next stage of complexity can be attempted. I think it is a mistake to try to start from a too realistic system in which everything is there." (Eugene M. Renkin, 1968, from a discussion of the paper).

A. THE PHYSIOLOGICAL MODEL

Figure 14 depicts in schematic form the physiological-anatomical relationship of elements involved in fluid exchange between the blood and tissue spaces. The solid arrows indicate the net direction of flow of fluid and colloids in an animal at rest. The dashed arrows represent fluid movement within the tissues due to filtration and reabsorption according to the traditional Starling model. The capillary wall and surrounding basement membrane form a barrier inhibiting, but not eliminating, exchange of fluid and proteins between the blood and the interstitial fluid. The capillary is the only site of exchange between these two compartments, except at those points where lymph is returned. It is generally recognized that these latter sites are in the low pressure

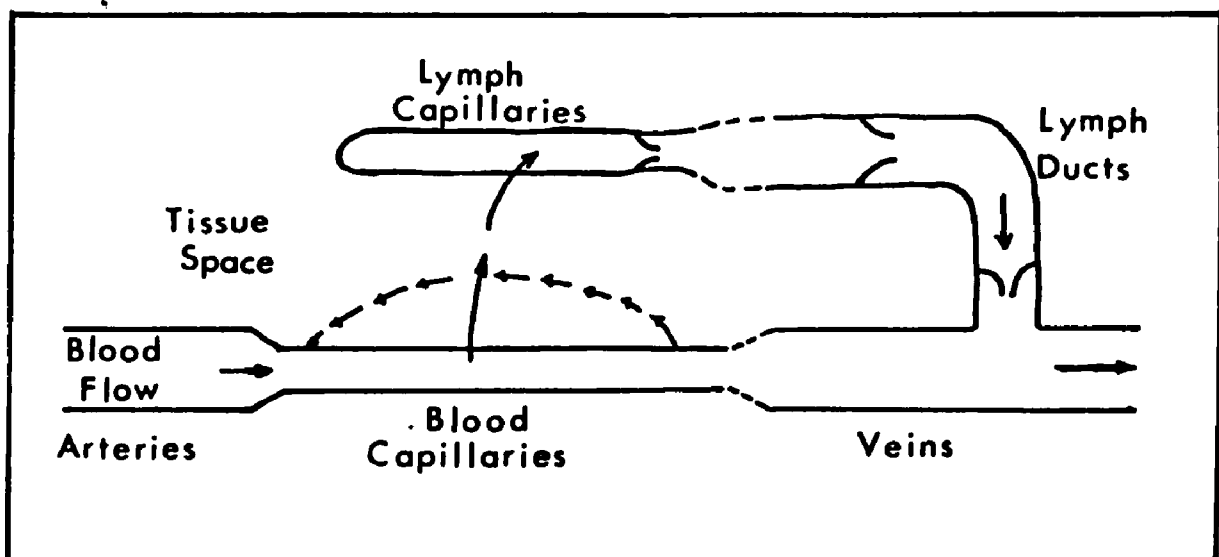


Figure 14. Schematic of Plasma-Interstitial-Lymph Fluid Circulation

venous side of the circulatory system. Also shown are the dead-ended lymphatic capillaries that collect interstitial fluid before passing it into the lymphatic duct system.

B. THE COMPARTMENTAL MODEL

The physiological system can alternately be visualized as a two compartment system as illustrated in Figure 15. The extracellular space is shown divided into two distinct compartments -- the plasma compartment and the interstitial compartment -- separated by the capillary membrane of surface area, A . Each compartment is characterized by certain state variables: a fluid volume, V , a protein concentration, C , and a fluid pressure, P . The colloid osmotic pressures, π , are well known functions of the protein concentration.

Each of these compartments have volumes which are allowed to vary in accordance with physical laws that relate the state variables. These laws have already been discussed in Chapter II. The actual model that has been adopted for the purpose of mathematically simulating the infusion experiments will be summarized below. This will be a lumped parameter model in which regional differences in such parameters as capillary membrane permeability, tissue compliance and lymph flow are not considered, but rather average values are assumed for the entire body.

The arrows connecting the two compartments show the net directions of exchange. Fluid movement across the capillary membrane occurs by ultrafiltration which is described by the Starling relationship. Net filtration is proportional to the filtration conductivity coefficient, $K_w A$, and the differences between the hydrostatic and colloidal osmotic pressures of the two compartments. Plasma proteins -- the major macromolecules of the circulation -- will be considered to be transported across the capillary membrane by restricted diffusion which is proportional to the capillary protein permeability, $K_p A$. Both diffusion and ultrafiltration can theoretically transport material in both directions across the capillary wall. However, lymph flow returns fluid and protein only in one direction -- from the tissue spaces to the plasma. Lymph flow is assumed to be related to the volume of tissue fluid. The protein concentration of lymph is taken to be identical to that of the interstitium.

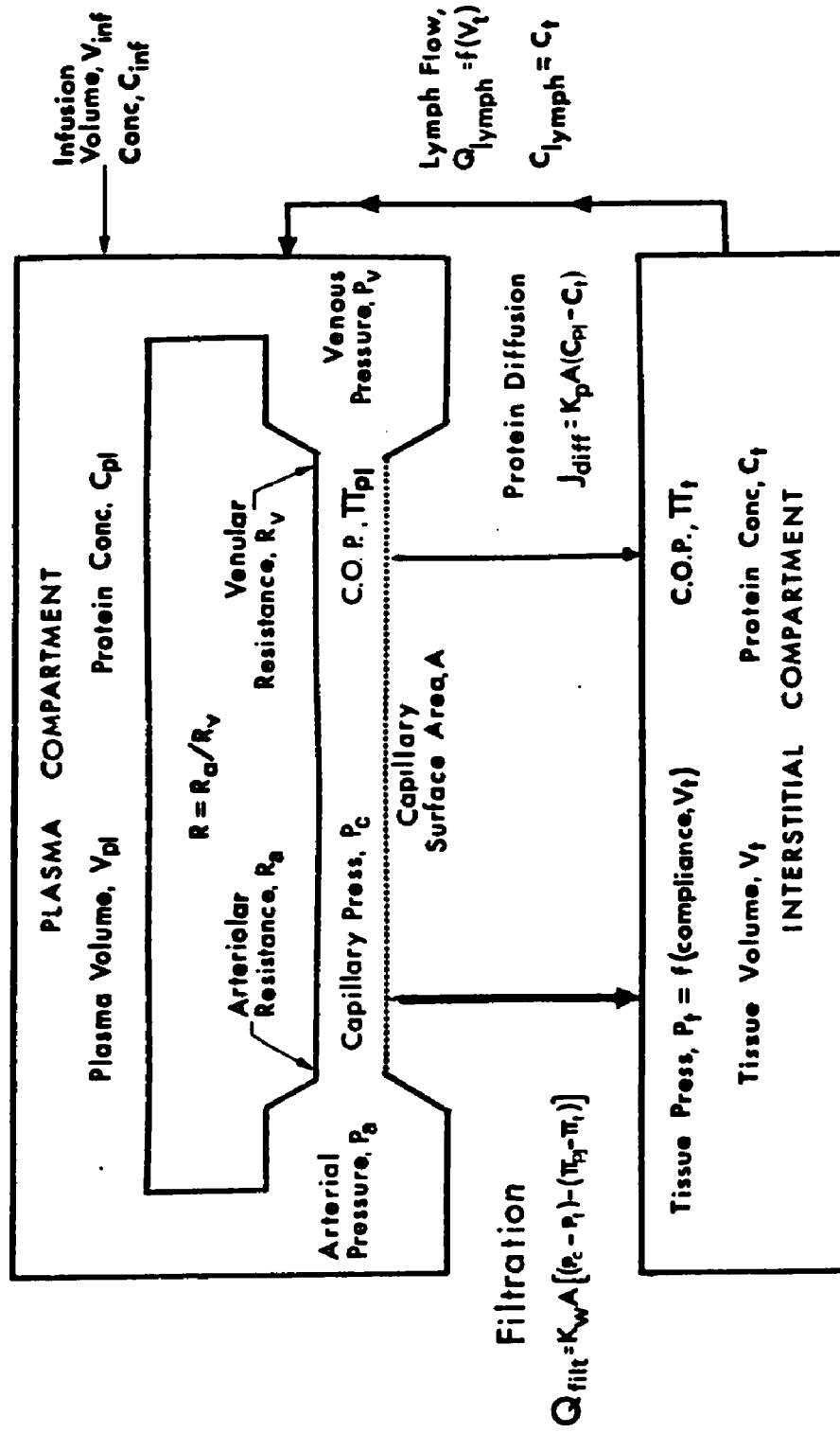


Figure 15. Compartmental model of the extracellular system.

Resistance upstream of the capillary bed, R_a , is located in the arterioles and is usually the major resistance element in the entire circulation. Downstream resistance, R_v , is located in the postcapillary venules. The capillary pressure at any time is dependent not only on the arterial and venous pressures, but also on the ratio of the pre-/postcapillary resistance ratio, R .

The infused fluid is administered intravenously and enters the plasma compartment. Infusion of Tyrode's solution decreases the colloidal concentration of plasma, thus favoring increased net ultrafiltration but decreasing the gradient for protein diffusion. In the case of iso-oncotic dextran solutions, the colloidal concentration remains essentially constant. In either case, there is a transient increase in circulatory pressures which enhances the flow of filtrate into the tissue spaces. As fluid and protein leave the circulation at rates dependent upon their respective permeabilities, tissue pressure increases, inhibiting further filtration. At the same time an increase in lymph flow limits the rise in tissue pressure and repletes, in part, the circulatory fluid and protein previously lost by capillary exchange. Fluid movement is affected by proteins to the extent that they affect the oncotic pressure gradient, while protein movement is affected by fluids to the extent that the fluid volumes determine the concentration gradient. During unsteady state conditions, changes in fluid volume and protein mass are dependent on the instantaneous exchange rates of fluids, Q , and proteins, J , across the capillaries and through the lymph system. Because of the numerous exchange pathways and interrelating mechanisms, prediction of the transient behavior following an infusion requires a rather complex analysis. Until recently this degree of complexity has not been recognized (Nims, 1966).

In previous attempts to simulate mathematically transcapillary exchange of fluids and proteins, two somewhat different concepts of the capillary were considered. Wiederhielm (1968) described the capillary as a two segment tube whose arterial and venous ends differ with respect to permeability, surface area and hydrostatic pressures. Although this concept of the capillary is in keeping with more recent physiological findings, it has not been demonstrated that it is clearly different with regard to simulation behavior than Guyton & Coleman's (1967) uniform cylinder with homogeneous characteristics. For this reason, as well as the conceptual simplicity of a uniform capillary, we will consider a basic capillary model of constant diameter, permeability and fluid pressure. A second

difference between these two studies is that Guyton & Coleman and Wiederhielm postulated different functional relationships for tissue compliance. The effects of both of these relationships on tissue pressure and lymph flow will be examined in our analysis.

The following section describes in detail the material balances, rate equations, empirical relationships and assumed parameter values necessary to translate the compartmental model just described into a working computer program for simulating the fluid changes following infusion. Time-varying experimental data, as well as assumed initial conditions, will be used as input data. Dynamic parameter optimization techniques will be used to simulate the behavior of the capillary surface area, A , and the pre-/postcapillary resistance ratio, R .

C. THE MATHEMATICAL MODEL

Figure 16 is a flow diagram depicting the mathematical operations for an analysis of short term plasma-interstitial fluid exchange dynamics. This diagram is similar to an analog computer circuit description and differs from the compartmental diagram (Figure 15) in that it depicts the flow of information rather than materials. The system can be conceptually divided into three sections: Blocks 1 - 10 are concerned with variable input data, Blocks 11 - 17 are concerned with fluid movement and Blocks 19 - 26 are concerned with protein movement. Each element can be described by a mathematical input/output function. In general, the input data consist of rate and duration of infusion as well as experimentally determined mean arterial and venous blood pressure data, while output data consist of the response of any of the variables shown, although we are particularly interested in the disappearance of plasma volume, V_{pl} .

Input Data

Blocks 1 and 2 represent the arterial and venous pressures, P_a and P_v , respectively. These are the time-varying responses of the cardiovascular system experimentally measured during the infusion. Blocks 3 and 4 are multiplying coefficients representing functions of the pre-/postcapillary resistance ratio, R , as described by Landis and Pappenheimer (1963).

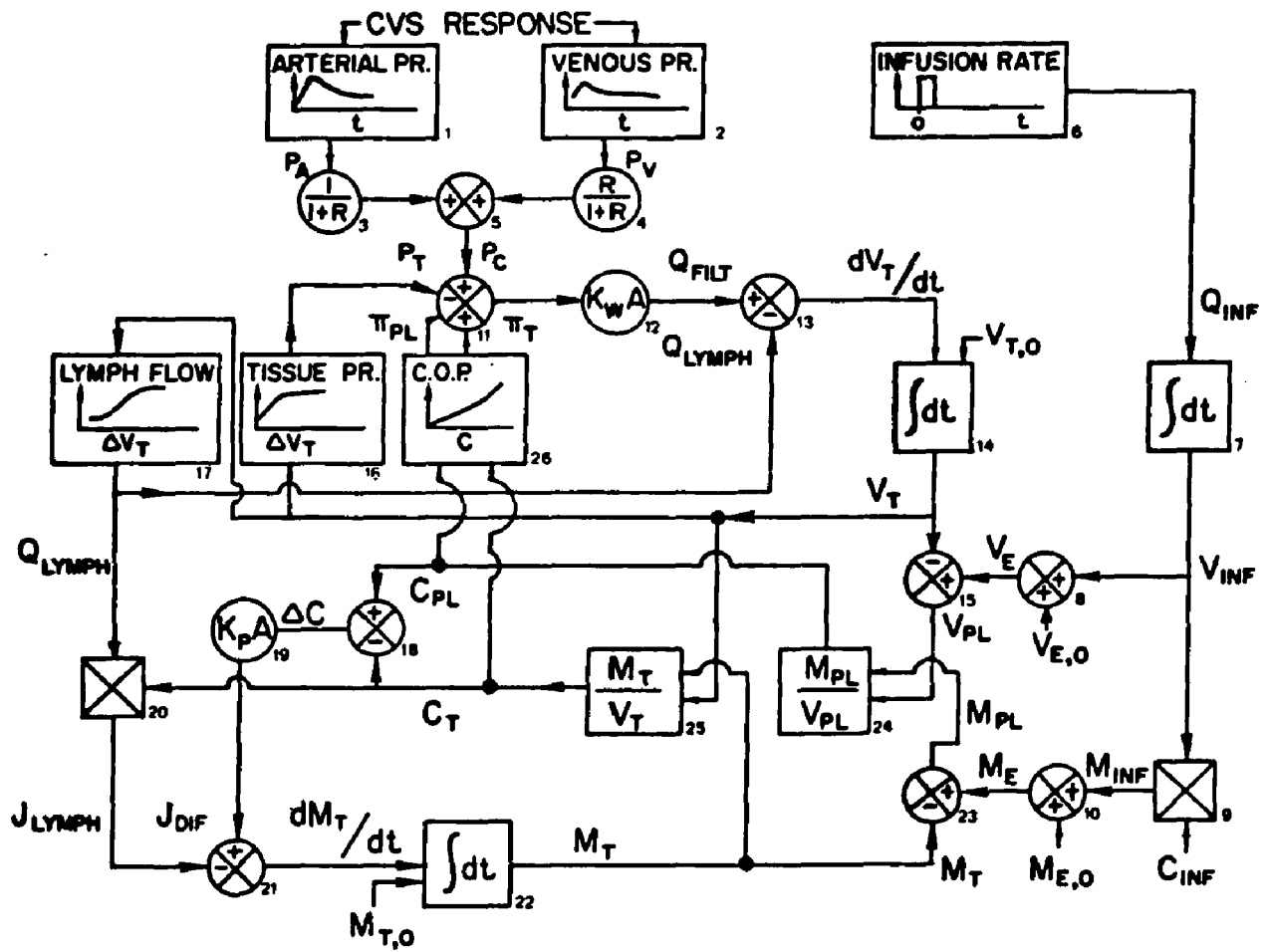


Figure 16. Operational analysis flow diagram of the plasma-interstitial fluid exchange model.

The capillary pressure, P_c , is estimated in Block 5 by the linear combination:

$$P_c(t) = \left[\frac{1}{1+R}\right]P_a(t) + \left[\frac{R}{1+R}\right]P_v(t) \quad (\text{VI-1})$$

Intravenous infusion is given by the infusion rate pulse function, Q_{inf} , of Block 6, which is integrated over time in Block 7 to produce an infusion volume, V_{inf} . The infusion volume is added to the original extracellular volume, $V_{e,o}$, in Block 8 and multiplied by the equivalent protein to give the mass of proteins infused, M_{inf}^1 , in Block 9. The latter in turn is added in Block 10 to the original mass of extracellular proteins, $M_{e,o}$. The effect of the infused solution on the system is to increase both the extracellular volume, V_e , and the extracellular protein mass, M_e , during the period of infusion, ΔT_{inf} . V_e and M_e are defined by the following expressions:

$$V_e = V_{e,o} + V_{inf} \quad (\text{VI-2})$$

where

$$V_{inf} = \int_0^t Q_{inf} \{h(t) - h(t - \Delta T_{inf})\} dt \quad (\text{VI-3})^2$$

and

$$M_e = M_{e,o} + M_{inf} \quad (\text{VI-4})$$

where

$$M_{inf} = V_{inf} \times C_{inf} \quad (\text{VI-5})$$

¹Note: $C_{inf} = 0$ for Tyrode's solution.

² $h(t) - h(t - \Delta T_{inf})$ is a pulse function whose value is zero for $0 < t < \Delta T_{inf}$ and 1.0 for the infusion period, $0 < t < \Delta T_{inf}$.

Fluid Exchange

The four pressures which determine the net driving force for fluid movement across the capillary wall, [capillary pressure, P_c , plasma oncotic pressure, π_{pl} , tissue pressure, P_t , and tissue oncotic pressure, π_t]³, are added in Block 11 in accordance with Starling's law. The resultant driving force for ultrafiltration is multiplied by the capillary filtration coefficient, $K_w A$, in Block 12 to obtain the rate of fluid filtration across the capillary, Q_{filt} :

$$Q_{filt} = K_w A (P_c - P_t + \pi_t - \pi_{pl}) \quad (VI-6)$$

The interstitial fluid compartment is assumed to exchange fluid with the plasma compartment via capillary and lymph exchange. There is considered to be no net exchange between the interstitial and intracellular compartment. Hence, a material balance on the fluid in the interstitial compartment, shown in Block 13, is given by:

$$dV_t/dt = Q_{filt} - Q_{lymph} \quad (VI-7)$$

where

dV_t/dt = rate of change of tissue fluid volume,

Q_{lymph} = rate of lymph flow.

The output of Block 13 is integrated at Block 14 to give the value of the interstitial volume, V_t :

$$V_t = \int_0^t (dV_t/dt) dt + V_{t,o} \quad (VI-8)$$

where $V_{t,o}$ = interstitial fluid volume prior to infusion. Inasmuch as the extracellular fluid is distributed only between the plasma and tissue

³The prefix "tissue" will be used interchangeably with the term "interstitial", designated by subscript, "t". Similarly variables relating to the plasma compartment will be appendaged by subscript "pl". The symbol "t" used anywhere other than as a subscript refers to the independent variable, time (i.e. $P_a(t)$, dt , etc.).

compartments, the plasma volume may be computed in Block 15 as:

$$V_{pl} = V_e - V_t \quad (VI-9)$$

The pressure of the fluid in the tissue compartment is determined by the compliance, γ , (the change in volume per unit change in pressure) of that compartment and the volume of fluid filling the compartment, i.e.,

$$\Delta P_t = \frac{1}{\gamma} \Delta V_t$$

If we let $P_{t,o}$, the tissue pressure existing when the tissue fluid volume is $V_{t,o}$, be arbitrary reference values, we can write:

$$\Delta P_t = P_t - P_{t,o}$$

and

$$\Delta V_t = V_t - V_{t,o}$$

Hence,

$$\Delta P_t = \frac{1}{\gamma} (V_t - V_{t,o}) + P_{t,o} \quad (VI-10)$$

Block 16 represents this relationship in which the tissue pressure can be calculated from the change in interstitial volume. For convenience, $P_{t,o}$ and $V_{t,o}$ are taken as the pre-infusion values. The actual values of γ and $P_{t,o}$ are currently under considerable dispute. Although in the present analysis the changes in tissue pressure are more important than the absolute values, the absolute value of compliance is a critical factor affecting the course of transcapillary filtration.

There have been no correlations, either empirical or theoretical, that successfully describe the flow of lymph under all conditions, and in particular, as a result of infusions. Both Guyton & Coleman (1967) and Wiederhielm (1968) suggest that lymph flow is a function of tissue pressure, although the data from which they obtained their correlation is open to some question. Inasmuch as tissue pressure was not measured in the present study, but changes in tissue fluid volume, as well as lymph flow, were,

it was possible to make a correlation based on our own experimental observations, i.e.,

$$Q_{\text{lymph}} = f(\Delta V_t) \quad (\text{VI-11})$$

This relationship is represented in Block 17. The describing functions in Blocks 16 and 17 will be discussed in detail later in this chapter.

Protein Exchange

In the present study, we have made the following assumptions with regard to macromolecular exchange:

- 1) transcapillary exchange of proteins and dextran are due entirely to restricted diffusion, similar to the approach taken by Guyton & Coleman (1967) and Renkin (1964b),
- 2) the infusion solutions containing dextran were of a concentration sufficient to make them iso-oncotic with canine plasma (20 mm Hg) and were considered to act in all other respects like plasma,
- 3) endogenously introduced dextran has the same capillary permeability as the plasma proteins,
- 4) the macromolecular concentration of lymph is the same as that of interstitial fluid, i.e. $C_{\text{lymph}} = C_t$.

With these assumptions we can write the equations governing the movement of protein between plasma and interstitial compartments.

The rate of protein movement across the capillary wall, J_{dif} , is proportional, in the case of restricted diffusion, to the concentration driving force across the capillary membrane and to the membrane permeability:

$$J_{\text{dif}} = K_p A (C_{p1} - C_t) \quad (\text{VI-12})$$

where

$K_p A$ = membrane permeability-surface area product (ml/sec)

C_{pl} = concentration of protein in plasma, gm/ml

C_t = concentration of protein in tissues, gm/ml

This equation is represented by Blocks 18 and 19.

Protein and fluid in the interstitium is assumed to be perfectly mixed prior to entering the lymphatics. The flow of protein through the lymphatic system and back to the plasma compartment, J_{lymph} , is given in Block 20 as the multiplication of lymph flow and lymph (or tissue) protein concentration:

$$J_{lymph} = Q_{lymph} \times C_t \quad (VI-13)$$

A material balance on the mass of protein in the tissue compartment, M_t , gives the rate of change of M_t as shown in Block 21:

$$dM_t/dt = J_{dif} - J_{lymph} \quad (VI-14)$$

Integration of Equation (VI-14) is performed in Block 22 where $M_{t,0}$ is the initial mass of protein present prior to infusion.

$$M_t = \int_0^t (dM_t/dt) dt + M_{t,0} \quad (VI-15)$$

Having calculated M_t , it is now possible to find M_{pl} , the protein mass in the plasma, as well as C_{pl} and C_t , the protein concentrations in each compartment. These calculations are performed in Blocks 23, 24, and 25, respectively.

$$M_{pl} = M_e - M_t \quad (VI-16)$$

$$C_{pl} = M_{pl}/V_{pl} \quad (VI-17)$$

$$C_t = M_t/V_t \quad (VI-18)$$

Finally, the osmotic pressures due to protein, π_{pl} and π_t , of the plasma and tissue fluids respectively, can be obtained from the protein concentrations as given by a modification of Van't Hoff's law (Landis & Pappenheimer, 1963):

$$\pi_{pl} = 2.10 C_{pl} + 0.16 C_{pl}^2 + 0.009 C_{pl}^3 \quad (VI-19)$$

$$\pi_t = 2.10 C_t + 0.16 C_t^2 + 0.009 C_t^3 \quad (VI-20)$$

Block 26 depicts both of these relationships.

In the simulated experiments in which dextran was infused it was necessary to distinguish between exogenous dextran and endogenous proteins. For this reason Equations (VI-12) to (VI-18) were solved two times, once for each of these macromolecular species. In addition, the colloid osmotic pressures were considered to be functions not simply of the protein concentration but to be the sum of the protein concentration and of a protein concentration whose colloid osmotic pressure was equal to that of the dextran.

D. VARIABLES AND PARAMETERS OF THE MODEL

1. OUTPUT VARIABLES

The twenty equations just described (Equations VI-1 to VI-20) enable us to find solutions for the twenty time-varying quantities which appear on the left side of the equality sign.⁴ It would be especially useful, however, to obtain solutions for those variables for which we have experimental data. These include $V_{pl}(t)$, $C_{pl}(t)$, $C_t(t)$, $M_{pl}(t)$ and $Q_{lymph}(t)$. But we are particularly interested in the behavior of the plasma volume, since data about its short-term response were previously unavailable. Furthermore, a model capable of predicting the disappearance rate of infused fluids could have extended clinical application.

2. PARAMETER OPTIMIZATION

All the variables that describe this highly nonlinear system can either be related to each other by acceptable mathematical expressions

⁴Complete definitions and units of each variable can be found in section entitled Symbols and Notation (Appendix).

or are otherwise known directly from experimental results, with the exception of the pre-/postcapillary resistance ratio, R , and the capillary surface area, A . Both of these variables are under various degrees of homeostatic control, and are known to be time varying quantities.⁵ The effective capillary surface area, which in the living animal can be controlled by precapillary sphincter tone, and the resistance ratio, which depends upon the diameter of the small vessels, are both under strong central and local control (see Chapter II-A3). Their dynamic behavior is not well understood. Changes in these quantities, which are very difficult to obtain experimentally, were not measured in the recent study. Rather, R and A were estimated analytically by treating them as free parameters during an optimization procedure that will be described in a later section. This procedure permitted these parameters to be time variant during and after infusion. Pre-infusion control values, designated as R_0 and A_0 , were obtained from the literature.

3. INPUT DATA

The input data required by the model were comprised of either initializing constants, time-varying quantities (i.e. forcing functions), or describing functions. For purposes of discussion this data can be divided into three categories: 1) Experimental input data were obtained during the experimental phase of this study; 2) Assumed data were obtained from previous studies; and 3) Calculated initial conditions were derived from the steady state solution of the equations presented in the last section using certain data obtained from 1) and 2). The variables in each of these categories are summarized below.

EXPERIMENTAL INPUT VARIABLES:

Forcing Functions

$Q_{inf} h(t) - h(t - \Delta T_{inf})$ = infusion rate pulse function

$P_a(t)$ = mean arterial pressure

$P_v(t)$ = mean venous pressure

Initializing Constants

$V_{pl,o}$ = pre-infusion plasma volume

$C_{pl,o}$ = pre-infusion plasma protein concentration

⁵See page 121, footnote.

(Initializing Constants, cont'd)

C_{inf} = equivalent protein concentration of infusate

BW = body weight of animal

Describing Function

$$Q_{lymph} = f_1(\Delta V_t)$$

ASSUMED INPUT VARIABLES:

Describing Function

$$P_t = f_2(\Delta V_t)$$

Initializing Constants

K_w = capillary filtration constant

K_p = permeability constant for transcapillary protein transport

A_o = pre-infusion capillary surface area

R_o = pre-infusion pre-/post capillary resistance ratio

CALCULATED INITIAL CONDITIONS

$V_{t,o}$ = pre-infusion tissue fluid volume

$V_{e,o}$ = pre-infusion extracellular fluid volume

$M_{t,o}$ = pre-infusion mass of tissue proteins

$M_{e,o}$ = pre-infusion mass of extracellular proteins

$P_{t,o}$ = pre-infusion tissue pressure

4. DESCRIBING FUNCTIONS FOR TISSUE AND LYMPH PARAMETERS

Blocks 16 and 17 of Figure 16 describe two relationships defining respectively, the tissue pressure and lymph flow as functions of tissue fluid volume. Figures 17a and 17b describe these relationships in more detail.

a. Tissue Pressure Function

Values of tissue compliance were obtained from two sources as shown in Table IV, and these are illustrated in Figure 17a.

TABLE IV
VALUES OF TISSUE COMPLIANCE USED IN SIMULATION

<u>Tissue Compliance</u>	<u>Method Obtained</u>	<u>Reference</u>
A) 96 ml/mm Hg-kg BW	Micropipette Puncture	Wiederhielm (1968)
B) 4 ml/mm Hg-kg BW for increase in $V_{t,o} < 15\%$	Implanted capsules	Guyton (1965a)
96 ml/mm Hg-kg BW for increase in $V_{t,o} > 15\%$		

As can be seen from Figure 17a, there is a considerable difference between these relationships. Both functions were examined in our analysis. The resting tissue pressure, $P_{t,o}$, measured with the micropipette method is often up to several mm Hg above atmospheric in contrast with the -3 to -9 mm Hg observed with the implanted capsule technique. However, changes in capillary filtration rate are influenced by changes from resting tissue pressure levels rather than the absolute value. That is why the ordinate in Figure 17a has been normalized by using the function, $P_t - P_{t,o}$.

b. Lymph Flow Function

In the present study, lymph flow was measured simultaneously with the volume of plasma that disappeared from the circulation following Tyrode's or dextran infusions. In Figure 17b we have correlated relative lymph flow, measured at various time intervals, with the corresponding increases in tissue fluid volume (i.e. decreases in plasma volume). The solid line is drawn through the average values of this data. The scatter of data reflects the fact that following infusion, lymph flow may reach a maximum value, that is, it does not always continue to increase even though the tissue fluid volume is apparently increasing.

Also shown on this graph is data from Wasserman and Mayerson's study (1952a) in which they showed that the peak value of lymph flow is approximately proportional to the volume of saline infused. (Their original data was modified for this correlation by assuming that 60% of the volume infused had entered the interstitium at the time peak lymph flows were achieved.)

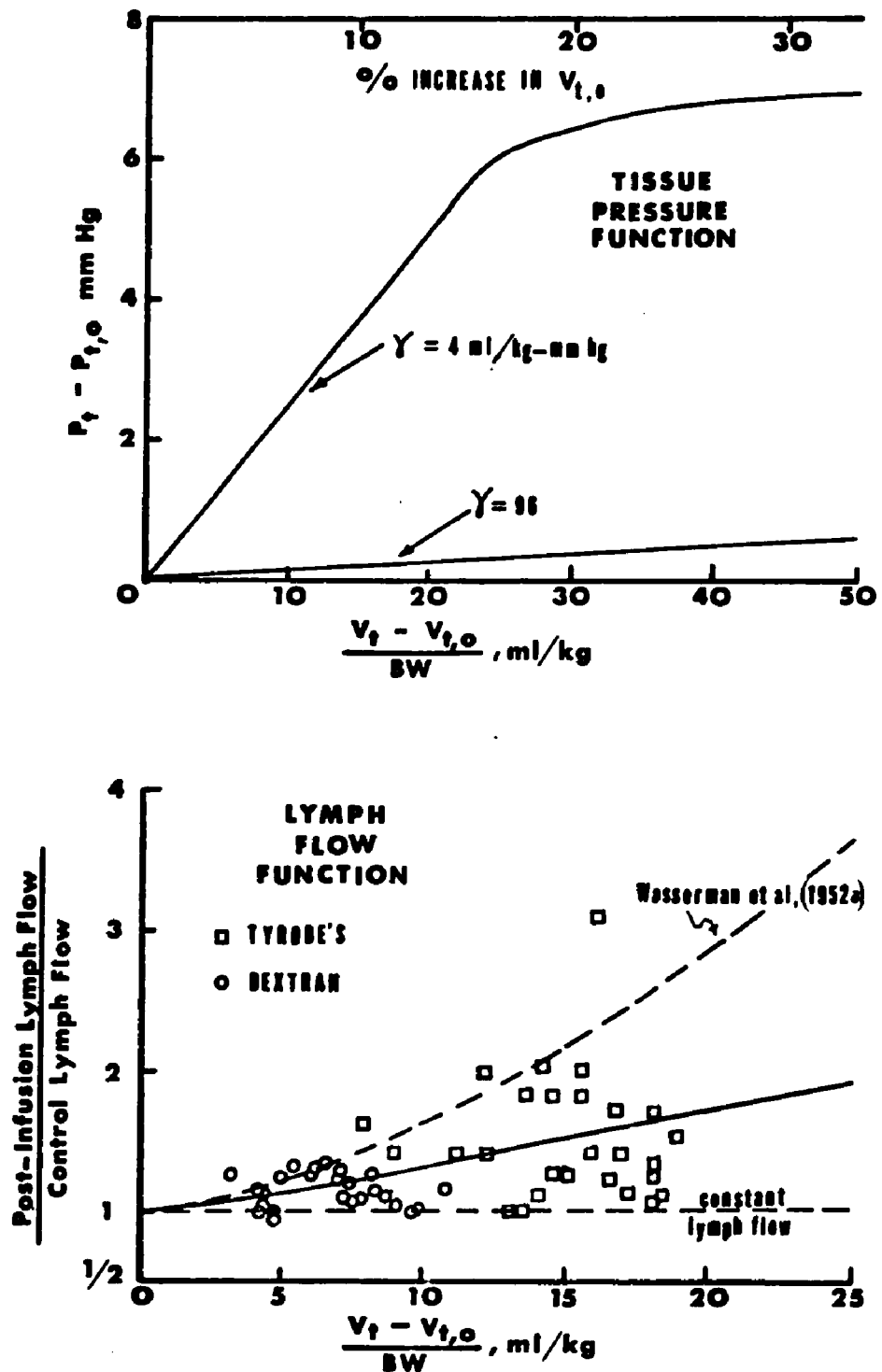


Figure 17. Describing functions for the model.

a) Top: Tissue pressure changes as a function of changes in tissue fluid volume.

b) Bottom: Relative lymph flow as a function of changes in tissue fluid volume. (Control flow = .55 ml/min).

Maximum lymph flows in the present study are comparable with Wasserman and Mayerson's data. However, it should be noted that the average lymph flow following infusion is much less than the maximum values. Guyton and Coleman (1967; 1968) have used this peak flow data to derive a correlation between lymph flow and tissue pressure, but the present data suggest that their relationship may greatly overestimate average lymph flow.

5. ASSUMED VALUES FOR INITIALIZING CONSTANTS: K_w , K_p , A_o , and R_o

There have been no studies reported in which $K_w A$, $K_p A$, A , or R have been measured during infusions, and very few of these parameters have been evaluated for the intact animal. Table V lists representative values that have been obtained in certain isolated tissues or in the resting animal. All values were originally reported in terms of unit weight of the test tissue. They have been modified for the present study by assuming a 20 kg dog is composed entirely of this tissue.

TABLE V
REPRESENTATIVE VALUES OF CAPILLARY PARAMETERS

Capillary Surface Area:

$A_o \times 10^{-5}$ (cm²/20 kg)

5 - 14	Skeletal Muscle	Pappenheimer (1953), Mellander, <u>et al</u> (1968)
1.2 - 4.6	Intact Dog	Attinger, <u>et al</u> (1966)
0.4 - 3.5	Intact Dog	Altman, <u>et al</u> (1971)

Capillary Filtration Coefficient:

$K_w A_o$ (ml/hr-mmHg-20kg)

68	Man, forearm	Landis, <u>et al</u> (1963)
73	Man, whole body	Landis, <u>et al</u> (1963)
70	Dog, hind leg	Guyton, <u>et al</u> (1966b)
168	Dog, hind leg	Pappenheimer (1953)
126	Cat, hind leg	Pappenheimer (1953)

(TABLE V, cont'd)

Protein Permeability Coefficient:

 $K_p A_o$ (ml/hr-20 kg)

>70	Dog, hind leg	Renkin (1966)
43	Dog, hind leg	Landis, <u>et al</u> (1963)
430	Dog, hind leg	Garlick, <u>et al</u> (1970)
20	Dog, intestine	Altman, <u>et al</u> (1971)

a. Capillary Surface Area

One of the few and often quoted measurements of capillary surface area in mammalian tissue was reported by Pappenheimer (1953) to be $70 \text{ cm}^2/\text{gm}$ skeletal muscle. However, this value was based on histological examination of frozen sections and thus represents the total surface area. Most investigators feel that only about 1/10 to 1/2 of the total number of capillaries are open to flow at any given time because of precapillary sphincter activity. Hence, the size of the functional capillary surface area is correspondingly smaller. The range of values for A_o shown in Table V reflects the referenced authors' estimates of the range between resting levels and maximum vasodilation. Presumably, during periods of maximum vasoconstriction, the values would be even lower. The values reported by Attinger, et al (1966) and Altman, et al (1971) were based on total blood flow and velocity measurements combined with assumptions about the geometry of an average capillary.

In the present study we have used Mellander and Johansson's (1968) estimate of $25 \text{ cm}^2/\text{gm}$ skeletal muscle. Their data suggests that the 55% of body mass which is non-muscular has three times more open capillaries at rest than does muscle. On this basis we estimated a value for $A_o = 10 \times 10^5 \text{ cm}^2$ in a 20 kg dog. Landis and Pappenheimer (1963) imply that the same area is available for both filtration and protein diffusion.

b. Capillary Filtration Coefficient

If capillary permeability or filtration coefficients are measured for tissues in which the capillary surface per unit weight of tissue is not known, the value obtained, $K_w A$ or $K_p A$, includes the area of the capillary walls, A . Hence, it is usually impossible to obtain K_w or K_p directly.

Mellander and Johansson (1968) show that values of $K_w A$ (per gm tissue) are at least several times greater in the capillaries of the skin and

splanchnic organs than those shown in Table V. However, it has been suggested that inasmuch as muscles comprise about 65% of the non-fat, non-skeleton body weight (fat and skeleton do not participate to a large extent in capillary exchange), it is likely that values measured in the hind leg preparation (70% muscle by weight) should be close to that of the whole body (Landis & Pappenheimer, 1963). The average value of $K_w A_o$ in Table IV is very close to 100 ml/hr-mm Hg-20kg which was adopted for this study.

c. Capillary Protein Permeability

The values shown for protein permeability (Table V) have been obtained for serum albumin. As can be observed, they vary over a large range. Our own calculations lead us to believe that the lower values are more reasonable. Equation (VI-25) expresses the steady state concentrations of protein in plasma and tissue fluid as a function of lymph flow and protein permeability for a diffusion process. This relationship was used by Renkin (1964b), who assumed that lymph and tissue fluid protein concentration are identical. Our experimental results showed an average control level of $C_{\text{lymph}}/C_{\text{plasma}} = 0.6$ and an average thoracic lymph flow rate of 30 ml/hr. Substituting these values into Equation (VI-25) we obtain a value of 43 ml/hr-20kg for $K_p A_o$, agreeing exactly with Landis and Pappenheimer. Furthermore, if the high value reported by Garlick and Renkin is substituted into Equation (IV-25), the resulting estimated whole body average of $C_{\text{lymph}}/C_{\text{plasma}}$ is 0.90. This is considerably higher than any previously reported values. The actual value for both protein and dextran which was used in this study was 25 ml/hr-20 Kg. Garlick and Renkin (1970) have demonstrated that dextran of MW greater than 60,000 exhibit permeabilities 1/10 to 1/5 that of albumin. It will be shown later that even large changes in values of $K_p A$ do not affect the simulated results for short-term infusion experiments.

d. Pre-/Postcapillary Resistance Ratio

Measurements of pre-/postcapillary resistance ratio in the resting state have been made by estimating the capillary pressure (usually by an isogravimetric procedure), measuring the arterial and venous pressures, and solving Equation (VI-1) for R (Pappenheimer & Soto-Rivera, 1948; Cobbold, 1963). Most of these studies have been made on the hind limb. A generally accepted value for R in skeletal muscle is 4.0 (Meilander & Johansson, 1968), although

values as high as 19.0 have been reported (Diana, 1970). Johnson's (1965) data on the isogravimetric intestine reveals a higher precapillary resistance and a lower postcapillary resistance than in muscle, resulting in an R_a/R_v value of about 10/1. Oberg (1964) assumed a value of 4.0 in the resting hind limb and intestine and was able to estimate changes from this value by measuring circulatory pressures and flows following hemorrhage. We shall follow this example and let $R_o = 4.0$. This means that 80% of the total peripheral resistance resides in the pre-capillary arterioles, or that 80% of the venous pressure is transmitted to the midpoint of the capillary. Values of R are independent of total body weight.

The table below summarizes the values for the initializing constants that were assumed during the simulation studies:

TABLE VI
ASSUMED VALUES FOR INITIALIZING CONSTANTS
(Values for a 20 Kg Dog)

Capillary filtration coefficient, $K_w A_o$	100 ml/hr-mm Hg
Protein permeability coefficient, $K_p A_o$	25 ml/hr
Capillary surface area, A_o	$10 \times 10^5 \text{ cm}^2$
Pre-/postcapillary resistance ratio, R_o	4.0

6. Calculation of Initial Conditions

The model requires a fairly complete description of the extracellular system during the steady state period prior to infusion. In particular, the following parameters are required: $V_{t,o}$, $V_{e,o}$, $M_{t,o}$, $M_{e,o}$, $P_{t,o}$. None of these values were experimentally determined in the present study. Thiocyanate space was measured, but it was thought that this value was a gross overestimation of the volume of distribution of extracellular protein, which is really the crucial variable (see Chapter II, Section A-4d). If we assume that normal steady state values of the plasma and interstitial volumes are 4.5% and 16% of BW respectively (Pitts, 1968), then we have:

$$V_{t,o} = V_{pl,o} \times 3.6 \quad (\text{VI-21})$$

where $V_{pl,o}$ is an experimentally determined constant. The remainder of the initial conditions can be estimated by solving the equations of the dynamic

model subject to steady-state restrictions that exist at $t = 0$, just prior to infusion, i.e.,

$$dV_t/dt|_{t=0} = 0 \quad (\text{VI-22a})$$

$$dM_t/dt|_{t=0} = 0 \quad (\text{VI-22b})$$

$$V_{\text{inf}}|_{t=0} = 0 \quad (\text{VI-22c})$$

The initial extracellular volume can be obtained from Equations (VI-2, 9 and 22c),

$$V_{e,o} = V_{pl,o} + V_{t,o} \quad (\text{VI-23})$$

In order to find the initial compartmental protein masses, M_{pl} and M_t , we utilize the experimental input variables, $V_{pl,o}$ and $C_{pl,o}$ and Equation (VI-17):

$$M_{pl,o} = C_{pl,o} \cdot V_{pl,o} \quad (\text{VI-24})$$

and then solve Equations (VI-12, 13, 14, and 22b) for the value of tissue protein concentration,

$$C_{t,o} = \frac{C_{pl,o}}{(1 + Q_{\text{lymph}}/K_p A)} \quad (\text{VI-25})$$

which can finally be used to find $M_{t,o}$ from Equation (VI-18)

$$M_{t,o} = C_{t,o} \cdot V_{t,o} \quad (\text{VI-26})$$

and $M_{e,o}$ from Equations (VI-4, 5, 16, 22c, 24, and 26)

$$M_{e,o} = M_{pl,o} + M_{t,o} \quad (\text{VI-27})$$

The value of the pre-infusion tissue pressure is found by assuming that the pressure gradients causing filtration are exactly balanced at steady state so that the rate of filtration is just equal to the rate of lymph flow.

Mathematically, the solution entails combining Equations (VI-6, 7, 22a) and solving for $P_{t,o}$:

$$P_{t,o} = P_{c,o} - \pi_{pl,o} + \pi_{t,o} - Q_{lymph}/K_w A \quad (VI-28)$$

where Q_{lymph} at steady state is taken as 2 ml/kg-hr (Mayerson, 1963). The value of $\pi_{pl,o}$ and $\pi_{t,o}$ can easily be determined from Equations (VI-19 and 20), using initial concentrations. $P_{c,o}$ is determined from Equation (VI-1) for $t = 0$.

The initial calculations just outlined (Equations VI-21 to VI-28) are incorporated into the computer simulation program before dynamic simulation of infusion begins. (Conceptually, it is included within the block labelled "Model..." in Figure 20). Thus a complete picture of the initial state of the system is obtained.

E. METHODS OF DYNAMIC SIMULATION

1. PURPOSE OF SIMULATION

The simulation consists of finding the time-varying solution to the idealized model described in the previous section. There are many variables involved in this system, but the solution we are particularly interested in is the response of the plasma volume as it initially expands during infusion and then decreases. The purpose of the simulation will be to perform the very same type of experiments on the computer that were done with animals. Hopefully, the simulated results will compare favorably with the results obtained on animals. If they do not, we may be obliged to readjust our thinking about the assumptions leading to the theoretical model. Alternatively, parameter optimization techniques can be used to bring simulated and experimental results into closer agreement. However, unless the model is a good representation of reality, there is no a priori assurance that even parameter optimization will bring about a successful outcome.

2. DESIGN OF SIMULATION EXPERIMENTS

Four different situations were simulated paralleling the experimental design: 1) slow Tyrode's infusion, 2) rapid Tyrode's infusion, 3) slow

dextran infusion, and 4) rapid dextran infusion. The primary goal was to obtain simulated plasma volume responses similar to the experimental responses we have already presented, and which are redrawn in Figure 18. The experimental arterial and venous pressure responses for the four groups which are used as inputs into the model have been likewise redrawn in Figure 19. Experimental input constants were determined by averaging the necessary factors for each experimental group. These are listed in Table VII. Additional initializing constants that were not measured but were assumed have been shown previously in Table VI.

3. THE SIMULATION PROCEDURE

The diagram in Figure 20 summarizes the method used to combine the experimental data, mathematical model, and parameter optimization techniques. Time-varying input functions consisted of the infusion rate and the experimentally determined blood pressures. Initializing constants were either obtained experimentally or from average values found in the literature as previously described. At the end of a simulated experiment the plasma volume response was compared to the experimental response by a least squares function. An optimization subroutine, consisting of a direct pattern search and steepest ascent method, incremented the optimizing parameters, R and A , in a direction that quickly resulted in a minimum of the least squares function. When this occurred the time varying results for any desired variable could be printed or plotted.

Ideally, only one forcing function should be necessary for this model, i.e. the infusion rate pulse function Q_{inf} . From this a more complete model could predict the arterial and venous pressures which are needed in Equation (VI-1).⁶ However, circulatory pressure changes cannot be accurately estimated because little is known about the dynamic behavior of vessel compliance, or about the redistribution of blood volume between various parts of the circulation following infusion. For this reason, the blood pressure responses were taken directly from the data obtained in our infusion experiments.

Three types of simulation studies were performed. The first consisted of a series of parametric studies in which we examined the effects of certain

⁶For two attempts at such an analysis see Beneken & DeWitt (1967) and Guyton & Coleman (1967).

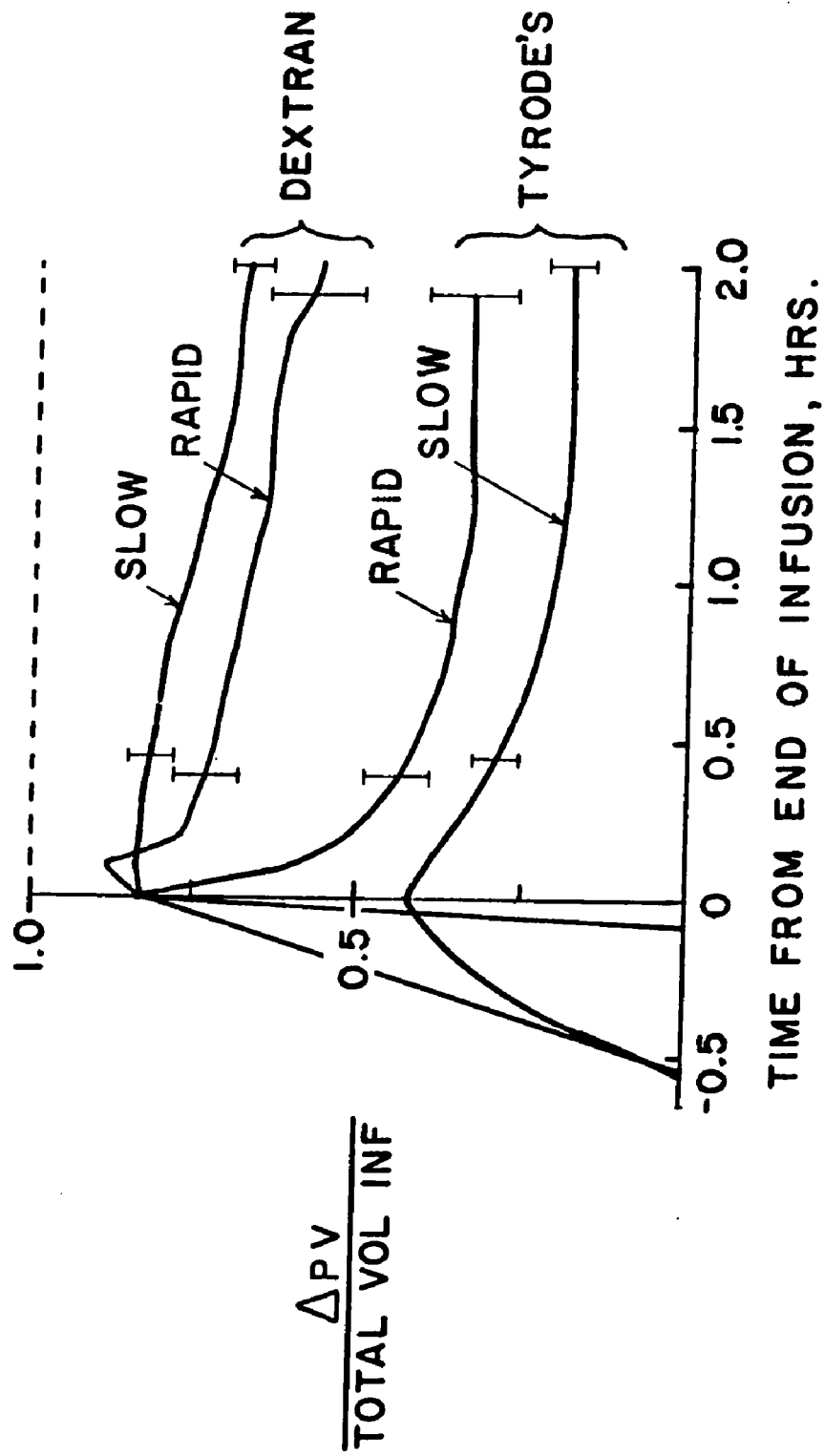


Figure 18. Normalized plasma volume responses for the four experimental groups.

BLOOD PRESSURE RESPONSE

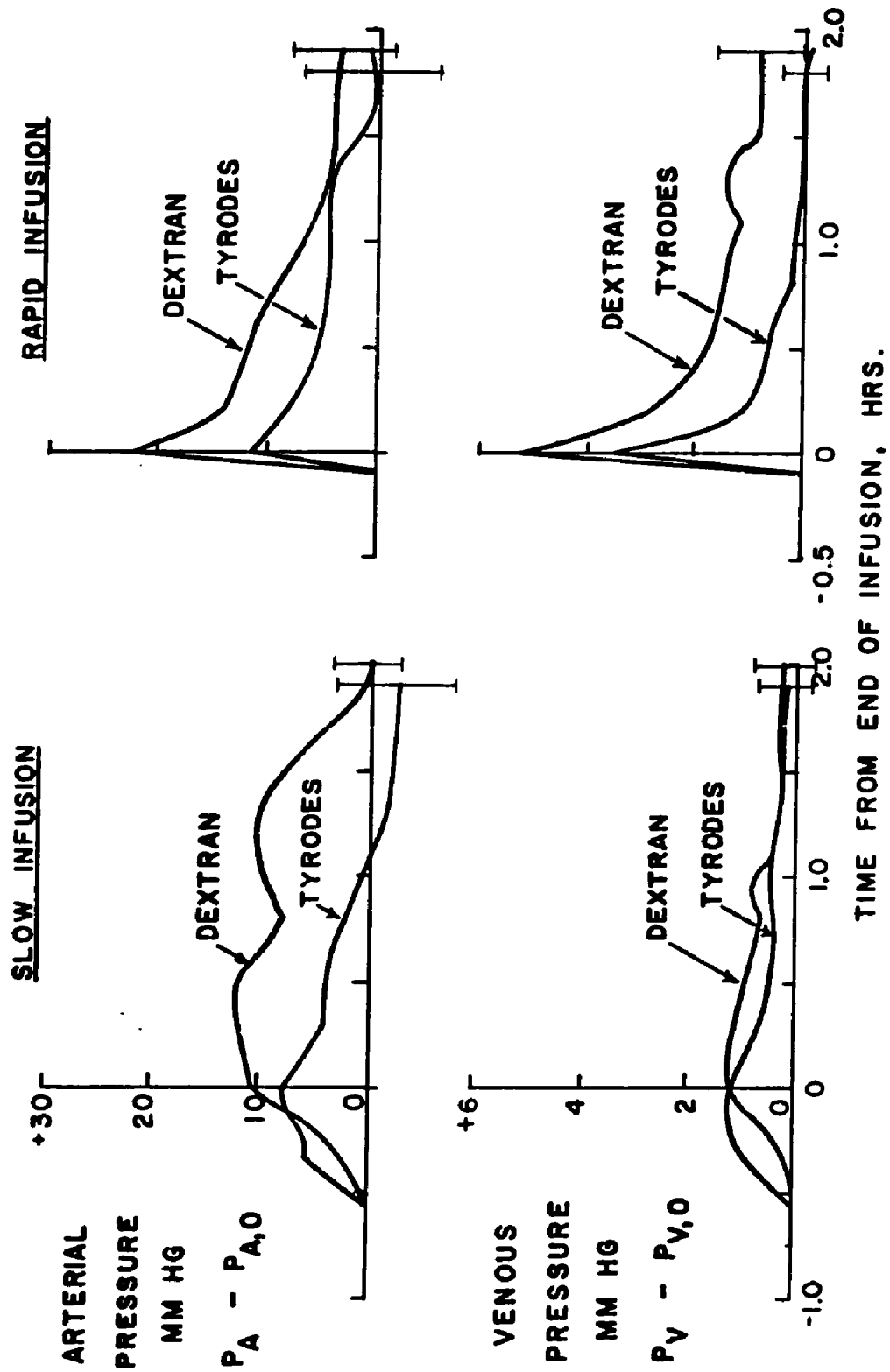


Figure 19. Blood pressure responses for the four experimental groups. Control values appear in Table VII.

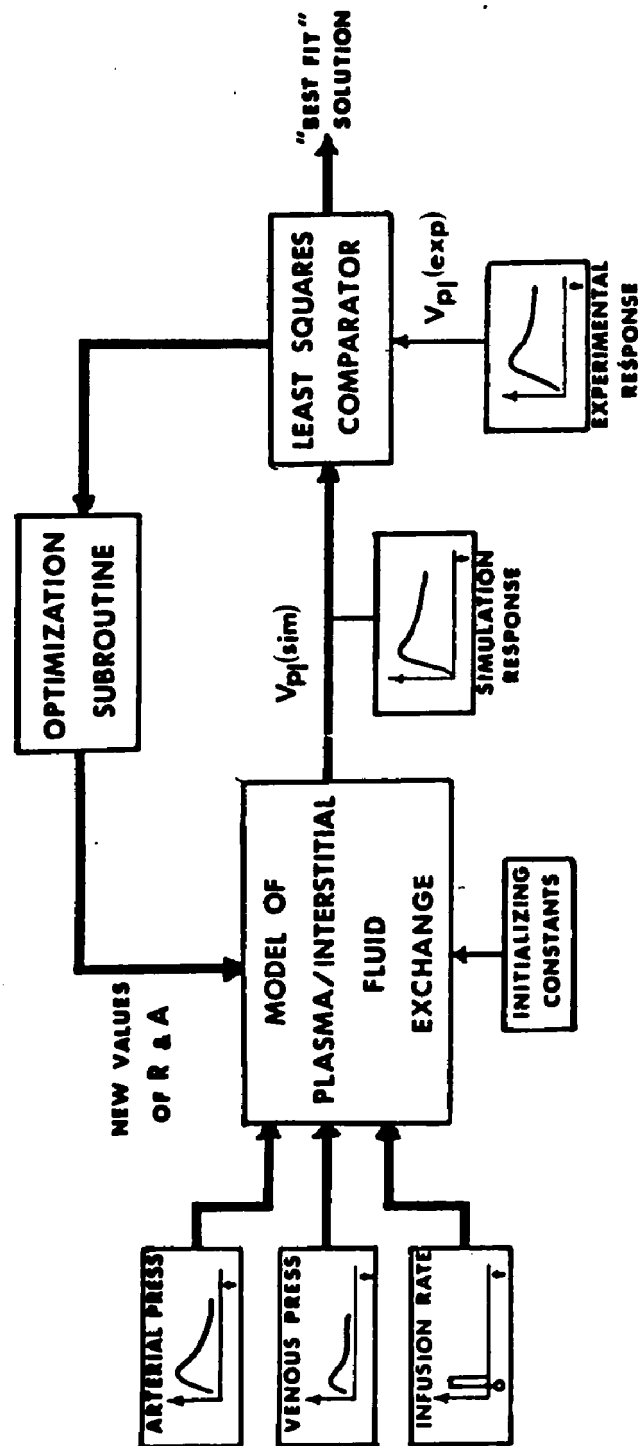


Figure 20. Flow chart of simulation procedure.

TABLE VII

EXPERIMENTAL INPUT CONSTANTS FOR SIMULATION STUDIES

Infusion Group	V_{inf} ml	ΔT_{inf} hr	C_{inf}^* gm/ml	$V_{pl,o}$ ml	$C_{pl,o}$ gm/ml	BW kg	$P_{a,o}$ mmHg	$P_{v,o}$ mmHg	$P_{c,o}^{**}$ mmHg
1) Slow Tyrode's	488	0.563	0.0	986	0.0615	19.4	67.0	3.22	16.0
2) Rapid Tyrode's	485	0.0818	0.0	1198	0.0514	19.3	62.0	2.62	14.5
3) Slow Dextran	502	0.542	0.060	950	0.0598	20.1	61.5	0.85	13.0
4) Rapid Dextran	506	0.0928	0.060	1295	0.0518	20.3	58.4	1.23	12.7

* Dextran solutions were formulated to have a COP = 20mm Hg. The equivalent protein concentration required to produce such a pressure is 0.0595 gm/ml

** Pre-infusion capillary pressure based on $R_o = 4.0$

important parameters on the dynamic behavior of the model. In these studies no attempt was made to obtain agreement with the experimental data and therefore the optimization subroutine was bypassed. In the second method, time-invariant values of R and A were obtained for the entire simulated run using the optimization subroutine. These were designated as constant parameter optimization studies. The third method of analysis, piecewise optimization studies, treated the optimizing parameters as time-varying quantities and a more rigorous "fit" between observed and simulated results was attempted throughout the entire run. By dividing the time course of the experiments into several segments we optimized the entire run in a piecewise fashion, using the optimized results of one segment as initial conditions for the next segment. In this way we obtained new values of R and A for each segment, that is, a time profile of these parameters which is in keeping with the concept that, in the living animal, the resistance ratio and surface area are known to be time-varying.

4. THE COMPUTER AND SIMULATION LANGUAGE

The basic tools of our simulation experiments were the digital computer (IBM 360/67 computing system operating under the Michigan Terminal System (MTS)), and a general purpose simulation language program (Continuous Systems Modeling Program - CSMP).⁷ This language is especially designed to solve sets of differential equations describing continuous physical systems easily and efficiently, as well as to facilitate data input and output displays (Brennan, et al, 1970). Numerical integration was performed by a fixed step 4th-order Runge-Kutta algorithm using a step size of about three minutes which represents 1/10 of the average slow infusion period and 1/2 of the average rapid infusion period.

5. LEAST SQUARES COMPARATOR AND OPTIMIZATION SUBROUTINE

The "Least Squares Comparator" in Figure 20 consists of a single function that computes a value relating to the goodness of fit between simulated and experimental responses. There is no universally agreed upon definition

⁷IBM Application Program Manuals: 1) "System/360 Continuous System Modeling Program, Application Description", #H20-0240-2; 2) "User's Manual", H20-0367-2, 3rd Edition, 1968, White Plains, N.Y.: IBM Technical Publications Dept.

with which to compare two distinct curves to determine how different or how identical they are. A common method, however, is to use a least squares function (see e.g. Abbrecht & Prodany, 1971) and this is the approach we have followed. Letting "F" be the value computed by this optimizing function and Vs_i and Vx_i designate the simulated and experimental plasma volumes respectively evaluated at time t_i , we compute the expression:⁸

$$F = \frac{\sum_{i=1}^N (Vs_i - Vx_i)^2}{\left(\sum_{i=1}^N Vx_i\right)^2} \quad (VI-29)$$

F is a measure of the total relative dispersion between the two curves $Vs(t)$ and $Vx(t)$ evaluated at t_i , $i = 1, \dots, N$. In practice, the time interval, $t_{i+1} - t_i$, represents multiples of the numerical integration step. An entire infusion-response period must be simulated (approximately 2 hours real time) before a final value of F is obtained. The problem of determining the best fit between experimental and simulated results is thus reduced to locating the values of R and A that make the function, F, a minimum.

In order to reduce the number of trials necessary to find the desired optimizing parameters, and to facilitate the use of the scheme on the computer, an optimization subroutine has been incorporated into the simulation program. The particular algorithm chosen is a combination of the "direct pattern search" and "steepest ascent" methods as described, for example, by Carnahan & Wilkes, 1970. The purpose of this program is simply to change R and A by predetermined step sizes so that the optimization function, F, continues to decrease. If a value of F is computed which is greater than

⁸The relative dispersion of a series of observed values of x_i , $i = 1, \dots, n$ may be given by the coefficient of variation, C_v , where:

$$C_v = \frac{\text{standard deviation of } x}{\text{average value of } x} = \frac{\sqrt{\sum (x_i - \bar{x})^2 / n}}{\sum x_i / n} \quad \text{or} \quad C_v^2 / n = \frac{\sum (x_i - \bar{x})^2}{(\sum x_i)^2} \quad . \quad \text{If}$$

we let $x_i = Vs_i$ and the expected value of x , $\bar{x} = Vx_i$, then the optimizing function $F = C_v^2 / n$.

that obtained in the previous run, the prior values of the rate parameters are first restored and then readjusted in a new direction. The program has the ability to increase the step increments once a path toward the minimum value has been established. Conversely, when the values of the rate parameters are in the immediate vicinity of their optimum values, the step size is automatically decreased. The two rate parameters are always incremented one at a time, never simultaneously. Between each increment, of course, an entire infusion response period is simulated.

In general, the lower the value of F the better the 'fit' between predicted and experimental curves. There is no guarantee, however, that the simulated response curve that corresponds to a minimum value of F will have the same general shape as the experimental curve. Agreement will only exist if the model is a good representation of reality and if the input data is accurate. Even then, the optimization program does not insure a "good fit" but only the "best fit" possible with this system of equations. Furthermore, if the mathematical system is not "well behaved" it is likely that the optimization subroutine we have chosen may locate local, rather than global, minimums of F . Although there are many other techniques, some more and some less sophisticated than those we have used, there are few iron-clad rules regarding optimization procedures. They should be regarded merely as devices to help evaluate the model. It is very likely that the parameters of this model can be adjusted so that simulated and experimental results compare favorably. But the model can only be said to have theoretical leverage if the values of these adjustable parameters are physiologically reasonable. It is on this basis that we have judged our results

See footnote 5, this chapter:

⁹The capillary filtration coefficient, K_A , and the protein permeability coefficient, K_P , both contain the capillary surface area term. They have both been measured at rest and are known to vary under conditions of stress. No previous data have been obtained to describe their behavior during infusions. It is believed by many investigators that the permeability terms, K_w and K_p , are relatively constant and that the capillary surface area is the factor that causes the $K-A$ term to vary during exercise, standing, hemorrhage, etc. (See footnote 2, Chapter II.) This will be the view adopted in the present study. Mathematically, however, the results would be nearly identical if we assumed K_w as well as A to be time-varying quantities.

VII. RESULTS OF COMPUTER ANALYSIS

"...data can always be fitted 'satisfactorily' by some small set of equations containing a few adjustable parameters (but) it is only the biological plausibility of a particular model which justifies preferring it to all others." D.S. Riggs (1970)

A. PARAMETRIC STUDIES

In this part of our analysis we have not sought an adequate representation of our data. We rather wish to investigate the effects of changing certain parameters on the dynamics of the simulation model. In particular we have examined the following parameters: pre-post capillary resistance ratio, R ; capillary surface area, A ; capillary filtration coefficient, $K_w A$; capillary protein permeability, $K_p A$; lymph flow, Q_{lymph} ; and tissue compliance, γ . The results of this parametric study are illustrated in Figures 21-25. We have chosen the rapid Tyrode's infusion case to illustrate the parametric effects. Other cases were also studied and will be mentioned when necessary.

Several important conclusions can be reached from this study:

1) The optimizing parameters, R and A , are seen to affect both the shape and the absolute values of the plasma volume response to a great extent (see Figures 21 and 22). It is especially important that parameters which are used to optimize a particular response function exert a sensitive influence on that function. The effect of varying R has its most direct effect on the capillary pressure, which decreases for increasing values of R . Changes in A affect both $K_w A$ and $K_p A$. As seen in Figure 23 the effect of increasing either of these coefficients is the same - to increase the net filtration rate and thereby decrease the retention of infusate in the plasma.

2) As can be seen in Figures 22 and 23, higher values of capillary surface area, A , results in higher filtration rates at the early portion of the infusion response, but lower values at longer times. The initial higher filtration rate occurs primarily as a result of the direct multiplying effect of the capillary filtration coefficient as demanded by the Starling relationship (Equation VI-6). However, because of this

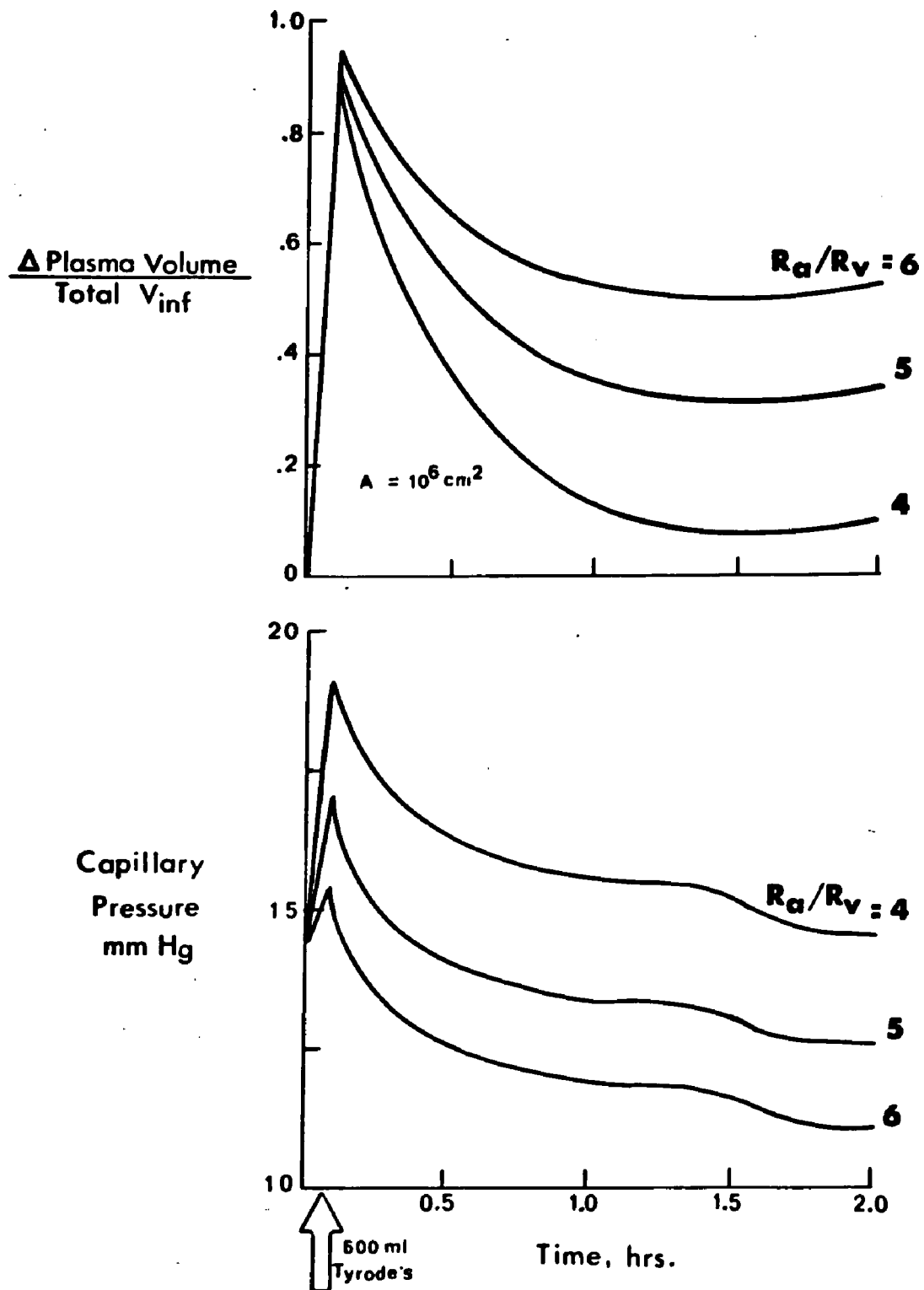


Figure 21. Effect of pre-/postcapillary resistance ratio on capillary pressure and simulated plasma volume responses.

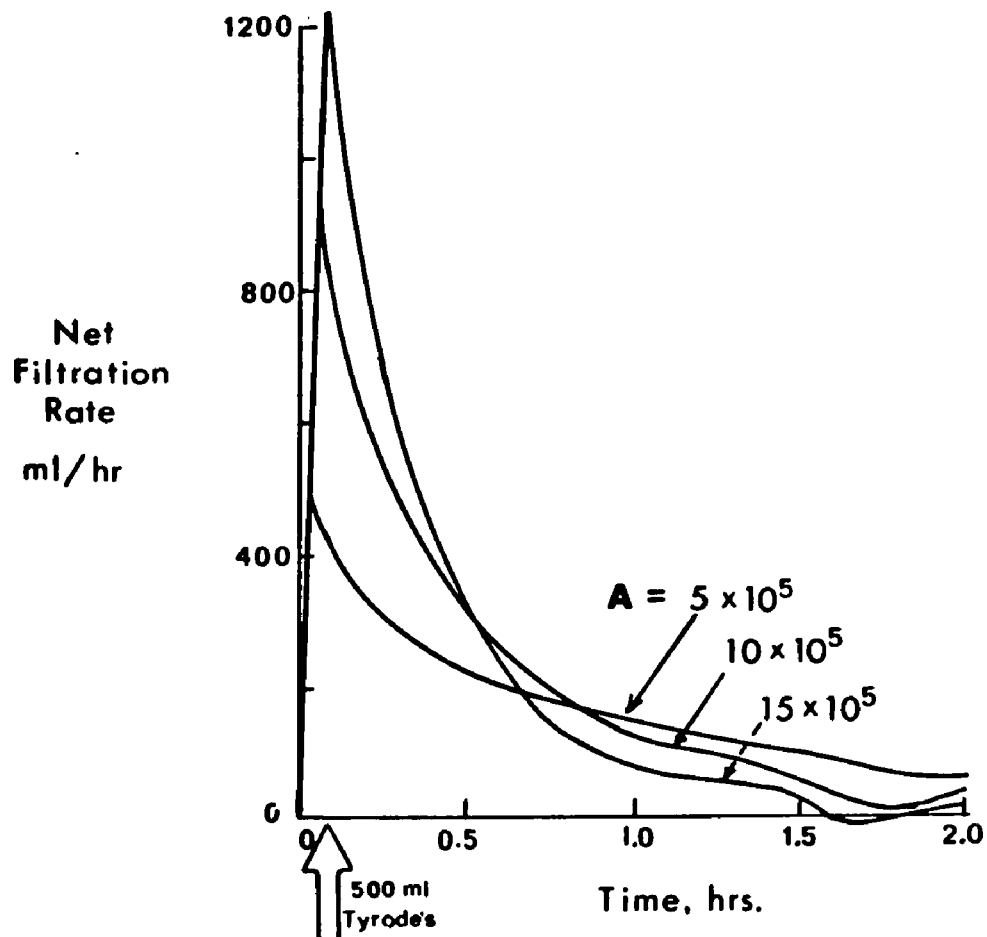
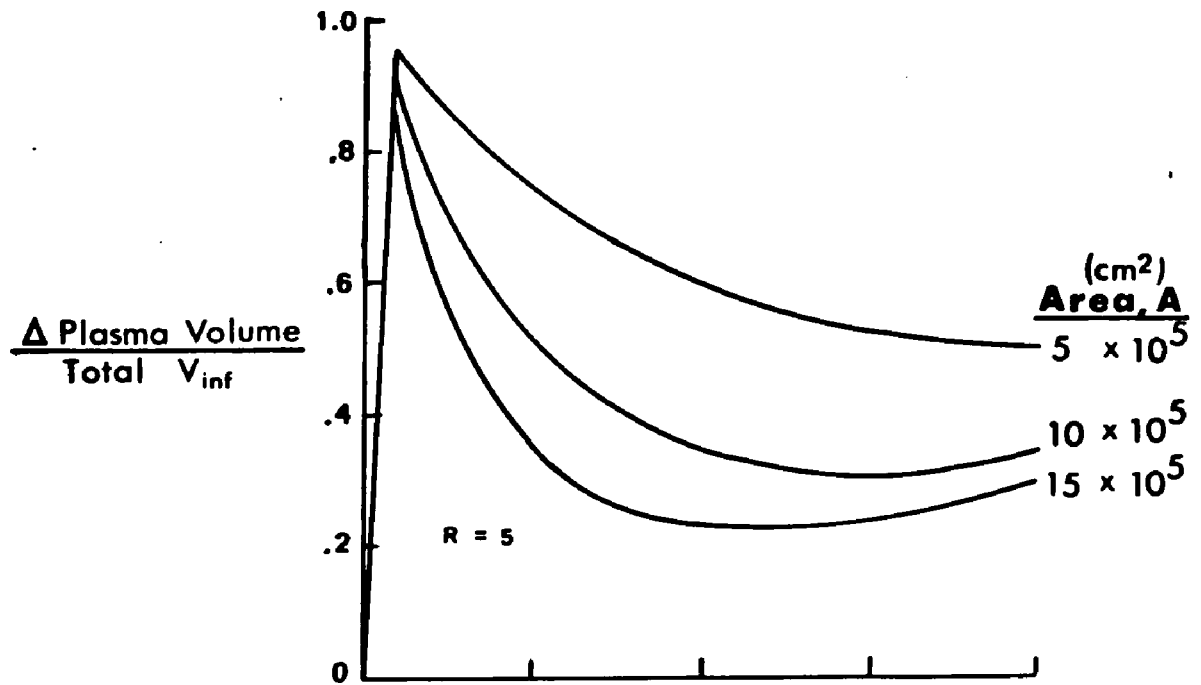


Figure 22. Effect of capillary surface area on simulated plasma volume and transcapillary filtration responses.

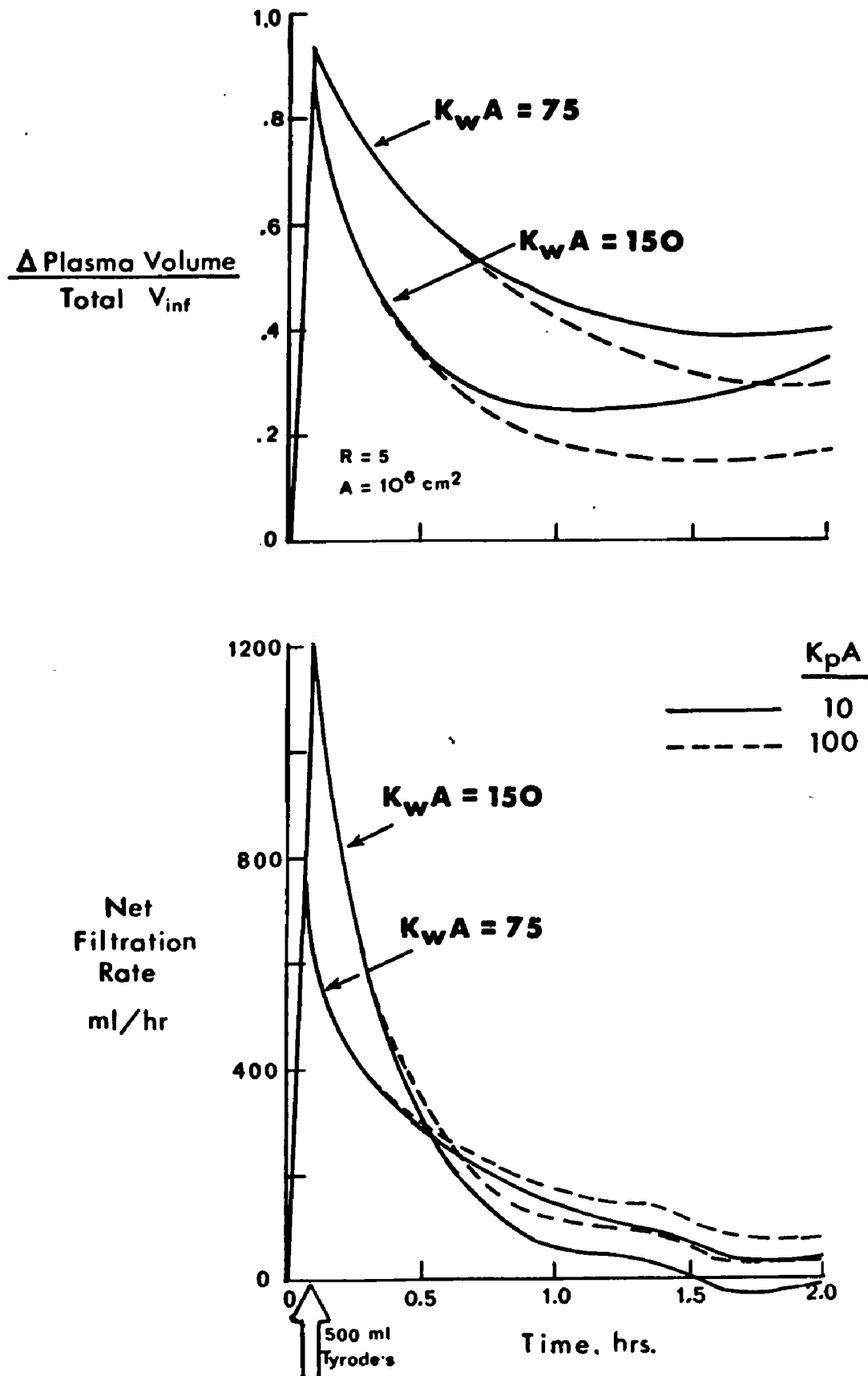


Figure 23. Effect of $K_w A$ and $K_p A$ on simulated plasma volume and transcapillary filtration responses.

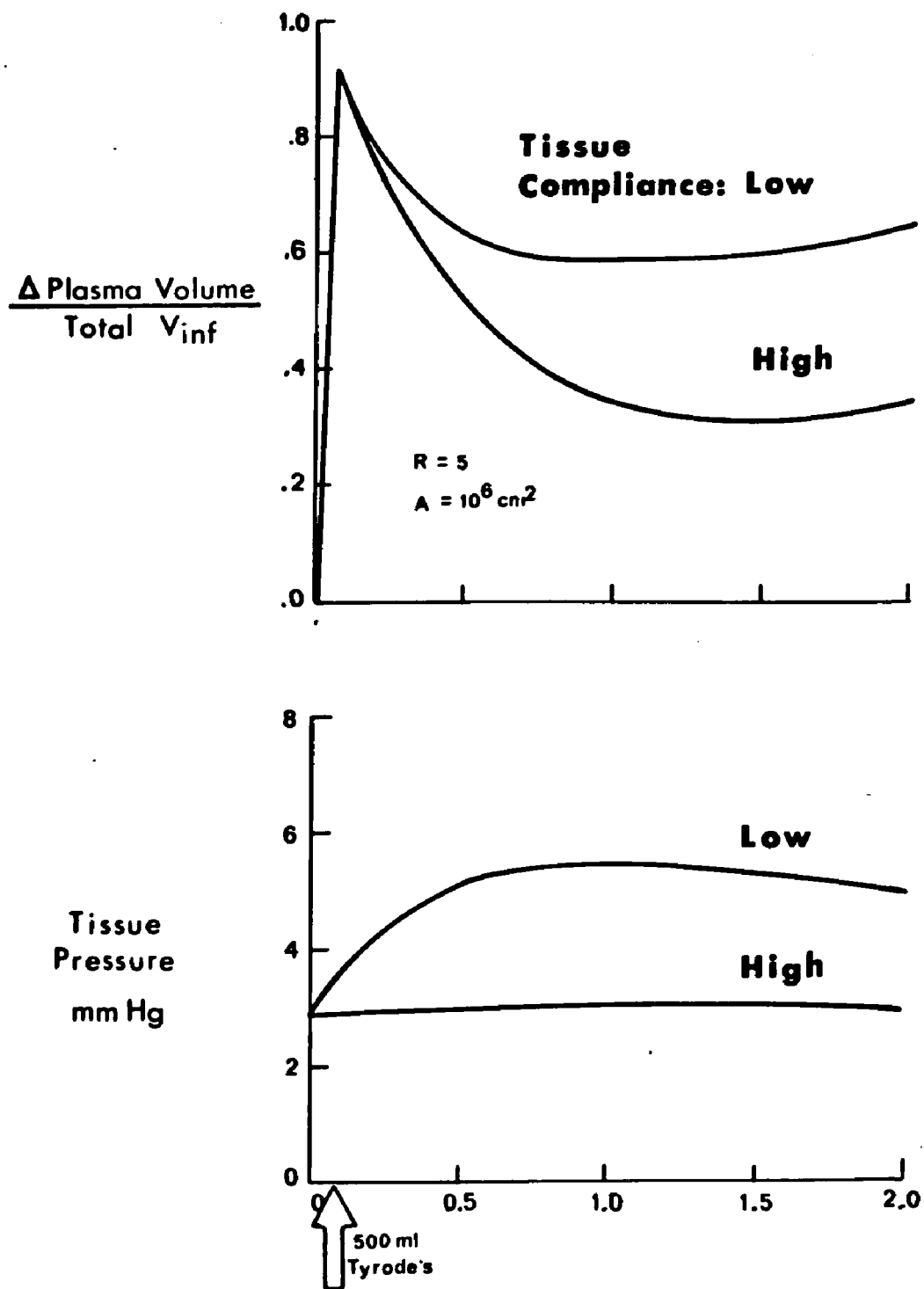


Figure 24. Effect of tissue compliance on simulated plasma volume and tissue pressure responses.

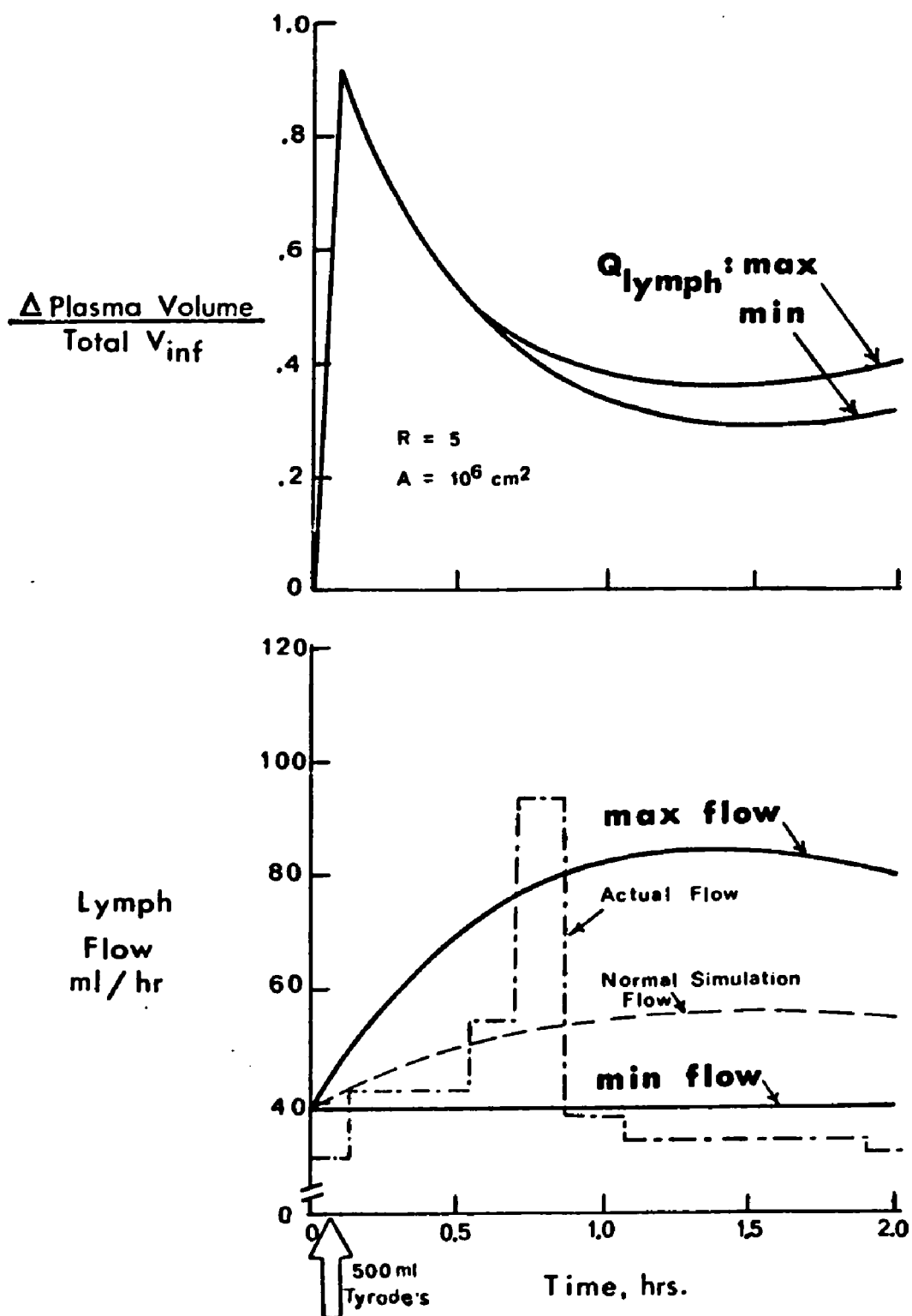


Figure 25. Effect of lymph flow on the simulated plasma volume response.

high initial filtration rate, the plasma proteins, diffusing relatively slowly from the plasma, are concentrated much faster than otherwise. Ultimately the increasing plasma COP (and decreasing capillary pressure) decreases the filtration rate to such an extent that the transcapillary flow might even reverse direction for a short time (i.e. net reabsorption). Whenever net filtration rates become less than lymph flow rates (approximately 40-60 ml/hr) the plasma volume will increase rather than decrease.

3) The effects of varying the filtration coefficient, $K_w A$, are much greater than those observed for varying the protein permeability, $K_p A$ (see Figure 23). Water tends to follow proteins into the tissues due to osmotic gradients. Thus higher protein permeabilities increase the filtration rate. However, this effect is relatively minor because even at the higher values of $K_p A$ little protein leaves the capillary.

The plasma volume response is not affected by changes in varying $K_p A$ until the latter stages of the simulation experiment when the integrated effect of the low protein exchange rate becomes noticeable. A similar analysis during dextran infusions shows much less of an effect on the plasma volume response following an increase in protein permeability.

4) As suggested in Figure 24 the effect of tissue compliance on plasma volume is quite significant. In this study we have investigated the effects of two extreme cases: a relatively low compliance of 4 ml/kg-mm Hg as suggested by Guyton and a relatively high compliance of 96 ml/kg-mm Hg as used by Wiederhielm (1968) and others (see Figure 17a). The direct effect of varying the compliance parameter is on the tissue pressure. Thus for a low compliant tissue a given increase in capillary pressure which enhances filtration is quickly counteracted by a relatively high increase in the tissue pressure. This results in a much lower net filtration rate and a greater retention of the infusate in the circulation than is observed for a tissue that is more compliant. All the results presented in this chapter were obtained by assuming a highly compliant tissue (96 ml/kg-mm Hg).

5) In general, the effect of lymph flow on the simulated plasma volume response is shown to be minimal in the case of Tyrode's

infusion (see Figure 25) and almost negligible in the case of dextran (not shown). The curves marked "maximum lymph flow" were obtained using the data of Wasserman and Mayerson (1952a), and represent peak values of lymph flow obtained during infusions into intact dogs. We have discussed elsewhere (Chapter V, Section F) that it is highly unlikely that these peak values are sustained for any great length of time. The curve labelled "minimum lymph flow" has been obtained assuming that lymph flow does not change at all during the simulated infusion response. Our simulation results have shown that lymph flow for both these extremes is minor compared to the average values of net filtration rates during these short-term experiments.

Also shown on the lower graph of Figure 25 for comparison are a) the simulated lymph flow response resulting from the experimental relationship between lymph flow and tissue fluid volume that was assumed throughout the remainder of these studies (see Figure 17b), and b) the data from one real experiment, labelled "actual flow". It can be seen that the time profile of the simulated and actual responses are quite different, although the area under the curves, representing the total lymph volume returned, is approximately the same. The lack of an adequate description of lymph flow does not really effect the conclusions we have made, due to the minor effect of lymph flow on short term experiments of this sort.

B. PIECEWISE OPTIMIZATION STUDIES

1. METHOD OF ANALYSIS

When the simulated plasma volume response is fitted to the observed response by piecewise optimization of the parameters R and A , the agreement between the two curves can be made nearly perfect. This is illustrated in Figures 26 and 27, where simulation results for the four experimental groups are shown. The responses of six time-varying quantities have been selected: 3 of them, the plasma volume response, plasma protein concentration, and plasma protein mass, are compared to the averaged experimental results, while 3 other variables, capillary

Figures 26 and 27. Results of simulated infusions for the four experimental groups using piecewise optimization procedure. Experimental data is shown for comparison.

Figure 26. Simulated results of slow and rapid Tyrode's infusions.

Figure 27. Simulated results of slow and rapid dextran infusions.

SIMULATION OF TYRODE'S INFUSION

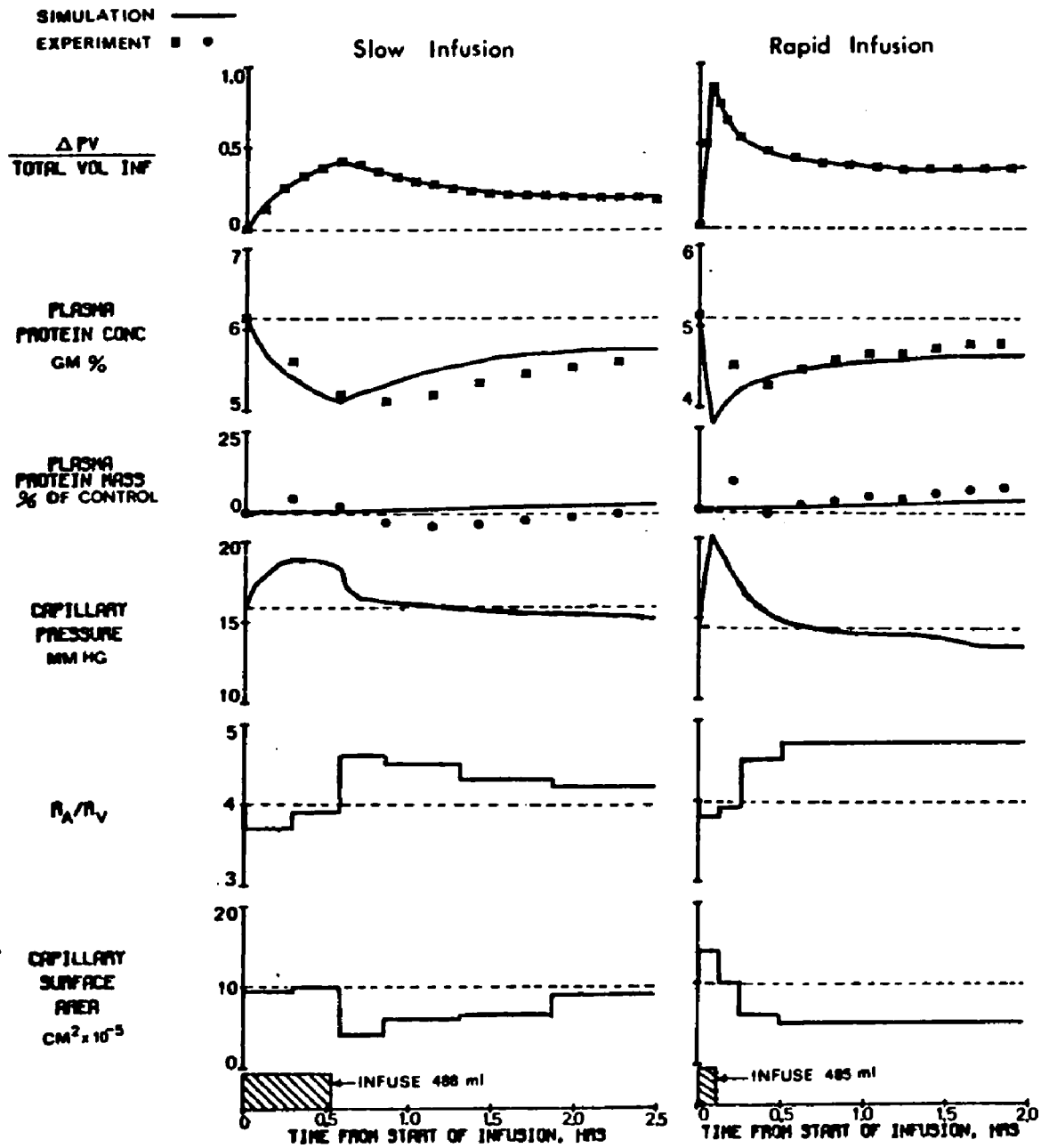


Figure 26.

SIMULATION OF DEXTRAN INFUSION

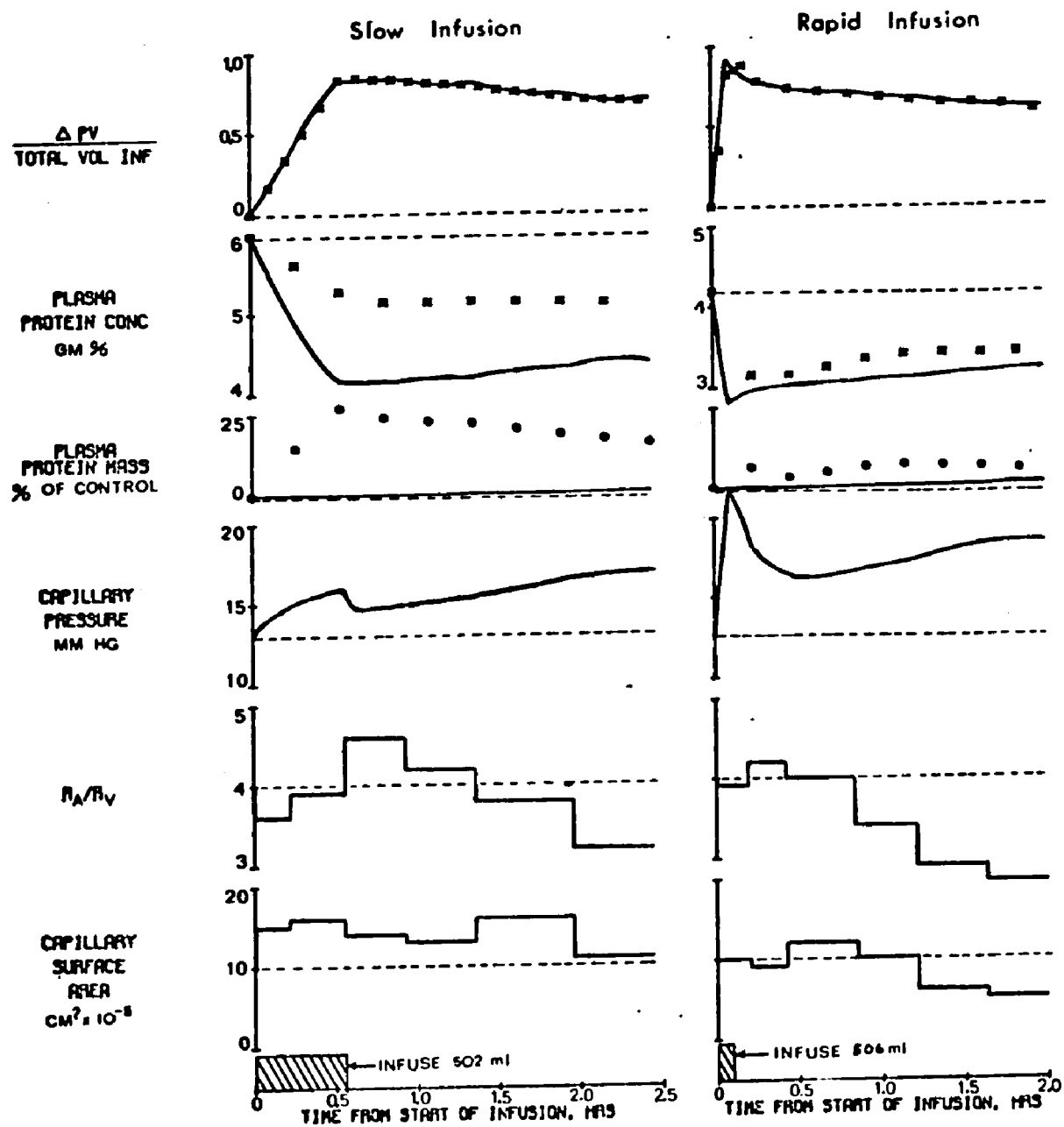


Figure 27.

pressure, pre-/postcapillary resistance ratio, and capillary surface area, are purely predicted responses with no comparable experimental results.

As outlined in the previous chapter, the resistance ratio, R , and capillary surface area, A , were assumed to be constant over small time periods and their numerical values were found by an optimization algorithm that minimized a least squares function comparing observed and simulated plasma volume response. The time course of each experiment was divided into six segments as indicated by the step changes in R and A . The model, once optimized in this manner, was able to predict the response of the plasma protein concentration, the plasma protein mass, and capillary pressure.

All other variables indicated in the flow chart of Figure 16 could also have been predicted, but the ones illustrated in Figures 26 and 27 have been chosen as being of special interest. Either they can be compared to experimental results, or else they are time-varying responses of quantities whose behavior is not well understood, and in most cases rarely measured.

2. COMPARISON OF RESPONSES BETWEEN THE FOUR GROUPS

In analyzing the piecewise optimization study, it is far more instructive to compare the results of the four separate groups of experiments, looking for similarities, differences, and trends rather than to discuss each group by itself. Some of the more important results are discussed below.

a) Response of the Optimizing Parameters

The early stages of the responses of R and A are very similar. There is a small initial decline and then an increase above control levels for the pre-/postcapillary resistance ratio. The effective capillary surface area either increases by about 40% or remains essentially constant. The responses for the remainder of the experiment are dependent upon both the rate of infusion and the type of solution. Thus, the Tyrode's infusion causes the resistance ratio to increase

above control while the dextran infusion causes a reduction in this parameter. These effects are enhanced at greater infusion rates. There is a dramatic contrast between the capillary area response of the slow Tyrode's compared to the slow dextran infusions. In both cases the area returns to near normal at the end of the experiment, but the transient response is always below control level for Tyrode's and above control for the dextran infusion.

The simulation predicts that in the post-infusion period the resistance ratio will be lower with Tyrode's infusion than with dextran, while the opposite is true for surface area. Increasing the rate of infusion seems to exaggerate the early post-infusion responses of Tyrode's and the late behavior of Dextran. Thus, considering only the rapid infusions, the responses of R and A following Tyrode's is stabilized within 1/2 hour, but dextran causes delayed changes that do not become significant until the latter half of the experiment. These responses may be due to passive increases in transmural pressure caused by the infusion, which may in turn induce local and central mechanisms that actively affect vessel tone.

b) Capillary Pressure Response

The simulated capillary pressure waveform is derived directly from the optimized response of the resistance ratio and the experimental arterial and venous blood pressure responses (see Equation VI-1). It should be noted that the responses for the four groups are quite consistent: in the case of both slow and rapid Tyrode's infusion the capillary pressure declines rapidly after infusion until it is just slightly below control, while for dextran infusions the capillary pressure is prevented, by the lowered resistance ratio, from returning to control levels. The physiological implications of this difference will be explored in the next chapter. The accompanying simulated rise in capillary pressure might have been expected from the experimental data alone, which shows plasma volume still decreasing at 2 hours in spite of a plasma COP that was slightly above control.

c) Response of Plasma Volume

The simulated plasma volume responses are found to be in excellent agreement with the experimental data. Although this may not be surprising, given the piecewise method which we used, it nevertheless cannot be assumed a priori that any one or more parameters of any model can be used in a similar manner to obtain similar agreement and still obtain parameter values within reasonable physiological limits. Such agreement is usually found only if the basic form of the model is a reasonable representation of reality. The model will be evaluated on this basis in the next chapter.

d) Response of Protein Concentration and Mass

One serious deficiency with the analysis, up to this point, is the lack of independent experimental observations with which to compare many of the model's predictions. However, two variables that were directly measured are the plasma protein concentration and the plasma protein mass (the product of experimental protein concentration and experimental plasma volume).

Our analysis predicts that over the course of the experiment there will be a slight increase in plasma protein mass, while even a greater increase is actually observed in most cases. The experimental results show that the increase of protein mass is much larger following the slow dextran infusion (as much as 25% above control) than for the three other groups (less than 7%). Simulation predicts that the increase over the two hour period should be less than 2% for all groups. Thus agreement between observed plasma protein concentration and mass time-profiles is reasonable except for the slow dextran infusion case. Lack of agreement is also obvious early in the simulation in the case of both rapid infusions, most likely due to inadequate mixing of the proteins in the plasma compartment during the minutes following infusion.

C. CONSTANT PARAMETER OPTIMIZATION STUDIES

In this section we examine the relative trend of certain variables that were not previously discussed, such as the relative contributions

of capillary filtration and lymph flow to plasma volume changes during infusion. The most efficient way of accomplishing this is to assume that the optimizing parameters, R and A , are constant throughout the experimental response period. This will also give us an opportunity to demonstrate the discrepancy that exists between experimental and simulated plasma volume responses when one does not assume time-varying behavior for these parameters, as was done in the last section.

As examples, we have chosen to simulate the responses following a rapid infusion of either Tyrode's or dextran solutions. Initializing constants were identical to those used for the piecewise optimization studies. In Figure 28 the optimizing function was weighted to ensure a good least squares fit between simulated and experimental plasma volume response at the end of the experimental period. Similar analyses were performed, but not shown here, in which equal weight was given to the entire time period. The use of constant values for the pre-/postcapillary resistance ratio and the capillary surface area resulted in relatively poor agreement between the observed and simulated responses. While the discrepancy at the early stages following infusion (see Figure 28) might be acceptable, we have shown in the previous section that we could account for this difference by assuming time-varying behavior for the optimizing parameters.

Nevertheless, we can use the model, optimized with constant parameters to predict changes in the other important variables shown. It can be seen that changes in both capillary pressure and plasma colloid osmotic pressure are much greater than the changes occurring in the tissue compartment as reflected by the tissue pressure and interstitial colloidal osmotic pressure. The rise in tissue pressure is small compared to what would have occurred if we had assumed a low instead of a high tissue compliance (see Figure 24). The analysis predicts an almost imperceptible decline in tissue oncotic pressure during both Tyrode's and dextran infusions. This behavior is the result of a complex series of events, reflecting on the one hand filtration and protein diffusion into the interstitium and on the other, protein and fluid removal through lymph ducts.

CONSTANT PARAMETER SIMULATION

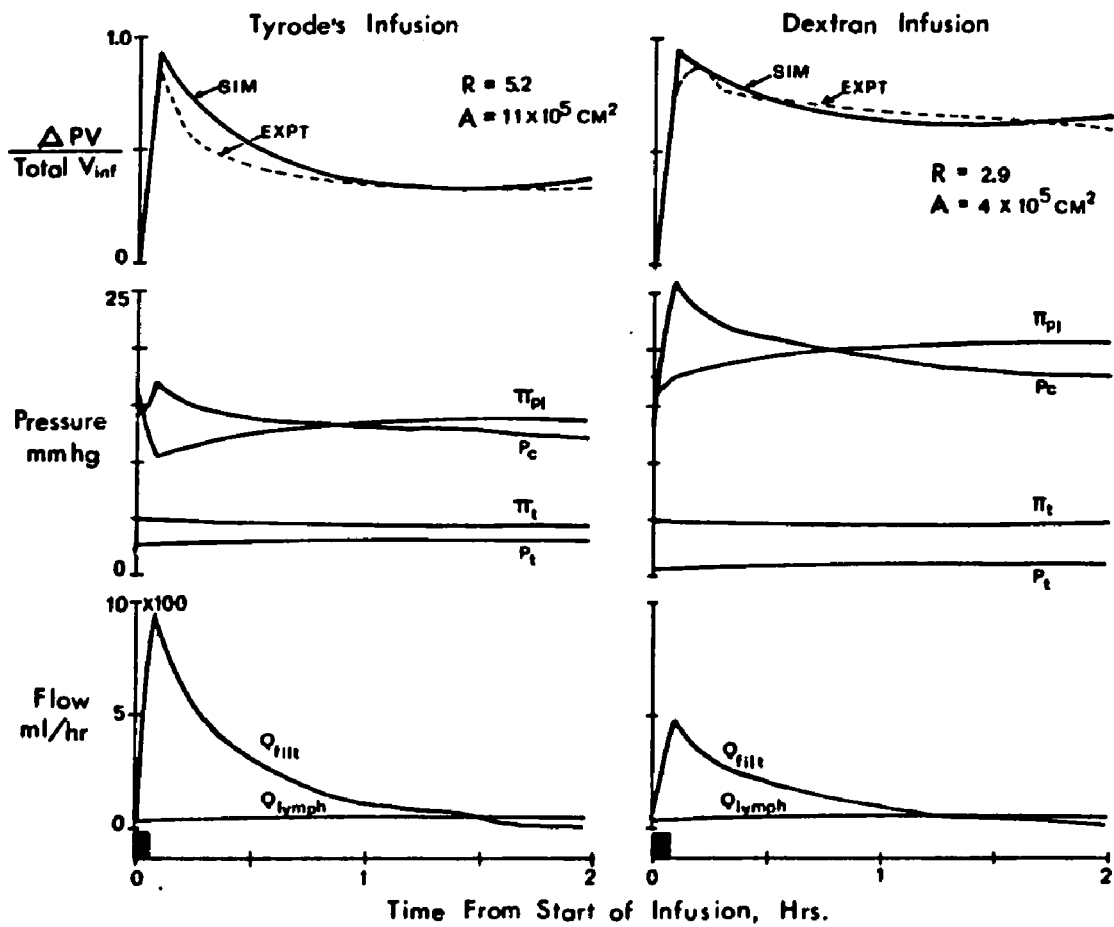


Figure 28. Simulation of rapid Tyrode's and dextran infusions using constant parameter optimization procedure.

During Tyrode's infusion each pressure variable changes in a direction that first increases and later decreases filtration. During infusion, capillary pressure increases and plasma COP decreases thus enhancing net filtration. After infusion, however, the capillary pressure declines, plasma COP increases, tissue pressure increases and tissue fluid COP decreases, all of which tend to counteract the initial effect. It is reassuring, if not humbling, to find that this behavior, described by modern computing simulation techniques, is in agreement with the principles set down by Starling more than 70 years ago.

A qualitatively similar response is seen in the case of dextran infusion with the important exception that the plasma COP increases slightly at the time of infusion (control value of π_{pl} for this group was slightly lower than the COP of the infusate) and continues to increase throughout the experiment as protein-poor fluid filters into the interstitium. The initial increase in filtration driving force is due mainly to the increase in capillary pressure and as a result the maximum filtration rate is about one-half that predicted during Tyrode's infusion.

It is apparent from this study that of the two fluid pathways that connect the plasma and interstitial compartments, transcapillary filtration plays a much more important role than lymph flow, at least during the major portion of the experiment. However, near the end of the experiment, the filtration rate declines to values approximately equal to that of the lymph, and may in fact actually decrease below it as shown. When this occurs, there is a net flow of fluid into the plasma compartment, and the plasma volume is seen to increase very slightly. This behavior was actually observed in several individual experiments as seen in Figures 5 - 8. The present analysis shows that this effect can be due to either of two events: a) the outward filtration rate can decline to a level less than that of lymph flow, or b) net filtration may actually reverse direction (i.e. net reabsorption) and fluid can enter the plasma directly across the capillary wall.

VIII. PHYSIOLOGICAL IMPLICATIONS OF THE SIMULATION STUDY

"...the aim of a mathematical model ought to be to raise more questions than it answers...A corroboration of a model never proves that the model represents reality but only that it can be taken to represent the phenomenon studied (Anatol Rapoport and Albert M. Chammah, 1965)

A. SIMULATED BEHAVIOR OF R, A, AND P_c

Although there is no direct evidence to support the simulated behavior of R, A, and P_c that was obtained during the piecewise optimization studies, it is possible to show that the degree to which they changed is well within physiological limits. Mellander (1968) has summarized the limits of variation that have been observed in several important parameters that control blood flow in skeletal muscle. These data were obtained during studies of hemorrhage, standing, exercise, hypoxia, etc. Table VIII compares these values with those obtained during the present simulation study.

TABLE VIII

VARIABILITY OF MICROCIRCULATORY PARAMETERS:
EXPERIMENTAL VS SIMULATION

Parameter	Known Physiological Variation			Variation During Simulation		
	Min	At Rest	Max	Min	Control	Max
P_c (mm Hg)	10	25	40	12	15	22
A (cm^2)	2000	10000	40000	5000	10000	18000
R*	1.7	4	18	2.8	4	4.8

*Known resistance ratio limits were calculated by assuming constant arterial pressure = 100 mm Hg, venous pressure = 5 mm Hg, resting R = 4 and variation of P_c as shown in first line, left side.

In all cases it can be observed that the absolute magnitude and the

relative variation of the simulated responses are within the expected range. This is an extremely important finding, for it furnishes proof that the model we have constructed is capable of predicting reasonable values for quantities that could not be experimentally obtained. Whether or not the precise dynamic behavior of these predicted parameters are correct must ultimately await further experimental studies. However, certain experimental data that are already available will allow us to reach several preliminary conclusions regarding the validity and physiological significance of the dynamic response.

Before discussing this, however, it is worth pointing out that each consecutive value of R and A , obtained from the piecewise optimization study, is related to the previous value in a manner that indicates a continuous relationship (see Figures 26 and 27). This was not a necessary outcome of the method used, since each segment was optimized independently. If optimizing parameters are chosen which do not exert a sensitive influence on the model's response, then the dynamic responses obtained for these parameters can be almost random in nature. For example, when the protein permeability (which is an insensitive parameter) was used as an optimizing parameter, it was found that widely different values produced a reasonably good fit with the data, but the dynamic behavior of $K_p A$ so obtained did not have any physiological significance.

One might expect that during an infusion of any solution, the effective capillary surface area would increase due to passive increases in transmural pressure and reflex mechanisms, and that afterwards the area would return to normal or below due to both lessening of the distending pressure forces and local myogenic activity. Furthermore, one might expect this effect to be exaggerated for the higher infusion rate and with dextran more than Tyrode's. Essentially, this is what was predicted.

An important result of the simulation study is that the predicted responses for Tyrode's and for dextran infusions were qualitatively and quantitatively different with respect to the pre-/postcapillary resistance ratio and capillary pressure. In the case of Tyrode's there is a sustained increase in the resistance ratio while dextran causes a delayed but dramatic decline (Figures 26 and 27). The capillary

pressure declines to control values following Tyrode's but is increasing above control levels at the end of the dextran response period. If this predicted behavior does indeed occur in the living animal, it can most readily be explained by: a) a high degree of autoregulation during Tyrode's infusion, and 2) in the case of dextran, a failure to autoregulate brought about by a heretofore unsuspected delayed increase in postcapillary resistance. These conclusions will be discussed below.

B. EFFECT OF TISSUE COMPLIANCE ON THE SIMULATED BEHAVIOR OF THE PRE-/POSTCAPILLARY RESISTANCE RATIO

1. TYRODE'S INFUSIONS

The results of the optimization studies presented in the last chapter were obtained by assuming a tissue compliance of 96 ml/kg-mm Hg as suggested by Wiederhielm (1968), or about twenty times that of the vascular bed compliance (Gauer, et al, 1970). If one were to assume a compliance about that equal to the vascular bed (4 ml/kg-mm Hg) in keeping with the findings of Guyton, et al (1965a) we would obtain quite different results as shown in Figure 29.

These graphs compare the simulated results of a rapid Tyrode's infusion assuming either a high or a low compliance. The results for the low compliance case were obtained using the same piecewise optimization procedure as that used for the high compliance case, previously presented in Figure 26. The agreement with the experimental plasma volume response, while not shown, is identical in both cases. For a tissue space of low compliance the resistance ratio is predicted to decrease rather than increase, causing the capillary pressure to be higher than before. Physically, this increase in pressure is necessary to counteract the effect of increased tissue pressure and still maintain the required filtration rate. Thus, the analysis indicates that a substantial qualitative difference exists in the behavior of the pre-/postcapillary resistance ratio for the cases of high vs low tissue compliance.

Is there any reason to suspect that one of these simulated cases is closer to reality than the other? While no one has yet measured the

TYRODE'S INFUSION

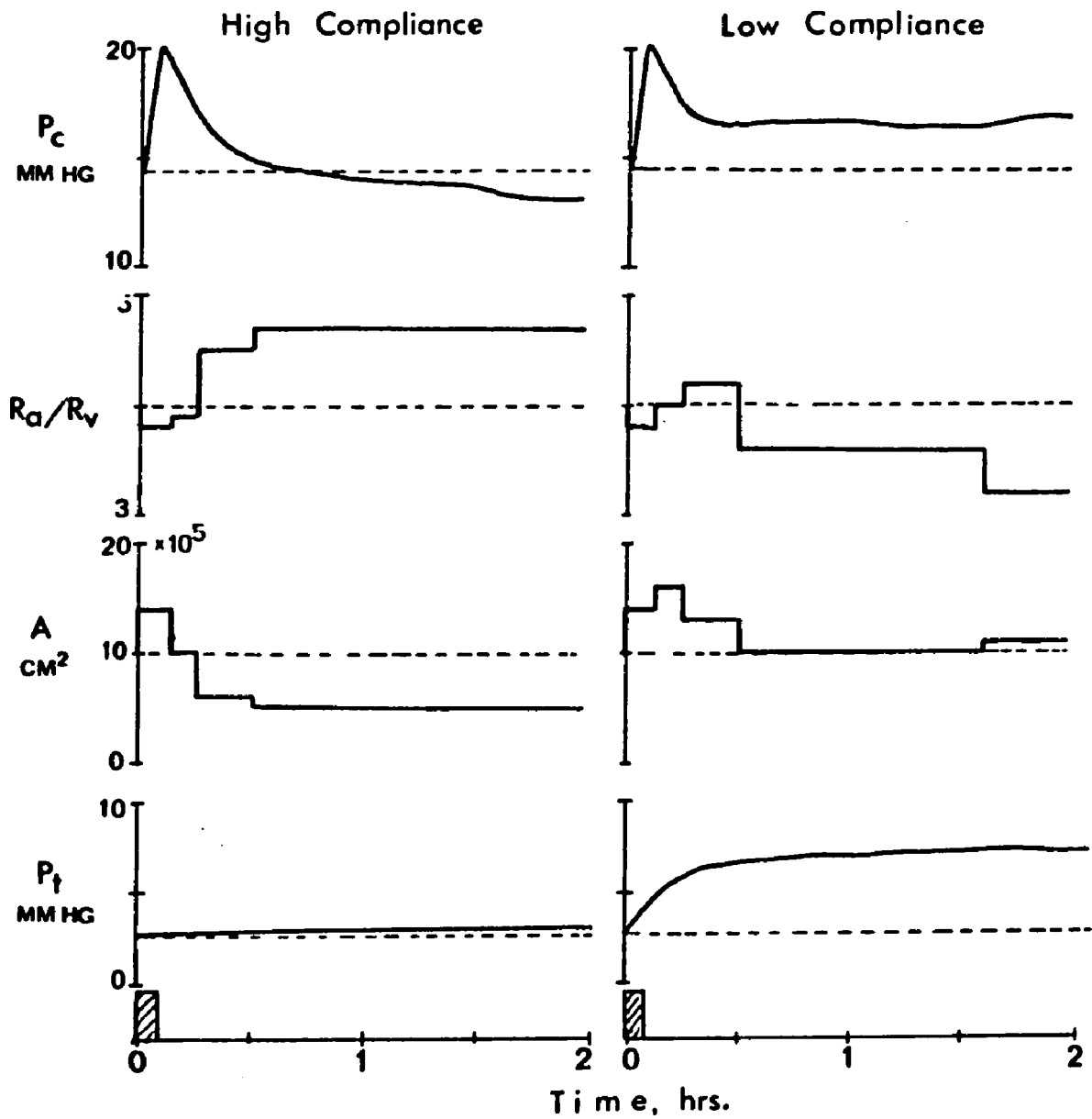


Figure 29. Effect of tissue compliance on microcirculatory variables during simulation of rapid Tyrode's infusions.

resistance ratio, R_a/R_v , during infusions, a technically difficult procedure in the intact animal, it is possible to measure the total peripheral resistance, $R_a + R_v$. Some preliminary findings have already been presented (Figure 12) and the TPR results for the rapid infusion are redrawn in Figure 30 along with the simulated R_a/R_v responses of Figure 29.

The dramatic and sustained increase in TPR (subsequent to an initial decline) shown here has been observed previously following infusions (Conway, 1966; Carey, et al, 1965) and during experimental hypertension (Coleman & Guyton, 1969). Although the increase in TPR has been postulated to be due entirely to local autoregulation (Guyton, et al, 1971), this theory does not have solid substantiation.

It is generally believed that under many conditions the changes in TPR are primarily due to changes in R_a , with R_v remaining relatively constant (e.g. Renkin, 1966; Mellander, 1960; Johnson & Hanson, 1965; Pappenheimer & Soto-Rivera, 1948). If this is true, then the values of TPR may be assumed to vary in the same direction as R_a/R_v . Comparison of the curves in Figure 30 reveals a much stronger similarity between these experimental results and the predicted resistance ratio for the high, rather than the low, compliance case. This analysis suggests that the assumption of an overall highly compliant tissue is more accurate than one of low compliance.

An alternative hypothesis is that the observed increase in TPR is primarily due to an increase in R_v , and in that case the resistance ratio would be expected to decline as predicted for the low compliant tissue. However, this interpretation, while possible, is unlikely in the case considered here. This will be discussed below in more detail.

2. DEXTRAN INFUSIONS

Figure 31 illustrates the simulated effects of assuming either high or low tissue compliance during a rapid dextran infusion. In contrast to the Tyrode's results, the value of R is predicted to decrease especially late in the experimental period for both high and low compliance cases. (It should be noted that the values for both

TOTAL PERIPHERAL RESISTANCE

$$TPR = \frac{P_a - P_v}{CO} = R_a + R_v, \quad \frac{\text{mm hg}}{\text{l/min}}$$

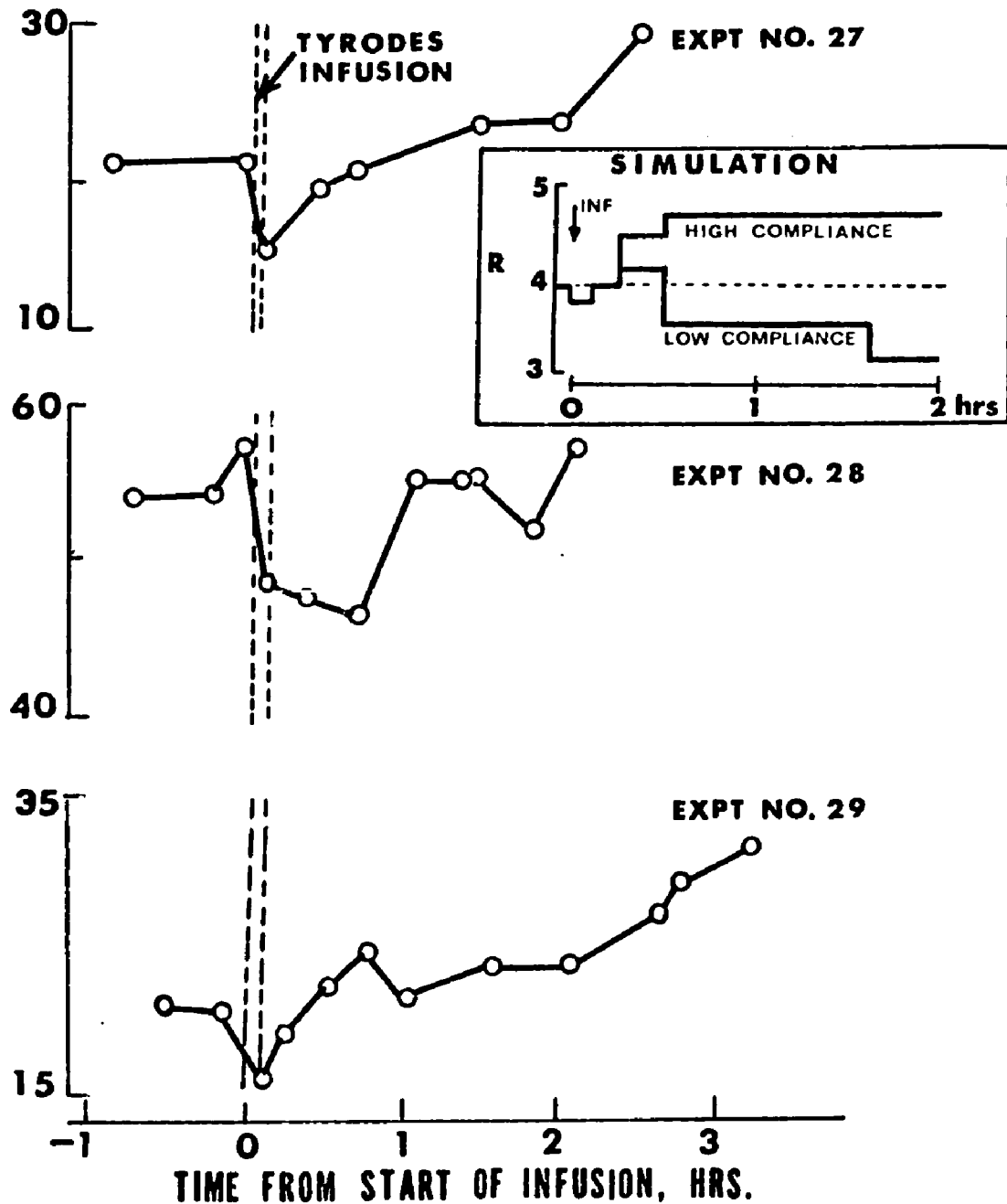


Figure 30. Comparison of experimental total peripheral resistance and simulated pre-/postcapillary resistance ratio during rapid Tyrode's infusions.

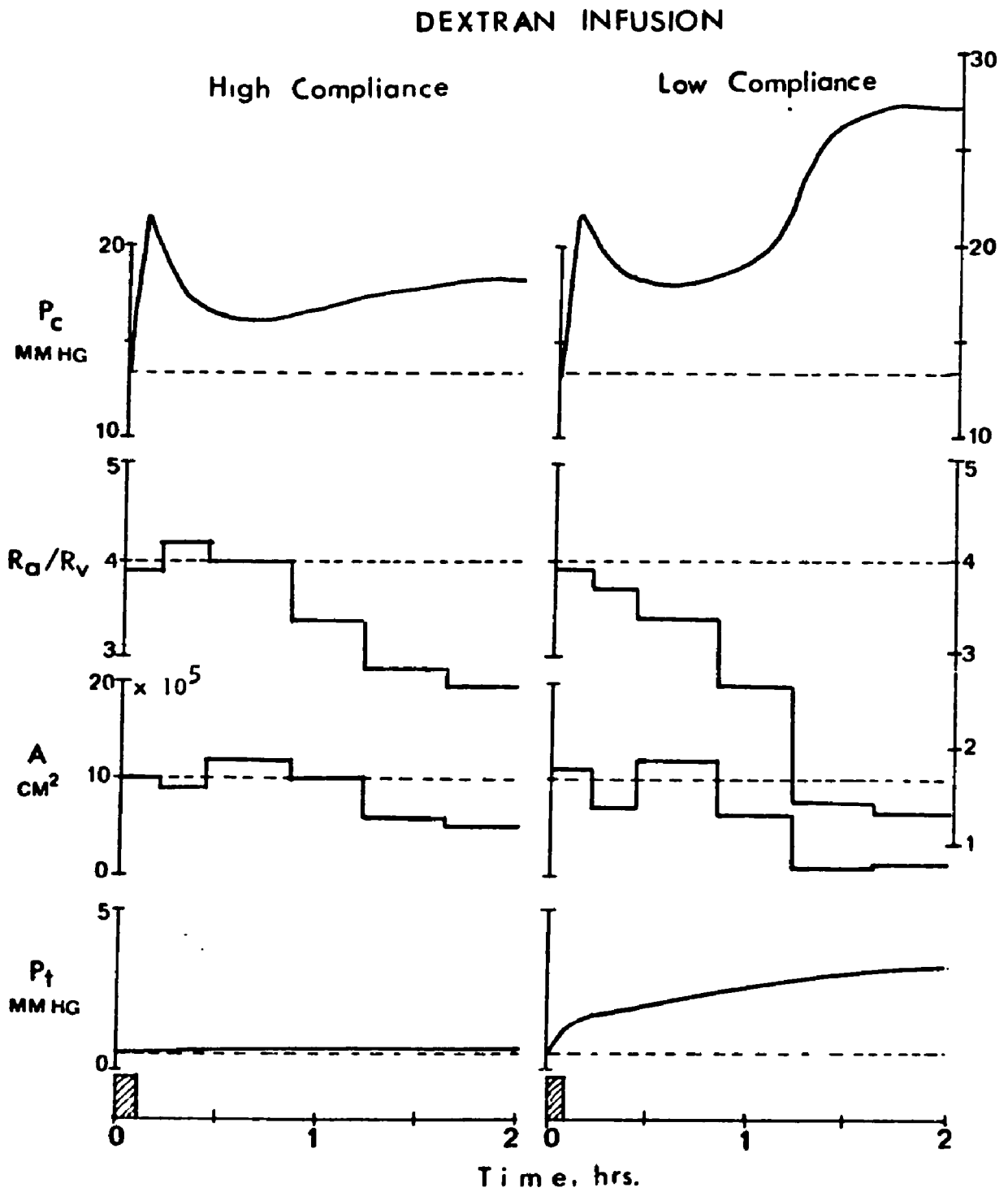


Figure 31. Effect of tissue compliance on microcirculatory variables during simulation of rapid dextran infusions.

the resistance ratio and capillary surface area, obtained when assuming a low compliance, are below the known physiological range -- see Table VIII.) TPR measurements were not performed during dextran infusions in the present study, but Conway (1966) has established that TPR exhibits a delayed increase (1.5 hours post-infusion) following administration of dextran plus whole blood, a behavior similar to our observations for Tyrode's.

The fact that R was predicted to decrease following dextran infusions was unexpected. The ratio of R_a/R_v can show a delayed decrease if: a) R_a decreases relatively more than R_v , b) R_a decreases while R_v increases, and c) R_v increases relatively more than R_a . The first two alternatives, which depend on a delayed decrease in pre-capillary resistance, are unlikely to occur in the present situation. If Conway's data applies here, then TPR would be expected to show a delayed increase and hypothesis (a) could not be correct.

There are several possible mechanisms known to cause a pre-capillary vasodilation that might be operative here. A passive distention of vessel diameters undoubtedly occurs due to the excess infused volume. However, it would be expected that the venules, having a higher compliance, would dilate more than the arterioles, resulting in an increase in R_a/R_v . Central reflexes are known to respond to an increase in arterial pressure by decreasing the pre- more than the postcapillary resistances (Mellander, 1968), but these reflexes are known to become fully effective within one minute following a pressure change. Therefore, they would not be expected to account for the delayed response in peripheral resistance. (Early decreases in R and TPR are observed in both the experimental and simulated results, Figures 29-31).

There are two poorly understood mechanisms, however, that are known to cause delayed changes in vessel tone. One of these is the myogenic autoregulatory response and the other is the delayed compliance or stress relaxation. Both of these are thought to be entirely mediated thru local mechanisms and they are known to be delayed, powerful and longlasting (Guyton, 1963; Guyton, et al, 1971). It is generally believed the myogenic response is operative in small peripheral vessels and causes vasoconstriction in response to increased

transmural pressure. In contrast, stress relaxation is usually said to take place in the larger veins and arteries and causes a vasodilation.

The simulated decrease in R_a/R_v could be directly attributed to these mechanisms if myogenic vasoconstriction affected the post- more than the precapillary resistance, or if stress relaxation occurred primarily in the pre-capillary vessels. All available evidence indicates that, if anything, just the opposite occurs. For example, many reports have indicated that increases in peripheral transmural pressure (especially in the small veins) cause a generalized myogenic vasoconstriction in which both the precapillary sphinctor tone and the R_a/R_v ratio increases (e.g. Folkow, 1964; Mellander, 1968). Thus the myogenic response can fully account for the simulated behavior of both R and A during the Tyrode's infusion (assuming a high compliant tissue) -- see Figure 29. It cannot however, explain the delayed decrease in R predicted for the dextran infusion.

In view of the above, it appears that delayed decreases in pre-capillary resistance are unknown to occur in the face of moderate pressure changes. Hypothesis (c) must, by default, be responsible for the simulated behavior. However, we have argued that the two most likely candidates to account for delayed changes in vascular tone, the myogenic response and stress relaxation, are by themselves incapable of increasing the postcapillary resistance more rapidly than the pre-capillary resistance. It appears that the simulated results cannot be simply explained by any of the mechanisms known to affect vessel tone.¹

Before discarding the model's predictions as anomalous, we would like to propose the following explanation for the predicted fall in resistance ratio: The increased circulatory pressure following dextran infusion causes a dilation of the great veins due to stress relaxation. This delayed increase in central vascular capacitance removes a large quantity of blood from the small post-capillary venules which, as shown by Wideman (1967), normally contains the greatest volume of fluid in the circulation. Redistribution of venular blood will then cause the venules to constrict passively. Myogenic activity, occurring simultaneously, adds to the postcapillary vasoconstriction.

¹The possibility of a direct chemical effect of dextran on the resistance vessels should not be ruled out but such a reaction has not been reported.

The predicted decrease in capillary area that accompanies this response may also be explained by a translocation of blood from the microcirculation to the larger vessels, allowing closure of certain portions of the vascular bed. This phenomena is postulated not to occur following Tyrode's infusions because it has been previously established, during similar infusion experiments, that stress relaxation is minimal in contrast to that observed after administering dextran (Prather, et al, 1969).

Figure 32 illustrates the proposed qualitative changes in R_a and R_v that would account for the changes in the resistance ratio. Thus the unexpected results predicted by the simulation can be explained by a combination of sequential factors: local reflexes distending large vessels, redistribution of blood away from the periphery, and passive decreases in vessel diameter. This mechanism depends on a greater degree of stress relaxation occurring in larger vessels than in the post-capillary resistance veins. Stress relaxation occurring in the arteries would be expected to have relatively little effect on R_a compared to the effect of the veins on R_v since capacitance of the arteries is much less than that of the veins (Guyton, et al, 1959).

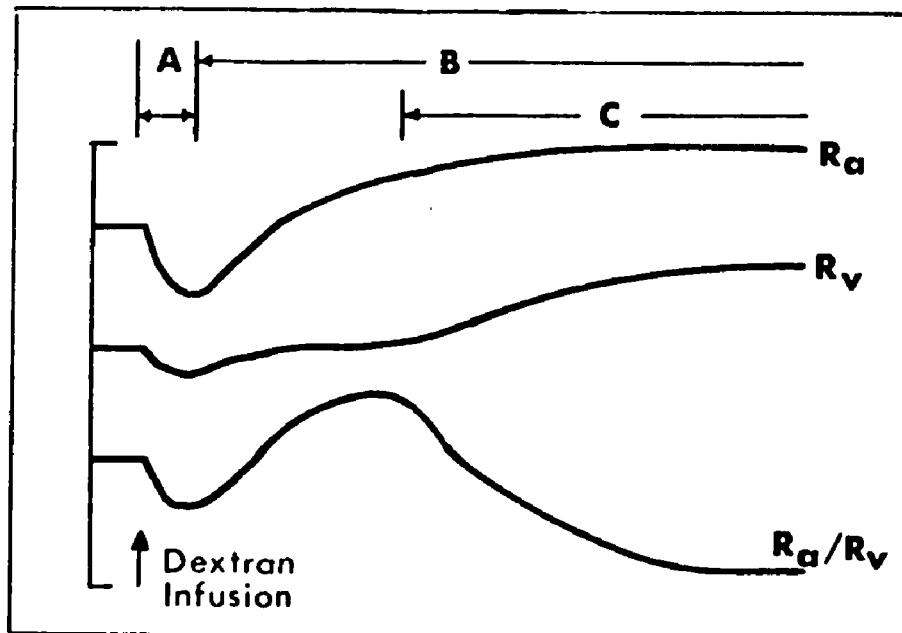


Figure 32. Postulated changes in pre- and postcapillary resistances during dextran infusion. The primary mechanisms effective during the times are indicated: A = central reflex response and passive distension of vessels, B = myogenic response, C = stress relaxation and redistribution of blood away from the periphery.

Surprisingly little is known about changes in venous tone elicited locally or directly by changes in intraluminal pressure (Herd, 1970). According to Folkow and Mellander (1964) a major difficulty in studying the postcapillary vessels, lies in our inability to quantitatively distinguish between the two main functions of the venous system: the resistance function and the capacitance function. Whether there is a dissociation between the control of these components and how they may be separately controlled is not known (Renkin, 1966). Due to the relatively low hydraulic resistance of the postcapillary veins, small changes in either their resistance or in venous pressure can have profound effects on the regulation of intravascular/extravascular volume. The venous system, because of its visco-elastic properties, and by virtue of the smooth muscles in its walls, can both passively and actively change its capacitance, and hence, is fundamentally important in the distribution of blood within the cardiovascular system. Our hypothesis takes into account both of these specialized functions of the veins.

3. ANALYSIS OF TISSUE COMPLIANCE

An important result of the present analysis is that a relatively high value for tissue compliance consistently produced more reasonable physiological responses for the microcirculatory parameters, R and A , than did a much lower value. It can be asked if this invalidates Guyton's (1965a) concept of a normally low compliant tissue space. The question seems to be answered by Guyton himself (1965b) who suggests that animals which are anesthetized for several hours tend to accumulate tissue fluid due to reduced lymph flow and osmotic forces. In the present study, the animals were anesthetized several hours prior to the first infusion and then several more hours during the infusion response period. If tissue fluid progressively accumulated during this period it is possible that the tissue compliance would decrease, as the upper curve in Figure 18 (after Guyton, 1965a) suggests. Furthermore, each infusion would increase the amount of tissue fluid. Thus, it appears that on experimental and theoretical

grounds, both Guyton's and Wiederhielm's (1968) relationship for tissue compliance lead to the same conclusion: that the anesthetized animal used in this study had a relatively high tissue compliance. An alternative explanation is that although Guyton determined a low tissue compliance for selective areas of the body, the overall body tissue compliance may be much higher.

C. ROLE OF PRE-/POSTCAPILLARY RESISTANCE RATIO AND SPHINCTER TONE IN THE REGULATION OF PLASMA-INTERSTITIAL FLUID BALANCE

The choice of optimizing parameters in this study was far from arbitrary. The precapillary and postcapillary resistances and sphincter elements of the periphery play a crucial role in controlling fluid balance, capillary exchange, and blood flow. They are directly responsible for changes in: 1) total peripheral resistance, which is a major factor in controlling total blood flow to individual organs, 2) the pre-/postcapillary resistance ratio, which affects capillary hydrostatic pressure and thereby aids in controlling ultrafiltration, and 3) the capillary surface area available for exchange, which not only determines the distribution of total blood flow within a capillary network, but along with the resistance ratio is capable of controlling total blood volume as well as intra- and extravascular fluid balance. Although control of these peripheral elements appears to be coordinated with cardiac performance and pressure regulation via central mechanisms, it is possible for the local autoregulatory elements to override central adjustments to satisfy the needs of an individual tissue. In light of the sometimes competing demands for controlling total blood volume, organ perfusion, capillary pressure, and local fluid balance, it is certainly not clear how these mechanisms might interact during plasma volume expansion to produce a beneficial effect to the animal. In particular, the integration of control mechanisms affecting pre- and postcapillary resistance, sphincter tone and vessel capacitance, still remains an unsolved problem.

The predicted peripheral resistance changes that accompanied the Tyrode's infusions appeared to satisfactorily readjust the capillary

pressure to pre-infusion levels. In the case of dextran, however, the resistance ratio was adjusted in a direction that progressively increased capillary pressure. At the same time, the effective capillary surface area during the response to dextran appeared, on the average, to be higher than that predicted for Tyrode's. These changes might be interpreted as an attempt to control primarily total blood volume, and secondarily to control local fluid balance.

The initial rise in capillary pressure and decrease in plasma COP following Tyrode's infusion may have been sufficient to reduce the plasma volume to levels that were within the normal range of homeostatic control of central volume and pressure. (Blood volume was expanded about 10% after one hour). Fluid balance on the local level could then best be insured by maintaining a normal capillary hydrostatic pressure. In fact, when the volemic stresses are least acute (as in the slow Tyrode's infusion) simulation predicts that capillary pressure, resistance ratio and capillary area all return to nearly normal levels by the end of the experiment. However, increases in plasma volume due to dextran infusions (about 25% blood volume expansion after one hour) were not so easily counteracted by the initial passive rise in circulatory pressures. It would be reasonable then for the resulting hypervolemic stress to invoke delayed changes that would increase capillary pressure and surface area. This would have the effect of eliminating excess volume from the circulation. Thus, local and central control of transcapillary filtration may first ensure adequate regulation of total blood volume. Only if this can be accomplished do readjustments occur which return peripheral parameters to normal levels.

It must be emphasized that much of the evidence on which the above discussion is based has been derived from our simulation studies, and not experimental observation. Nevertheless, there is evidence that supports this interpretation. Zweifach (1971) has observed that the capillary pressure is remarkably stable (± 2 mm Hg) despite wide variations in arterial pressure. Except for the minutes immediately following infusion, the simulated capillary pressure remains within these same limits. Guyton, et al (1971) have established that autoregulation of blood flow occurs not only in individual organs, but in the total

systemic circulation as well. They have also found that the ability of the total circulation to autoregulate diminishes with increasing arterial pressure. This observation gives credibility to the present study where it appears that the relatively small increases in blood volume expansion following Tyrode's may permit better autoregulation than with the larger volume changes observed with dextran. The importance of the pre-/postcapillary resistance ratio and the capillary surface area in autoregulating transcapillary filtration during exercise, standing, and hemorrhage has previously been discussed by Lundvall, et al (1967) and Mellander (1968). The present analysis can be interpreted as demonstrating a similar kind of autoregulation during the hypervolemic states following infusion.

D. INCREASE IN PLASMA PROTEINS FOLLOWING INFUSIONS

Both our experimental studies and theoretical analysis indicated that plasma proteins increased following infusions in all groups. The experimental and simulated results agreed well except for the slow dextran infusions (Figure 27). In that group the protein mass increased to 25% above control levels.

There are many contradictory reports in the literature that show both increases and decreases in plasma protein quantity following infusion. For example, after infusing dextran in dogs, Davies, et al (1963) reports up to a 15% increase, Prather (1968) observed a very small transient increase that returned to control, and Wasserman and Mayerson (1952b, 1954) found either no change, or up to a 30% decrease. Similarly, for saline infusions in humans, Moore, et al (1966) measured an "unexpected" 10% increase in plasma proteins, while Prather (1968) infused 2 liters Tyrode's in dogs and found a 30% decrease. Although the results for dextran do not seem to be correlated with the volume infused, Wasserman and Mayerson (1952a) showed that small saline infusions (25 ml/kg) tended to increase plasma proteins as much as 25%, while there was a decrease for larger infusions.

The origin of the protein that enters the plasma upon infusion is still not clear. Both Moore, et al (1966) and Wasserman and Mayerson

(1952a) rule out the possibility that the rate of hepatic synthesis of albumin is great enough to account for the observed increases. Wasserman and Mayerson ascribed the increase of proteins following smaller saline infusions, to a greater rate of protein return thru the lymphatics than the rate of protein escape from the capillaries. However, calculations (based on our experimental data) on the amount of lymph protein returning thru the thoracic duct show that it is unlikely that this source can account for the larger increases observed in the present study. Moore, et al (1966) support this view. In addition to the thoracic duct, however, there are other lympho-venous shunts that might be involved (Rusnyak, 1967). One must assume the majority of this new protein is preformed and previously present in some other area of the extracellular fluid, possibly in the hepatosplanchnic lymphatics (Moore, et al, 1966). The data of Greenway and Stark (1971) indicate, however, that the interstitial fluid of the dog's liver contains only about 4 gms of protein, which is too small to account for the 7-15 gm increase we observed following the slow dextran infusion. It has been suggested that interstitial protein can enter the circulation thru other pathways than the lymphatics, namely thru sites in the capillaries that are anatomically separate from those involved with transcapillary filtration (Moore, et al, 1966). Casely-Smith (1971) has described fenestrations equipped with diaphragms in the capillary wall, thru which it is speculated that proteins are able to enter the circulation but are prevented from returning to the tissues.

The fact that this large protein release was observed for the first but not the second infusion of dextran in the same animals indicates that the postulated store of preformed proteins became depleted after the stress of the first infusion. It is not readily apparent why a smaller degree of protein release was observed during Tyrode's infusion although this solution was administered in a different group of animals than those receiving dextran.

E. EVALUATION OF THE MODEL

1. VALIDITY OF MODEL

Mathematical theorems describing abstract systems can be proven true or false. In contrast, there is no comparable formalism to test the mathematical model of a real system. However, there are certain guiding principles, some of which have been discussed by Rapoport and Chammah (1965).

The aim of a mathematical model ought to be to raise more questions than it answers, that is, point the way for further experimentation. A model should be able to account for known behavior of the most crucial variables under consideration. The fewer free parameters a model has and the greater the range of situations to which it applies, the stronger it is. The value of a model increases with its ability to explain observed behavior and to predict beyond the data with which it was generated.

While the present model should be evaluated in terms of these criteria, no attempt will be made to do so here. The model and the analysis must stand on its own merits. However, it may be mentioned that the predictive capability of our model is restricted somewhat by the input data necessary to stress it. That is, if blood volume and central circulatory pressure changes are known, it is possible to predict certain microcirculatory parameters. If the predictions regarding efficient overall autoregulation of capillary pressure during Tyrode's infusion are indeed correct, then the disappearance of infused fluid from plasma can be accurately simulated with the model without using optimization, rather than measuring plasma volume and blood pressure changes and fitting parameters. Unfortunately, it appears that effective autoregulation does not occur with dextran infusion and the assumption of a constant capillary pressure would be erroneous. It has already been mentioned that the excellent agreement between simulated and experimental plasma volume responses might not have occurred, even using optimization techniques, unless the model was a reasonable representation of reality. In addition, the reasonable and well ordered behavior of the time-varying responses of the optimizing parameters

add support to the model's validity. Thus, it may be stated that the model, which centers around a lumped-parameter Starling relationship for the whole body, appears to satisfactorily account for short-term acute hypervolemic changes in the nephrectomized dog.

2. IMPLICATIONS OF THE LUMPED-PARAMETER CONCEPT

The model considered in this study is a "two-compartment lumped-parameter model", so called because we have treated each resistance, capacitance or mass element as if it were concentrated in either the plasma or interstitial compartments. We have neglected regional differences which are known to occur for such factors as lymph flow, capillary permeability, capillary pressure, tissue compliance, and peripheral resistance. Thus the splanchnic organs are known to have a higher capillary permeability and a greater degree of autoregulation than the muscles (Mayerson, et al, 1960; Mellander & Johansson, 1968), the liver has a higher effective compliance than the kidney or skeletal muscle (Greenway & Stark, 1971), and it has been established that lymph flow at rest is derived primarily from the liver and intestines (Rusnyak, et al, 1967). However it is quite normal and reasonable to take a simplified approach when first studying a complex system especially since little quantitative information is available regarding the regional differences cited above.

How might these simplifications affect the results of our analysis? As an example, consider the transcapillary exchange mechanisms for fluid and protein. Deviations between simulation and experimental results might be attributed to the existence of more than one interstitial compartment that exchanges protein and fluid with the plasma. The additional compartments might have different mass transfer coefficients and therefore different time constants for the response behavior of filtration or diffusion. The sum behavior of several such compartments might result in a more accurate representation of the data.

Another example concerns the values obtained for the microcirculatory variables: R , A , and P_c . We should consider their simulated behavior to be representative average values for the entire systemic

circulation and not necessarily that of an individual tissue like muscle or intestine. On the other hand, Mellander (1968) and Oberg (1964) have suggested that the major portion of any acute plasma fluid shift occurs within the muscle rather than other areas. Inasmuch as most of the body tissue is muscle, the results we have obtained for the microcirculatory variables might reflect changes in this tissue.

In spite of the two-compartment simplification, most of the results appear to be understandable. While they may point the way to further experimentation for confirmatory evidence, the present study does not indicate that a model with additional fluid compartments need be considered.

3. DEFICIENCIES OF THE MODEL

No model is complete, for that would imply a complete understanding of the system under consideration and obviate the need for mathematical approximations. The deficiencies of the present model can be divided into two categories: a) incomplete understanding or relationships included in the model, and b) relationships so poorly understood that they cannot even be approximated and thus were not included.

Deficiencies in the first category include a poor understanding of macromolecular transport across capillaries and thru the lymph system, inadequate knowledge of factors that propel lymph fluid, and incomplete data regarding tissue pressure and tissue compliance. We have been able to show that the first two of these factors play a relatively unimportant role in short-term infusion experiments or else, in the case of tissue compliance, the model was able to distinguish between two alternate relationships.

Deficiencies in the second category are more extensive since they can include factors which affect the entire fluid balance regulating system, i.e. control of renal function, cell-tissue exchange, and body fluid solute concentrations. However, if we limit the model to its original purpose, that of describing plasma-interstitial fluid exchange, there are at least two major areas which are wholly incomplete; viz., the relationships describing short-term behavior of both

a) the central blood pressures, and b) the hydraulic resistance of the microcirculation. These variables have been accounted for in the present model by either using experimentally determined arterial and venous blood pressures or parameter optimization-simulation techniques that determined suitable values for the resistance ratio and capillary surface area. By so doing we have avoided the need to describe the role of factors which affect vessel tone, such as, vasoactive chemicals, baroreceptor feedback pathways, circulating hormones, intravascular blood distribution, and the even more poorly understood local autoregulatory reflexes.

F. SIGNIFICANCE AND APPLICATIONS

The results of the present investigation can be applied on several levels to the study of body fluid regulation: a) experimental methods to study transcapillary filtration in the whole body, b) clinical investigation of physiological events during the treatment of body fluid imbalances, and c) theoretical techniques for analyzing the dynamics of the plasma-interstitial fluid exchange system.

Transcapillary fluid exchange has been studied previously by many different techniques, including: direct observation of single capillaries (Landis, 1927; Zweifach & Intaglietta, 1966, 1968); isogravimetric procedures (Pappeheimer & Soto-Rivera, 1948; Johnson & Hanson, 1962, 1966; Diana, 1970), isovolumetric methods (Mellander, 1960; Folkow, et al., 1963), and double isotope techniques (Levine, et al., 1967) applied to isolated organs; and pressure plethysmographic methods for studying filtration rates in the intact human forearm (Krough, et al., 1932). In most whole organ studies, transcapillary movement is estimated by indirect measurements of changes in tissue volume. One aspect of the present investigation, as seen in this perspective, was the measurement of filtration rates in the whole animal by eliminating renal excretion and determining continuous changes in blood volume using a single-injection isotope dilution technique.

In another sense, our study may have pertinence to the clinician engaged in treating imbalances in body fluid regulation, using such

techniques as fluid replacement and hemodialysis therapy. Until this study, there has been virtually no work in attempting to quantitatively relate the post-infusion responses of blood volume and blood pressures. For example, in reporting on a series of infusion experiments, Wasserman and Mayerson (1952a) observed that "blood pressure changes in all (saline infusion) experiments were minimal and could not be related to the changes in the distribution of saline." We have shown that the relationship is far from obvious and that it may in fact contain information which would be extremely difficult to measure directly. The theoretical analysis performed in this study has predicted changes in the microcirculation which, if true, support previously postulated mechanisms with which the circulatory system can protect itself against hypervolemic stresses. In addition, the model may prove to have utility in predicting the disappearance of infused solutions or investigating peripheral vascular behavior during therapy.

There is no reason why the techniques used in this study on dogs could not be extended to the human subject. The method of measuring blood volume changes continuously with the extracorporeal shunt-isotope dilution technique can easily be adapted to human subjects. Furthermore, while we have been presently concerned with infusion studies, the reverse process, that of hemodialysis, can be studied in an entirely analogous manner. It is recognized that problems related to fluid and pressure changes, occurring within the patient during dialysis, are far from completely understood (Abbrecht & Prodany, 1971). Computer simulation-optimization techniques, as used here, utilizing dynamic experimental data with a rational-parameter model and perhaps on-line computer operation, show promise as a means to approach these problems.

Perhaps the most proper way to regard the present study is to consider it as furthering the understanding of the extremely complex plasma-interstitial fluid exchange system. By combining simulation and optimization techniques we have examined this system for the case of unsteady state fluid distribution following infusions. A major contribution of this study was the prediction of the time-varying behavior of microcirculatory resistance elements using readily obtainable blood volume and blood pressure data. Some of the simulation

predictions were not expected, but all could be explained in terms of known physiological relationships. Analysis of the model has served the heuristic purpose of suggesting additional confirmatory experiments. Mathematically, our approach was not wholly original, but these techniques have just recently come into use to estimate constant parameter values connected with the peripheral vasculature. (Sato, et al, 1971; Rothe, 1971; Yamashiro, et al, 1971; and Lee, et al, 1971.)

IX. SUMMARY

The purpose of this investigation was to study, both experimentally and theoretically, the non-steady state distribution between the plasma and interstitial compartments of intravenously infused solutions. We have attempted to ascertain if certain well known physiological relationships, such as Starling's description of transcapillary fluid movement, which have been proven correct for the steady state in individual organs, can account for the dynamic disappearance of infused isotonic fluids in the nephrectomized dog.

In the theoretical portion of this study, a non-linear model was developed to simulate infusion experiments on the digital computer using IBM's CSMP language. The following factors were considered in the model: 1) transcapillary fluid exchange and protein diffusion, 2) lymph flow and tissue pressure expressed as functions of interstitial fluid volume, and 3) an accepted relationship between the capillary, arterial and venous pressures and the pre-/postcapillary resistance ratio. Two different relationships for tissue compliance that have been previously postulated were examined in the analysis. Experimental arterial venous blood pressure responses and the infusion rate were used as forcing functions. A special subroutine optimized two parameters, the pre-/postcapillary resistance ratio, R , and the effective capillary surface area, A , by minimizing the least squares difference between the simulated plasma response with the experimental response. The time-varying behavior of these parameters was obtained using a piece-wise optimization procedure. This approach extended the capabilities of previously developed models describing transcapillary exchange.

The model was tested with experimental data collected during 25 infusion experiments, lasting 2 - 3 hours each, performed nephrectomized dogs. Plasma volume expansion was accomplished by dividing the experiments into four groups according to the type of solution and rate of infusion: a) Tyrode's solution infused at 15 ml/min, b) Tyrode's solution infused at 100 ml/min, c) dextran solution infused at 15 ml/min, and d) dextran solution infused at 100 ml/min. The dextran solutions were isotonic and iso-oncotic with respect to canine plasma, and the dextran molecular weights ranged from 60,000 to 300,000. The

volume infused was 2.5% of body weight (approx. 500 ml). The variables that were measured, either continuously or by frequent sampling and analysis of blood and lymph, included: blood volume, mean arterial pressure, mean venous pressure, thoracic duct lymph flow rate, protein concentrations of plasma and lymph, and total peripheral resistance. Blood volume was measured continuously using a single injection of ^{51}Cr labelled red cells and monitoring the blood radioactivity as it passed thru an extracorporeal shunt.

Significant experimental results were the following: at the end of two hours the blood volumes of the Tyrode's and dextran groups were expanded to 10% and 24% respectively, while 27% and 65% of the respective infused volumes were retained in the plasma. Also at this time the plasma volume had reached a steady state value following Tyrode's infusions, but was still declining in the dextran infused animals. The rate of infusion did not affect these results to any significant extent. The averaged arterial and venous blood pressures were somewhat greater in magnitude and more sustained for dextran than for Tyrode's infusions. Total peripheral resistance, measured only during Tyrode's infusion experiments, showed no change on the average following the slow infusions. However, the response to the rapid infusion was an initial decline followed by a dramatic rise. The plasma protein quantity increased by several percent following all infusions except for the group receiving the slow infusion of dextran. In that case there were increases as high as 25% above control which could not be explained by protein returning from the thoracic lymph duct. Lymph flow did not change dramatically in three of the four groups, but following the rapid Tyrode's infusions there were transient increases up to three times control values.

The major results of the simulation study can be summarized as follows:

1. Excellent agreement was obtained between predicted and experimental plasma volume responses for the four experimental groups during a two hour simulations. It was found that agreement was improved considerably if the pre-/postcapillary resistance ratio and capillary surface area were allowed to assume time-varying, rather than constant

values.

2. Experimental data were unavailable concerning the effects of infusion on the microcirculatory variables, R , A , and capillary pressure. However, the behavior of these quantities as predicted by the simulation, were all within reasonable physiological limits as established in previous studies on exercise, hemorrhage, tilting, etc. In addition, the dynamic nature of the responses could be explained in terms of known physiological relationships.

3. The model predicted qualitatively different responses for the pre-/postcapillary resistance ratio (and thus for the capillary pressure as well), depending upon the type of solution infused and the value assumed for tissue compliance. Simulation predicts that the capillary pressure rapidly returns to normal after an initial rise, following infusions of Tyrode's solution. In contrast, following dextran infusions, the capillary pressure is predicted to rise, fall slightly, and then gradually increase to several mm Hg above control at the end of 2 hours. Thus, effective homeostatic control of capillary pressure may occur after moderate blood expansion by Tyrode's solution, but not during the more severe expansion by dextran solution.

4. Comparison of the simulation results with experimental data indicates that the assumption of an overall highly compliant tissue space (approx. 100 ml/mm Hg-kg body weight) is more accurate than that of the lower compliances previously reported for muscle and skin (approx. 4 ml/mm Hg-kg BW). This discrepancy with other investigators' results may be due in part to the anesthesia's effect of reducing lymph flow.

5. The pre-/postcapillary resistance was predicted to increase above control following Tyrode's infusion (assuming a high compliance tissue compartment) which was consistent with experimental TPR data and the myogenic hypothesis of autoregulation. In contrast, following dextran infusions, the simulated pre-/postcapillary resistance ratio exhibited a delayed decrease for either a high or low tissue compliance. This was attributed to an unexpected delayed increase in the postcapillary resistance. A postulated mechanism that would account

for this phenomena involved stress relaxation in the larger veins followed by redistribution of venular blood and a passive decrease in postcapillary vessel diameter.

6. The behavior of the simulated responses of R, A, and capillary pressure was interpreted as representing two types of circulatory responses to a volume load: 1) an attenuation of increases in total blood volume, and if this could be accomplished, 2) readjustment of microcirculatory resistances and exchange area to insure control of intra-extravascular fluid balance on the local organ level.

7. Parametric studies revealed that neither changes in lymph flow or transcapillary protein movement play important roles in determining the short-term plasma retention of infused fluids. On the other hand, changes in the capillary filtration surface area, the pre-/postcapillary resistance ratio, and tissue compliance do exert a major influence on this process.

8. We have shown that it is possible to relate the post-infusion responses of blood volume and blood pressure using a suitable model, and by so doing determine the time-varying behavior of certain difficult to measure microcirculatory variables. In general, it appears, that this model, centering around a lumped parameter whole body representation of the Starling relationship, is capable of satisfactorily accounting for short-term hypervolemic changes in the nephrectomized dog.

A P P E N D I C E S

APPENDIX A.

SYMBOLS, NOTATIONS AND UNITS

"Nomenclature should not be used to complicate relations which are in themselves difficult enough." (August Krogh, 1959)

Variables and Parameters

a	= molecular radius, angstroms
A	= effective capillary surface area of body, cm^2
BV	= blood volume, ml
BW	= body weight, kg
C	= concentration of colloids, gm/ml
CO	= cardiac output, l/min
COP	= colloid osmotic pressure, mm Hg
h	= step function
J	= net flow rate of proteins, gm/hr
$K_p A$	= capillary protein permeability-surface area product, ml/hr
$K_w A$	= capillary filtration coefficient, ml/hr-mm Hg
M	= compartmental protein mass, gm
MW	= molecular weight
P	= hydrostatic pressure, mm Hg
[Pr]	= experimentally measured protein concentration, gm %
PV	= experimentally measured plasma volume, ml
Q	= net flow rate of fluid, gm/hr
R	= R_a/R_v , pre-/postcapillary resistance ratio
RCV	= total circulating red cell volume, ml
t	= time, hr
TPR	= total peripheral resistance, mm Hg/l/min
V	= Compartmental fluid volume, ml

Greek Symbols

γ	= compliance of interstitial compartment, ml/mm Hg-kg BW
π	= oncotic pressure of compartmental fluid, mm Hg
ΔT_{inf}	= period of infusion, hr

Subscripts

a	refers to arterial or precapillary circulation
c	refers to capillary
dif	refers to diffusion of colloids across capillary wall
e	refers to extracellular compartment
filt	refers to transcapillary filtration
inf	refers to the infusion solution of infusion process
lymph	refers to lymph fluid
o	refers to control period or initial conditions
pl	refers to plasma compartment
t	refers to tissue (or interstitial) compartment
v	refers to venous or postcapillary circulation

APPENDIX B.

TABLE A-1

CONTROL VALUES FOR EACH EXPERIMENTAL ANIMAL

EXPT #	BW	V _{inf}	SOL'N	F _{cell}	RCV	BV	PV	[Pr] _{pl}
	kg	ml			ml	ml	ml	gm %
18	19.2	500	TYR	0.853	308	1165	845	7.1
19	21.0	525	TYR	1.055	556	1458	862	7.3
20	20.5	515	TYR	0.800	592	1721	1106	5.8
21	20.0	500	D250	0.876	577	1508	951	5.8
22	19.1	480	D250	0.948	508	1139	903	5.8
23	20.1	505	D250	0.870	477	1344	887	6.0
24	22.5	565	D80	0.940	698	1423	1105	5.6
25	21.0	525	D80	0.836	559	1561	1022	5.3
26	18.0	450	D80	0.904	475	1242	787	7.3
27	19.5	490	TYR	0.925	549	1707	1135	5.1
28	17.8	415	TYR	0.878	307	1191	859	6.0
29	19.3	480	TYR	0.961	594	1730	1109	5.6
MEAN	19.8	496		0.904	517	1432	964	6.1
STD DEV	±1.3	±38		±0.068	±114	±219	±124	±0.8

RESULTS EXPRESSED AS PERCENTAGE OF AVG BODY WEIGHT:

$$BV = 7.2\% \pm 1\%(sd) \quad PV = 4.86\% \pm 0.54\%(sd) \quad RCV = 2.6\% \pm 0.5\%(sd)$$

APPENDIX C.

TABLE A-2

CONTROL VALUES ASSOCIATED WITH INDIVIDUAL EXPERIMENTS
(See Figures 5 to 8)

EXPT #	PV _o	P _{a,o}	P _{v,o}	[Pr] _o
	ml	mm Hg	mm Hg	gm %
18 S	845	60	4.9	7.1
18 R	922	35	4.0	6.5
19 S	862	74	3.8	7.3
19 R	1006	74	3.2	6.8
20 S	1106	71	1.6	5.8
20 R1	1255	55	1.8	5.0
20 R2	1410	57	2.0	4.8
21 S	951	50	2.6	5.8
21 R	1211	52	3.4	-
22 S	903	55	0.6	5.8
22 R1	1148	51	0.6	5.4
22 R2	1499	52	1.2	-
23 S	887	64	-0.4	6.0
23 R	1191	66	0.1	4.8
24 S	1105	64	2.0	5.6
24 R	1368	77	2.3	5.2
25 S	1022	58	-0.5	5.3
25 R	1353	52	-0.2	4.9
26 S	787	78	0.8	7.3
27 S	1135	53	3.1	5.1
27 R	1219	52	3.9	4.8
28 S	859	80	3.9	6.0
28 R	889	86	3.6	5.6
29 S	1109	64	1.8	5.6
29 R	1218	60	2.0	5.5

S = slow infusion

R1 = 1st rapid infusion

R2 = 2nd rapid infusion

REFERENCES

- Abbrecht, P.H. and N. Prodan. 1971. A model of the patient-artificial kidney system. *IEEE Trans. Biomed. Eng.* 18: 257-264.
- Albert, S.N., W.A. Spencer, M. Finkelstein, J. Shibuya, S. Alpert and C. Coakley. 1956. A plastic coil simplifying radioactivity liquid phase counting. *J. Lab. & Clin. Med.* 48: 471-475.
- Albert, S.N. 1963. *Blood Volume*. Springfield, Ill: Charles C. Thomas.
- Albert, S.N., W.A. Spencer, J. Shibuy, C.S. Coakley, and R.J. Thistlewaite. 1957. Observations on fluctuations in blood volume as determined with radioactive isotopes. *Anesth. & Analg.* 36: 54-61.
- Altman, P. and D. Dittmar, (Editors) 1971. *Respiration and Circulation*. Bethesda: Fed. Amer. Soc. Expt. Biol.
- Areskog, N.H., G. Arturson, and G. Grotte. 1964. Studies on heart lymph I. Kinetics of I-131 albumin in dog heart-lung preparations. *Acta Physiol. Scand.* 62: 209-217.
- Arturson, G. and I. Kjellmer. 1964. Capillary permeability in skeletal muscle during rest and activity. *Acta Physiol. Scand.* 62: 41-45.
- Attinger, E.O. and A. Anné. 1966. Simulation of the cardiovascular system. *Ann. N.Y. Acad. Sci.* 128: 810-829.
- Beneken, J.E.W. and B. DeWit. 1967. A physical approach to hemodynamic aspects of the human cardiovascular system. In *Physical Bases of Circulatory Transport*. Chapter 1. (See Reeve & Guyton, 1967).
- Bohr, D.F., R.L. Verrier, J. Sobelski. 1971. Non-neurogenic tone in isolated perfused resistance vessels. *Circulation Res. Suppl.* 28 and 29: 159-164.
- Boyd, R.D.H., J.R. Hill, P.W. Humphreys, I.C.S. Normand, E.O.R. Reynolds, and L.B. Strang. 1969. Permeability of lung capillaries to macromolecules in foetal and new-born lambs and sheep. *J. Physiol.* 201: 567-588.
- Bradham, G.B., L.R. Bennett, J.C. DeHaven, E.C. Deland, M.B. Wolf and J.V. Maloney. 1964. Isotope dilution and thermodynamics in the study of intercompartmental body fluid exchange. *Surg. Gyn. & Obst.* 119: 1062-1068.
- Brennan, R.D., C.T. DeWit, W.A. Williams and E.V. Quattrin. 1970. The utility of a digital simulation language for ecological modeling. *Oecologia (Berl.)* 4: 113-132.
- Carey, J.S., R.S. Brown, N.W. Woodward, S.T. Yao and W.C. Shoemaker. 1965. Comparison of hemodynamic responses to whole blood and plasma expanders in clinical traumatic shock. *Surg. Gyn. & Obst.* 121: 1059-1065.

- Carnahan, B. and J.O. Wilkes. 1970. *Numerical Methods, Optimization Techniques and Process Simulation for Engineers*. Ann Arbor: The University of Michigan Engineering Summer Conference.
- Casley-Smith, J.R. 1967. The functioning of the lymphatic system under normal and pathological conditions. In *Progress in Lymphology*. A. Ruttiman (Ed.). p 348-349. Stuttgart: Georg Thieme Verlag.
- Casley-Smith, J.R. 1968. How the lymphatic system works. *Lymphology* 1: 77-80.
- Casley-Smith, J.R. 1971. Endothelial fenestrae in intestinal villi: differences between the arterial and venous ends of capillaries. *Microvasc. Res.* 3: 49-68.
- Cobbold, A., B. Folkow, I. Kjellmer, and S. Mellander. 1963. Nervous and local chemical control of pre-capillary sphincters in skeletal muscle as measured by changes in filtration coefficient. *Acta Physiol. Scand.* 57: 180-192.
- Coleman, T.G. and A.C. Guyton. 1969. Hypertension caused by salt loading in the dog. *Circulation Res.* 25: 153-160.
- Collete, J.M., G. Jantet, and E. Schoffeniels (Editors). 1967. *New Trends in Basic Lymphology*. Stuttgart: Birkhauser Verlag Basal.
- Conway, J. 1966. Hemodynamic consequences of induced changes in blood volume. *Circulation Res.* 18: 190-198.
- Corssen, G., P. Chodoff, E.F. Domino and D.R. Kahn. 1965. Neurolept analgesia and anesthesia for open-heart surgery. *J. Thor. & Card. Surg.* 49: 901-920.
- Courtice, F.C. 1968. The origin of lipoproteins in lymph. In *Lymph and the Lymphatic System*. Chapter 5. (see Mayerson, 1968).
- Davies, J.W.L., C.R. Ricketts and B.N. Williams. 1963. Plasma volume expansion by rapid infusion of a low molecular weight dextran. *Brit. J. Pharmacol.* 21: 220-225.
- Deland, E.C. and G.B. Bradham. 1966. Fluid balance and electrolyte distribution in the human body. *Ann. N.Y. Acad. Sci.* 128: 795-809.
- DeLangen, C.D. 1961. *The Peripheral Circulation of Blood, Lymph and Tissue Fluid*. Part I. Haarlem: DeErven F. Bohn.
- Diana, J.N. 1970. Capillary pressure and filtration coefficient during isoproterenol infusion. *Am. J. Physiol.* 219: 1574-1584.
- Dixon, S.H., S.P. Nolan, S.S. Stewart and A.G. Morrow. 1970. Neuroleptanalgesia: effects of Innovar on myocardial contractility, total peripheral vascular resistance, and capacitance. *Anesth. & Analg.* 49: 331-335.

- Doemling, D.B. and F.R. Steggerda. 1960. Chronic thoracic duct-venous shunt preparation in dogs. *J. Appl. Physiol.* 15: 745-756.
- Donovan, J.C., C.J. Lund, and L. Whalen. 1964. Simultaneous determinations of blood volumes using Evans blue and sodium radiochromate. *Surg. Gyn. & Obst.* 119: 1031-1035.
- Drinker, C.K. and M.E. Field. 1931. The protein content of mammalian lymph and the relation of lymph to tissue fluid. *Am. J. Physiol.* 97: 32-39.
- Edelman, I.S. and J. Leibman. 1959. Anatomy of body water and electrolytes. *Am. J. Med.* 27: 261-277.
- Eder, H.A. 1951. Determination of thiocyanate space. In *Methods in Medical Research*. M.B. Visscher (ed). 4: 49-53. Chicago: Yearbook Pub. Co.
- Elkinton, J.R. and T.S. Danowski. 1955. *The Body Fluids*. Baltimore, M.D.: Williams and Wilkins.
- Folkow, B. 1964. Description of the myogenic hypothesis. *Circulation Res.* 15 (Suppl.): 1 279-1 287.
- Folkow, B., O. Lundgren and I. Wallentin. 1963. Studies on the relationship between flow, resistance, capillary filtrate coefficient and regional blood volume in the intestine of the cat. *Acta Physiol. Scand.* 57: 270-283.
- Folkow, B. and S. Mellander. 1964. Veins and venous tone. *Amer. Heart J.* 68: 397-408.
- Foldi, M. 1967. Origin and composition of lymph. In *New Trends in Basic Lymphology*. p 11-18. (see Collete, et al, 1967).
- Garlick, D.G. and E.M. Renkin. 1970. Transport of large molecules from plasma to interstitial fluid and lymph in dogs. *Am. J. Physiol.* 219: 1595-1605.
- Gauer, O.H., J.P. Henry and H.O. Sieker. 1956. Changes in central venous pressure after moderate hemorrhage and transfusion in man. *Circulation Res.* 4: 79-84.
- Gauer, O.H. and J.P. Henry. 1963. Circulatory basis of fluid volume control. *Physiol. Rev.* 43: 423-481.
- Gauer, O.H., J.P. Henry and C. Behn. 1970. The regulation of extracellular fluid volume. *Ann. Rev. Physiol.* 32: 547-595.
- Gibson, H. and K.A. Gaar. 1970. Dynamics of the implanted capsule. *Fed. Proc.* 29: 319ABS.
- Gillett, D.J. and D.F.J. Halmaggl. 1970. Accuracy of single label blood volume measurements before and after corrected blood loss in sheep and dogs. *J. Appl. Physiol.* 28: 213-215.

- Gilmore, J.P. 1965. Pentobarbital sodium anesthesia in dog. *Am. J. Physiol.* 209: 404-408.
- Gitlin, D. 1957. Distribution dynamics of circulating and extravascular I-131 plasma proteins. *Ann. N.Y. Acad. Sci.* 70: 122.
- Gornall, A.C., C.J. Bardawill and M.M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177: 751-766.
- Gray, A.F. and K. Sterling. 1950. The tagging of red cells and plasma proteins with radioactive chromium. *J. Clin. Invest.* 29: 1604-1613.
- Greenway, C.V. and R.D. Stark. 1971. Hepatic vascular bed. *Physiol. Rev.* 51: 23-65.
- Grotte, G. 1956. Passage of dextran molecules across the blood-lymph barrier. *Acta Chir. Scand. Suppl.* 211: 1-84.
- Gupta, P.D., J.P. Henry, R. Sinclair and R. VonBaumgarten. 1966. Responses of atrial and aortic baroreceptors to non-hypotensive hemorrhage and to transfusion. *Am. J. Physiol.* 211: 1429-1437.
- Guyton, A.C., H.M. Batson, and C.M. Smith. 1951. Adjustments of the circulatory system following very rapid transfusion or hemorrhage. *Am. J. Physiol.* 164: 351-359.
- Guyton, A.C., A.W. Lindsey, B.N. Kaufman, and L.B. Abernathy. 1958. Effect of blood transfusion and hemorrhage on cardiac output and on the venous return curve. *Am. J. Physiol.* 194: 263-267.
- Guyton, A.C., B. Abernathy, J.B. Langston, B.N. Kaufman, and H.M. Fairchild. 1959. Relative importance of venous and arterial resistances in controlling venous return and cardiac output. *Am. J. Physiol.* 196: 1008-1014.
- Guyton, A.C. 1963a. *Circulatory Physiology: Cardiac Output and its Regulation*. Philadelphia: W.B. Saunders Co.
- Guyton, A.C. 1963b. A concept of negative interstitial pressure based on pressures in perforated capsules. *Circulation Res.* 12: 399-414.
- Guyton, A.C. 1965a. Interstitial fluid pressure II. Pressure-volume curves of interstitial space. *Circulation Res.* 16: 452-460.
- Guyton, A.C. 1965b. Pressure-volume relationships in the interstitial spaces. *Invest. Ophthalmol.* 4: 1075-1084.
- Guyton, A.C., K. Scheel, D. Murphree. 1966a. Interstitial fluid pressure: III. Its effect on resistance to tissue fluid mobility. *Circulation Res.* 19: 412-419.

- Guyton, A.C., J. Prather, K. Scheel, and J. McGehee. 1966b. Interstitial fluid pressure: IV. Its effect on fluid movement thru the capillary wall. *Circulation Res.* 19: 1022-1030.
- Guyton, A.C. and T.G. Coleman. 1967. Long term regulation of the circulation: Interrelationships with body fluid volume. In *Physical Bases of Circulatory Transport*. Chapter 11. (see Reeve & Guyton, 1967).
- Guyton, A.C. & T.G. Coleman. 1968. Regulation of interstitial fluid volume and pressure. *Ann. N.Y. Acad. Sci.* 150: 537-547.
- Guyton, A.C. and T.G. Coleman. 1969. Quantitative analysis of the pathophysiology of hypertension. *Circulation Res.* 24 (Suppl 1): 11-119.
- Guyton, A.C., H.J. Granger, and T.G. Coleman. 1971. Autoregulation of the total systemic circulation and its relation to control of cardiac output and arterial pressure. *Circulation Res.* 29 (Suppl. 1): 193 - 197.
- Haddy, F.J., M. Fleishman, and D.A. Emanuel. 1957. Effect of epinephrine, norepinephrine and serotonin upon systemic small and large vessel resistance. *Circulation Res.* 5: 247-251.
- Haljame, H. and H. Freden. 1970. Comparative analysis of the protein content of local subcutaneous tissue fluid and plasma. *Microvasc. Res.* 2: 163-171.
- Hall, J.G. 1969. The flow of lymph. *N. Eng. J. Med.* 281: 721-723.
- Herd, J.A. 1970. Overall regulation of the circulation. *Ann. Rev. Physiol.* 32: 289-312.
- Hidale, K., R.W. Hopkins, and F.A. Simoene. 1968. Studies of thoracic duct lymph in hemorrhagic shock. In *Lymph and the Lymphatic System*. Chapter 8. (see Mayerson, 1968).
- Jacobs, M.H. 1934. The quantitative measurement of the permeability of the erythrocyte to water and solutes. *J. Cell and Comp. Physiol.* 4: 161-183.
- Johnson, J.A. 1966. Capillary permeability, extracellular space estimation and lymph flow. *Am. J. Physiol.* 211: 1261-1263.
- Johnson, P.C. 1965. Effect of venous pressure on mean capillary pressure and vascular resistance in the intestine. *Circulation Res.* 16: 294-300.
- Johnson, P.C. and K.M. Hanson. 1962. Effect of arterial pressure on arterial and venous resistance of intestine. *J. Appl. Physiol.* 17: 503-508.

- Johnson, P.C. and K.M. Hanson. 1966. Capillary filtration in the small intestine of the dog. *Circulation Res.* 19: 766-773.
- Kerr, A.R. and J.W. Kirklin. 1970. Effect of rapid increases of blood volume on atrial pressures and pulmonary blood volume. *Ann. Surg.* 172: 278-283.
- Kjellmer, I. 1964. The effect of exercise on the vascular bed of skeletal muscle. *Acta Physiol. Scand.* 62: 18-30.
- Krogh, A., E.M. Landis and A.H. Turner. 1932. The movement of fluid thru the human capillary wall in relation to venous pressure and to the colloid osmotic pressure of the blood. *J. Clin. Invest.* 11: 63-95.
- Krogh, A. 1959. *The Anatomy and Physiology of Capillaries.* New York: Hafner Publishing Co.
- Ladegaard-Pedersen, H.J. 1970. Measurement of the interstitial pressure in subcutaneous tissue in dogs. *Circulation Res.* 26: 765-770.
- Landis, E.M. 1927. Micro-injection studies of capillary permeability. II. The relationship between capillary pressure and the rate at which fluid passes thru the walls of single capillaries. *Am. J. Physiol.* 82: 217-238.
- Landis, E.M. 1934. Capillary pressure and capillary permeability. *Physiol. Rev.* 14: 404-481.
- Landis, E.M. and J.R. Pappenheimer. 1963. Exchange of substances thru the capillary walls. In *Handbook of Physiology.* Circulation II: 961-1034. W.F. Hamilton and P. Dow (Editors). Washington, D.C.: Amer. Physiol. Soc.
- Leandoer, L. and D.H. Lewis. 1970. The effects of 1-norepinephrine on lymph flow in man. *Ann. Surg.* 171: 257-260.
- Lee, J.S., L.H. Smaje and B.W. Zweifach. 1971. Fluid movement in occluded single capillaries of rabbit omentum. *Circulation Res.* 28: 358-370.
- Levine, O.R., R.B. Mellins, R.M. Senior and A.P. Fishman. 1967. The application of Starling's law of capillary exchange to the lungs. *J. Clin. Invest.* 46: 934-944.
- Lundgren, O. and S. Mellander. 1967. Augmentation of tissue-blood transfer of solutes by transcapillary filtration and absorption. *Acta Physiol. Scand.* 70: 26-41.
- Lundvall, J., S. Mellander and H. Sparks. 1967. Myogenic response of resistance vessels and precapillary sphincters in skeletal muscle during exercise. *Acta Physiol. Scand.* 70: 257-268.

- Marty, A. and B.W. Zweifach. 1970. The high oncotic pressure effects of dextrans. *Arch. Surg.* 101: 421-424.
- Mayerson, H.S. 1963. The physiologic importance of lymph. In *Handbook of Physiology. Circulation II*:1035-1073. W.F. Hamilton & P. Dow (Editors). Washington, D.C.: Amer. Physiol. Soc.
- Mayerson, H.S. (Chairman). 1968. *Proc. on the Conf. on Lymph and the Lymphatic System*. Springfield, Ill: Charles C. Thomas Co.
- Mayerson, H.S., C.G. Wolfram, H.H. Shirley, and K. Wasserman. 1960. Regional differences in capillary permeability. *Am. J. Physiol.* 198: 155-160.
- McNeill, I.F., J.P. Williams, and F.D. Moore. 1963. The effects of hemorrhage and hormones on the partition of body water: I. A method for measuring small sequential increases in the volume of extra-cellular fluid. *J. Surg. Res.* 3: 322-331.
- Mellander, S. 1960. Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat. *Acta Physiol. Scand.* 50 (Suppl. 176): 1-86.
- Mellander, S. 1968. Contribution of small vessel tone to the regulation of blood volume and formation of edema. *Proc. Roy. Soc. Med.* 61: 55-61.
- Mellander, S. and B. Johansson. 1968. Control of resistance, exchange, and capacitance functions in the peripheral circulation. *Pharmacol. Rev.* 20: 117-196.
- Meyer, B.J., A. Meyer, A.C. Guyton. 1968. Interstitial fluid pressure V. Negative pressure in the lungs. *Circulation Res.* 22: 263-271.
- Milhorn, H.T. 1966. *The Application of Control Theory to Physiological Systems*. Philadelphia: W.B. Saunders.
- Moore, F.D., F.J. Dagher, C.M. Boyden, C.J. Lee and J.H. Lyons. 1966. Hemorrhage in normal man: I. Distribution and dispersal of saline infusions following acute blood loss--chemical kinetics of blood volume support. *Ann. Surg.* 163: 485-504.
- Morrison, D. 1967. *Multivariate Analysis of Variance*. New York: McGraw Hill.
- Neuhausen, B.S. and D.M. Rioch. 1923. The refractometric determination of serum proteins. *J. Biol. Chem.* 55: 353-356.
- Nims, L.F. 1966. Biological barriers and material transfer. *Adv. Biol. & Med. Phys.* 11: 159-230.
- Oberg, B. 1964. Effects of cardiovascular reflexes on net capillary fluid transfer. *Acta Physiol. Scand.* 62 (Suppl. 229): 1-98.

- O'Connor, W.J. and D.J. Potts. 1969. The external water exchanges of normal laboratory dogs. *Quart. J. Expt. Physiol.* 54: 244-265.
- Pappenheimer, J.R. 1953. Passage of molecules through capillary walls. *Physiol. Rev.* 33: 387-423.
- Pappenheimer, J.R. and A. Soto-Rivera. 1948. Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hind limbs of cats and dogs. *Am. J. Physiol.* 152: 471-491.
- Pitts, R.F. 1968. *Physiology of the Kidney and Body Fluids*. 2nd Edition. Chicago, Ill: Yearbook Medical Publishers, Inc.
- Prather, J.W. 1968. The effect of blood volume expansion on circulatory dynamics. Ph.D. Thesis. University of Mississippi. No. 68-16,877. Ann Arbor, Mich: Univ. Microfilms.
- Prather, J.W., K.A. Gaar and A.C. Guyton. 1968. Direct continuous recording of colloid osmotic pressure of whole blood. *J. Appl. Physiol.* 24: 602-605.
- Prather, J.W., A.E. Taylor, and A.C. Guyton. 1969. Effect of blood volume, mean circulatory pressure and stress relaxation on cardiac output. *Am. J. Physiol.* 216: 467-472.
- Rapoport, A. and A. Chammah. 1965. *The Prisoner's Dilemma*. Ann Arbor, Mich: The Univ. of Michigan Press.
- Reeve, E.B., M.I. Gregersen, T.H. Allen and H. Sear. 1953a. Distribution of cells and plasma in the normal and splenectomized dog and its influence on blood volume estimates with P-32 and T-1824. *Amer. J. Physiol.* 175: 195-203.
- Reeve, E.B., M.I. Gregersen, T.H. Allen, H. Sear and W.W. Walcott. 1953b. Effects of alteration in blood volume and venous hematocrit in splenectomized dogs on estimates of total blood volume with P-32 and T-1824. *Amer. J. Physiol.* 175: 204-217.
- Reeve, E.B. 1952. The contribution of I-131 labelled proteins to measurements of blood volume. *Ann. N.Y. Acad. Sci.* 55: 351.
- Reeve, E.B. and A.C. Guyton. 1967. *Physical Bases of Circulatory Transport: Regulation and Exchange*. Philadelphia: W.B. Saunders Co.
- Remington, J.W. and C.H. Baker. 1959. Plasma volume changes accompanying reactions to infusions of blood or plasma. *Am. J. Physiol.* 197: 193-200.
- Remington, J.W. and C.H. Baker. 1961. Evaluation of blood volume measurement techniques. *Circulation Res.* 9: 60-68.

- Renkin, E.M. 1964a. Normal regulation of tissue circulation. In *Effects of Anesthetics on the Circulation*. H.L. Price and P.J. Cohen (Editors). Springfield, Ill: Charles C. Thomas Co.
- Renkin, E.M. 1964b. Transport of large molecules across capillary walls. *The Physiologist* 7: 13-28.
- Renkin, E.M. 1966. Capillary blood flow and transcapillary exchange. *The Physiologist* 9: 361-366.
- Renkin, E.M. 1968. Capillary permeability. In *Lymph and the Lymphatic System*. Chapter IV. (See Mayerson, H., 1968)
- Renkin, E.M. 1968. Transcapillary exchange in relation to capillary circulation. *J. Gen. Physiol.* 52: 96s-108s.
- Riggs, D.S. 1970. *Control Theory and Physiological Feedback Mechanisms*. Baltimore: Williams and Wilkins.
- Rothe, C.F. 1971. Measurement of microvascular compliance and conductance using simulation and parameter optimization techniques. *Fed. Proc.* 30: 698Abs
- Rusznayk, I., M. Foldi, and G. Szabó. 1967. *Lymphatics and Lymph Circulation*. 2nd Edition. Oxford, Eng: Pergamon Press.
- Ruttiman, A. (Editor) 1967. *Prog. in Lymphology: Proc. Int. Symp. on Lymphology*. Stuttgart: Geor Thieme Verlag.
- Sato, T., S.M. Yamashiro and F.S. Grodins. 1971. Estimation of capillary hydrostatic pressure and filtration coefficient using sinusoidal perturbation of isogravimetric state. *Fed. Proc.* 30: 661Abs.
- Scholander, P.F., A.R. Hargans and S.L. Miller. 1968. Negative pressure in the interstitial fluid of animals. *Science* 161: 321-328.
- Selkert, E.E. (Editor) 1963. *Physiology*. Boston: Little, Brown & Co.
- Shirley, H.H., C.G. Wolfram, K. Wasserman, and H.S. Mayerson. 1957. Capillary permeability to macromolecules: stretched pore phenomenon. *Am. J. Physiol.* 190: 189-193.
- Shoukas, A.A. and K. Sagawa. 1971. Total systemic vascular compliance measured as incremental volume-pressure ratio. *Circulation Res.* 28: 277-289.
- Smith, E.L., R.A. Huggins and S. Deavers. 1965. Effect of blood volume on movement of protein and volume distribution of albumin in the dog. *Am. J. Vet. Res.* 26: 829-836.
- Starling, E.H. 1896. On the absorption of fluid from the connective tissue spaces. *J. Physiol.* 19: 312-326.

- Starling, E.H. 1909. *The Fluids of the Body*. Chicago:W.T. Keener & Co.
- Strauss, M. 1957. *Body Water in Man*. Boston: Little, Brown & Co.
- Stromberg, D. and C.A. Wiederhielm. 1970. Effects of oncotic gradients and enzymes on negative pressures in implanted capsules. *Am. J. Physiol.* 219: 928-932.
- Swan, H. and A.W. Nelson. 1968. Canine trapped plasma factors at different microhematocrit levels. *J. Surg. Res.* 8: 551-554.
- Szabó, G. 1967. Pressure and flow in the lymphatic system. In *Progress in Lymphology*. p 365-367. (see Ruttiman, A., 1967).
- Szabó, G. and Z. Magyar. 1967. Effect of increased systemic venous pressure on lymph pressure and flow. *Am. J. Physiol.* 212: 1469-1474.
- Takaori, M., P. Safar and S. Galla. 1970. Changes in body fluid compartments during hemodilution with hydroxyethyl starch and dextran 40. *Arch. Surg.* 100: 263-268.
- Tarazi, R.C., H.J. Melsher, H.P. Dustan, and E.D. Frolich. 1970. Plasma volume changes with upright tilt studies in hypertension and in syncope. *J. Appl. Physiol.* 28: 121-126.
- Wallentin, I. 1966. Importance of tissue pressure for the fluid equilibrium between the vascular and interstitial compartments in the small intestine. *Acta Physiol. Scand.* 68: 304-315.
- Wasserman, K. and H.S. Mayerson. 1952a. Mechanisms of plasma protein changes following saline infusions. *Am. J. Physiol.* 170: 1-10.
- Wasserman, K. and H.S. Mayerson. 1952b. Plasma, lymph and urine studies after dextran infusions. *Am. J. Physiol.* 171: 218-232.
- Wasserman, K. and H.S. Mayerson. 1954. Relative importance of dextran molecular size in plasma volume expansion. *Am. J. Physiol.* 176: 104-112.
- Wasserman, K., J.D. Joseph, and H.S. Mayerson. 1956. Kinetics of vascular and extravascular protein exchange in unbled and bled dogs. *Am. J. Physiol.* 184: 175-182.
- Wiedeman, M.P. 1963. Dimensions of blood vessels from distributing artery to collecting veins. *Circulation Res.* 12: 375-378.
- Wiederhielm, C.A. 1966. Transcapillary and interstitial transport phenomena. *Fed. Proc.* 25: 1789-1798.

- Wiederhielm, C.A. 1967. Analysis of small vessel function. In *Physical Bases of Circulatory Transport*. Chapter 19. (see Reeve & Guyton, 1967).
- Wiederhielm, C.A. 1968. Dynamics of transcapillary fluid exchange. *J. Gen. Physiol.* 52: 29s-63s.
- Wiederhielm, C.A. 1971. Steady state capillary water balance, a computer simulation study. *Fed. Proc.* 30: 661Abs.
- Wilson, E.B., Jr. 1952. *An Introduction to Scientific Research*. New York: McGraw Hill.
- Witte, C.L., M.H. Witte, and W.R. Cole. 1971. A simplified method for cannulation of the normal canine cervical thoracic duct. *Lymphology* 4: 159-161.
- Wolf, M.B. and J. McNamee. 1971. A mathematical model of water and solute exchange between the body fluids. *Fed. Proc.* 30: 708Abs.
- Yamashiro, S.M., T. Sato and F.S. Grodins. 1971. Comparison of parameter identification methods applied to a study of peripheral vascular function. *Fed. Proc.* 30: 698Abs.
- Yoffey, J.M. and F.C. Courtice. 1956. *Lymphatics, Lymph and Lymphoid Tissue*. Cambridge: Harvard Univ. Press.
- Zuidema, G.D., N.P. Clarke, J.L. Reeves, O.H. Gauer, and J.P. Henry. 1956. Influence of moderate changes in blood volume on urine flow. *Amer. J. Physiol.* 186: 89-91.
- Zweifach, B.W. 1961. *Functional Behavior of the Microcirculation*. Springfield, Ill: Charles C. Thomas Co.
- Zweifach, B.W. 1971. Local regulation of capillary pressure. *Circulation Res.* 28 & 29 (Suppl.): 1 129 - 1 134.
- Zweifach, B.W. and M. Intaglietta. 1966. Fluid exchange across the blood capillary interface. *Fed. Proc.* 25: 1784-1788.
- Zweifach, B.W. and M. Intaglietta. 1968. Mechanics of fluid movement across single capillaries in the rabbit. *Microvasc. Res.* 1: 83-101.