THE EFFECTS OF EXPERIMENTAL ERROR AND FILTERS ON THE MEASURABILITY OF RELATIVE VOLUMES AND PERMEABILITY

by

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ABSTRACT

Multiple tracer experiments in intact organs are used in physiology in the study of transport processes through membranes and capillary walls. Several tracers, including one which can not pass through the capillary wall, are injected simultaneously and their concentration in the outlet is observed. From these experiments one can deduce the distribution volume or holdup for each of the tracers as well as the role of transport through the membrane. However, intact organ measurements suffer from the disadvantage that some strong assumptions are involved in extracting the required quantities from the experiments. Furthermore, their accuracy is limited.

The object of this thesis is to investigate the accuracy of such measurements, and their sensitivity to the assumptions involved. This was done by studying a variety of hypothetical models, both by analytical techniques and by simulation and comparing the results to simulate tracer experiments with the assumed model.

The two major parameters that this thesis deals with are R and λ . R is the ratio of the extravascular volume to the

capillary volume. λ is a rate parameter related to the membrane permeability by equation 2.1. The conclusions of this thesis can be summarized as follows:

A. VOLUME MEASUREMENTS

There are several conditions required for a reasonably accurate measurement of accessible extravascular volumes or tracer holdup.

1. The extravascular volume must be at least 20% of the total capillary blood volume between the injection and measuring point.

2. The extravascular volume must be well purged. If there are any regions in which slow diffusion processes occur their volume will not be included in the measurement.

Theoretically, the volume measurement is always correct, but here the contribution of the slowly purged regions is in the tail of the concentration curve, which is unaccessible. The thesis develops quantitative criteria for these values and gives ways of estimating the errors involved.

B. MEMBRANE PERMEABILITY

Measurement of permeability is much less accurate and estimates are derived for the accuracies of such measurements. Again, small values of λ (small as compared to the resistance in the capillaries) cannot be measured and the same is true for large values. The range of λ for which reasonable estimates can be obtained is established, which could be of considerable value to investigators in the field. Figure 5.2 gives us the range of accessible λ .

Several methods of evaluating λ from tracer curves have been compared and their applicability discussed. It is shown that comparison of the results of different methods may give some information about the system.

C. FILTERS

In most experiments of this type, there is a significant volume of large arteries or veins, between the injection point and the measuring point which contribute to the results of the tracer experiment, but are not permeable

to the tracer. Sometimes the main part of the measured sojourn time distributions may be due to these elements. It is shown that they can be considered as a linear filter and the evaluation of λ and R, in the presence of a linear filter is investigated. It is also shown that in the range in which λ and R can be estimated, the filter changes the absolute values of R and λ . However, the ratio between the relative volumes and permeability of different tracers, remains unaffected.

These ratios of R and λ contain the most important information. In filtered experiments, a large number of tracers are normally used with different properties and the ratio of permeabilities and relative volumes are used to produce the nature of the transport phenomenon.

The effect of the filter on reconstruction of the sojourn time distribution in the outer phase is also investigated. It was shown that theoretically, at least, there are situations in which this leads to fundamental indeterminacies, as there are multiple solutions. This indeterminacy is introduced solely by the presence of a filter.

The methods developed in the theoretical part were applied to practical tracer experiments. The results obtained from our approximations were very good.

CHAPTER 1. INTRODUCTION

Tracer experiments have long been used to estimate internal volumes. More recently, multiple-tracers have been used by physicians and chemical engineers to estimate internal volumes (capillary volumes), external volumes (extra-vascular volumes), and the permeability (λ) of the diffusing substances. The physicians' interest stems from the knowledge that living beings depend on the transfer of mass between the circulatory system and the various organs of the body. Indicator-dilution techniques have provided the means of studying the nature of mass transfer in organ systems, or tissues, without necessitating direct physical invasion of the system, or the removal of parts of it for destructive analysis.

The chemical engineers' interest lies in the area of catalytic reactors, where the sojourn time of the reactant from the bulk phase to the catalyst surface, and the return of the reacted material to bulk phase, determines the design of the reactor. Tracer techniques have been used to establish the reactor's design parameters which would otherwise not be accessible. Polymer separation is another area of interest

for the chemical engineer. A solution of polymer molecules of various sizes, is passed through a gelatinous bed, where the smaller polymers permeate the gel and have a larger sojourn time through the bed than the larger polymer molecules. Separation of the various sizes is accomplished by collecting the bed's outlet in multiple vessels. Tracer experiments are used to determine the sojourn time distribution of the various sized polymers, in order that the bed's size and the collection scheme may be determined.

Figure 1.1 shows a schematic representation of a biological multiple tracer experiment. A non-diffusible tracer along with one or more diffusible tracers are injected into the organ's major artery and samples are collected and analyzed from the organ's major vein. The blood flowrate (F) and the quantity of each tracer (Q) injected, is separately measured.

Figure 1.2 shows a typical two tracer experiment. $h_N(t)$ represents the sojourn time distribution, (or normalized concentration profile) for the non-diffusible and $h_D(t)$, the sojourn time distribution for the diffusible tracer. It is immediately apparent that $h_D(t)$ has a longer tail.

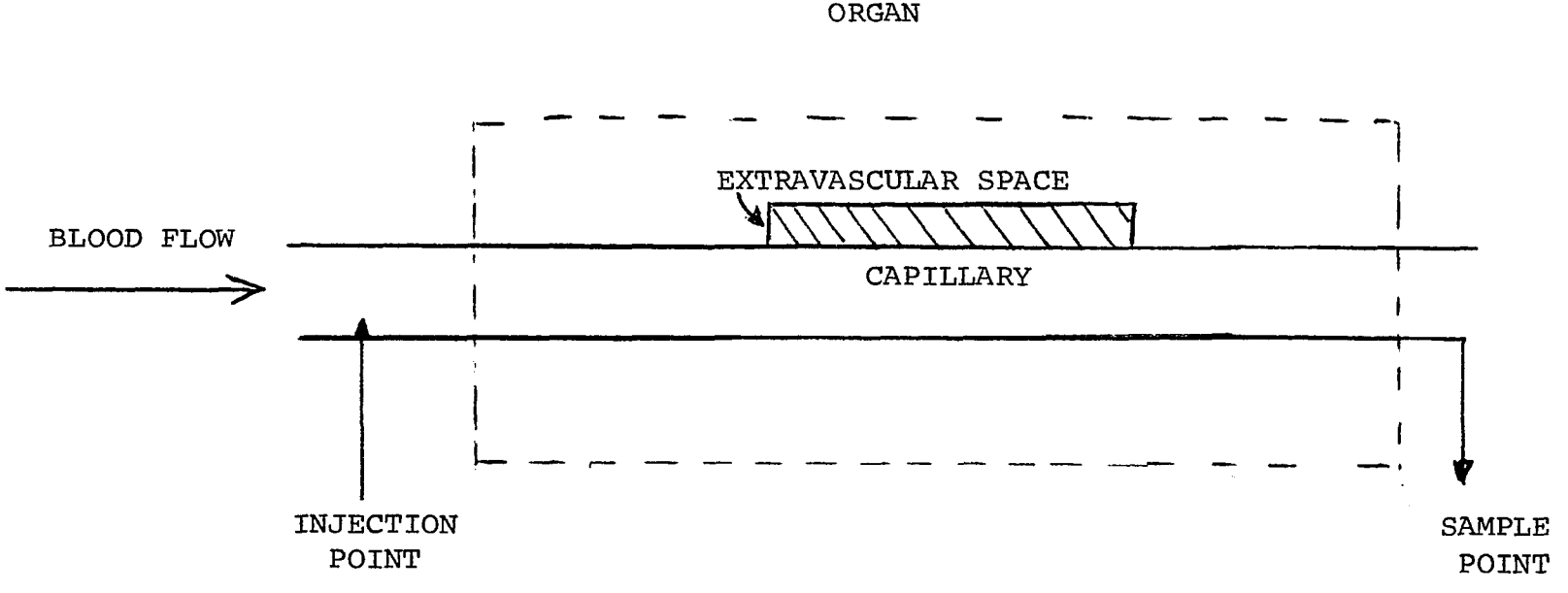


FIGURE 1.1 SCHEMATIC REPRESENTATION OF A BIOLOGICAL MULTIPLE TRACER EXPERIMENT

The tail corresponds to tracer particles which have left the first phase, and have jumped to the second phase, the extravascular space, (EVS). After spending some time in the second phase, they return to the first phase and are washed out. The washout, it should be noted, may occur after several excursions to the EVS.

Figure 1.2 also shows an area of immeasurability, whose fundamental cause is the lack of sensivity of our measuring instrument. There are a small number of experiments where the diffusible tracer has such a long tail that the experimenter does not have the time to measure it. Because of this area of immeasurability, errors in volume and permeability estimates, are inherent and one usually resorts to modelling to evaluate $h_N(t)$ and $h_D(t)$ in this range.

Zierler(36,37), Chinard(8,9,10), Crone(11), Bassingthwaighte (2,3,4,5,6), Shinnar(30), DeJulian and Yudilevich(34,35) are a few of the investigators who have proposed methods for estimating volumes, permeability, and extravascular behavioral information from the normalized concentration profiles. Volume estimates are obtained from the difference in the mean residence time of the diffusible and non-diffusible tracers. Permeability estimates can be obtained from the

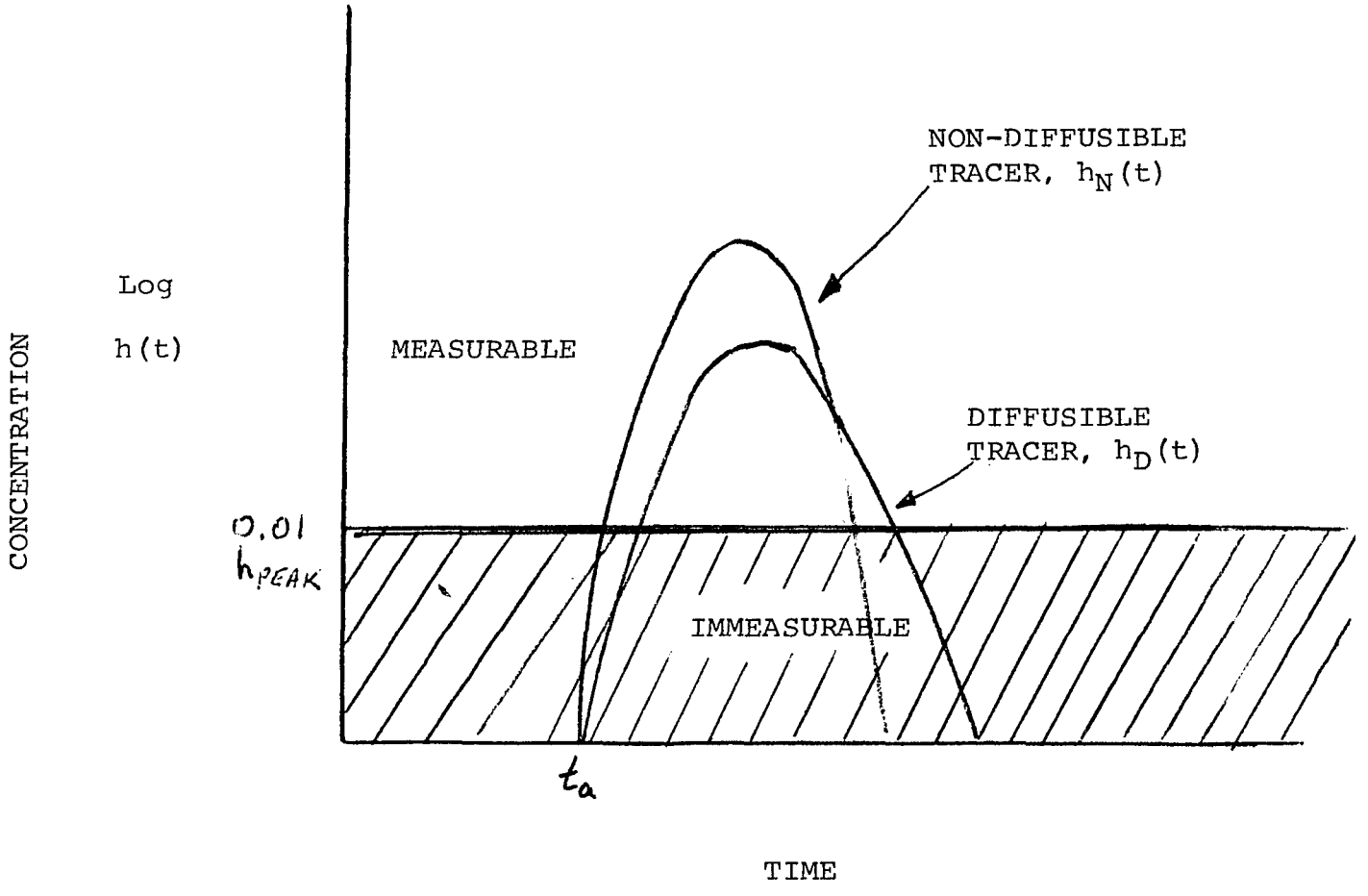


FIGURE 1.2 TYPICAL NORMALIZED CONCENTRATION RESPONSE OF A TWO TRACER EXPERIMENT

sojourn time distributions of the diffusible and non-diffusible tracers, at or near the appearance time. Extravascular behavioral and permeability information can be extracted from the Laplace transforms of the normalized concentration profiles.

Volume estimation, some techniques for permeability evaluation and the determination of the diffusion processes in the EVS, call for estimating higher moments of $h_N(t)$ and $h_D(t)$. We are faced by an immediate difficulty. No one can wait indefinitely to take all the measurements. Secondly, the measuring instrument has only a finite range of concentrations, usually two or three decades, which can be sensed. Both of these factors lead to estimates of integrals that are smaller than the integrals actually are. The moments of $h_N(t)$ and $h_D(t)$ are not truncated to the same degree further complicating the situation.

In addition to the truncation error described above, we encounter in all real experiments, what we will call "Experimental error". The "Experimental errors" involve errors in measurement, for example, the indistinguishability of two concentrations like 0.100 and 0.101. Other errors of measurement may include errors in fluid flowrate or errors in weighing the amount of tracer material. These

experimental errors, however, are equally applicable to the diffusible and non diffusible tracers. Furthermore, experimental errors are random in nature, and may as easily lead to over estimates of volumes as to underestimates, assuming the untruncated $h_N(t)$ and $h_D(t)$ were available.

Finally, it may not be possible to inject the tracer directly at the entrance of the diffusing bed, and sample directly at the exit of the diffusing bed. This is particularly true in biological systems where the diffusible portion of the organ is the capillary bed; this bed is preceded by arteries, arterioles and followed by venules and veins. The arteries, arterioles, veins and venules have a transfer function which distorts the concentration profiles used to estimate volumes and permeabilities. Furthermore, the measuring instrument has a transfer function which may or may not be separable from the required concentration profile. These errors will be referred to as errors caused by filters

In-vivo experiments cause one additional diffulty; that the concentration profile is distorted by recirculation. If the recirculation time is long in comparison to the mean scjourn

time of the organ, for example, three or four times, the experiment may be treated as if it were an open loop or in-vitro experiment. On the other hand for short recirculation times, as with those involving experiments with hearts, measured compositions must be modified to compensate for the effect of recirculation. Shinnar, Naor and many others have proposed methods dealing with recirculation.

In this thesis we will limit ourselves to open loop experiments where the blood flowrate is directly measurable. Our objective is to estimate volumes or relative volumes (EVS volume/Capillary volume), R, and permeabilities. Our discussions will center around three types of transfer functions:

1. Transfer functions where the entire concentration history is known, but may contain some measurement or experimental errors.
2. Transfer functions which are truncated because of lack of sensitivity of the measuring device.
3. Transfer functions which have been distorted by pre or post filters

For the last type of transfer function, it will be shown that for experiments with numerous diffusible tracers and one non diffusible tracer, only the ratios of R's and λ's are accessible, and not the absolute volumes and permeabilities.

CHAPTER 2- THESIS OBJECTIVE

This thesis can be divided into two parts as follows:

1. A study of Volume estimates
2. A study of Permeability estimates

In discussing volume estimates by diffusible tracers, we will in many instances, not deal with an absolute volume, but rather with the ratio of the extravascular volume to the capillary volume. This ratio will be designated by the letter R. Similarly, we will in most instances, not center our discussions on the conventional permeability (P), but rather, on a rate parameter λ which is related to P by:

$$\lambda = \frac{1}{M_N} \frac{PS}{F} \qquad (2.1)$$

where M_N= mean sojourn time

F = Flowrate

S = Area

The objective of this thesis is to establish bounds on the measurability of R and λ. From a theoretical viewpoint all R's and λ's should be measurable when one has perfect $h_N(t)$ and $h_D(t)$ curves. However, real curves have errors

which we classify in three ways:

1. Experimental erros
2. Truncation errors
3. Filter errors

Experimental errors are considered errors resulting from imprecise measurement. The imprecision may result from human error, an example being improperly weighing the tracer sample or improperly reading the measuring device. They also result from the indistinguishability of two concentrations e.g. 0.100 LBS/Ft.^{3} and 0.101 LBS/Ft.^{3}.

Truncation errors result mainly from the finite concentration range detectable by the measuring instruments. In many cases this is two or three decades. Truncation also occurs when the time span of the measurement is limited by practical considerations.

The third class error which we are considering is filter errors. In this class of error we will consider elements that alter $h_N(t)$ and $h_D(t)$. In biological systems, these elements are arteries, arterioles, veins and venules, which are non-diffusible elements, but contribute to the overall measured transfer functions, $h_{MN}(t)$ and $h_{MD}(t)$. The standard estimating techniques give underestimates of R and λ using $h_{MN}(t)$ and $h_{MD}(t)$ as

substitutes for $h_N(t)$ and $h_D(t)$. However, in many cases where multiple diffusible tracers are used, ratios of R and λ are maintained.

The bounds we will try to establish are in terms of values of R and λ, measurable directly from the imperfect $h_N(t)$ and $h_D(t)$. The bounds for the experimental errors, will be expressed in terms of the measurement error, E_r. Filters cause two types of errors:

1. Their mere presence results in underestimates of R and λ, because the filter's transfer function $h_f(t)$ is normally inseparable from the capillary space transfer function, $h_N(t)$; similarly the moments of the two transfer functions are inseparable

2. The second and more serious error results from distortion of the capillary space transfer function, by the filter. This results in multiple solution and indeterminacies.

In order to approximate the measurable ranges of R and λ , we will use simple transfer functions for both the capillary and extravascular space. The transfer function for the capillary space considered is a series of well mixed compartments with delays. For the extravascular space, we will use a reflected diffusor and its two degenerate cases, a well mixed compartment and a stretch. A summary of various $h_N(t)$ and $h_D(t)$ is shown in Table 2.1. A derivation of the three extravascular space transfer functions is given in Appendix B.

We realize that complicated blood flow patterns cannot be simulated by simple transfer functions. However simple transfer functions are valid indicators of limits on measurability of R and λ . In the last chapter of this thesis we will apply our techniques to experimental data supplied to us by Doctors Bassingthwaighte and Yipintsoi.

Table 2.1 SUMMARY OF ANALYTICAL TRANSFER FUNCTIONS

Capillary Space	Extravascular Space	Analytic Form For Non-Diffusible Tracer, $h_N(t)$	Analytic Form For Diffusible Tracer $h_D(t)$
1. General	Well mixed	$h_N(t) = \frac{F}{Q} c(t)$	$h_D(t) = e^{-\lambda t} + \int_0^t h_N(\tau) e^{-\lambda\tau} e^{-\frac{\lambda}{R}(t-\tau)} \sqrt{\frac{\lambda^2\tau}{R(t-\tau)}} I_1\left(2\sqrt{\frac{\lambda^2\tau(t-\tau)}{R}}\right) d\tau$
1a. Examples			
One well mixed compartment	well mixed	$h_N(t) = \gamma e^{-\gamma t}$	$h_D(t) = \gamma [C e^{-At} + D e^{-Bt}]$ $A = \frac{1}{2}\left[\beta + \left(\beta^2 - \frac{4\lambda\gamma}{R}\right)^{1/2}\right]$ $B = \frac{1}{2}\left[\beta - \left(\beta^2 - \frac{4\lambda\gamma}{R}\right)^{1/2}\right]$ $\beta = \frac{\lambda(R+1)}{R} + \gamma$ $C = (A - \lambda/R)/(A-B)$ $D = \left(\frac{\lambda}{R} - B\right)/(A-B)$

Table 2.1- Continued

Capillary Space	Extravascular Space	Analytic Form For Non-Diffusible Tracer, $h_N(t)$	Analytic Form For Diffusible Tracer, $h_D(t)$
1b. Two equal well mixed compartments in series	well mixed	$h_N(t) = (2\gamma)^2 t e^{-2\gamma t}$	$h_D(t) = (2\gamma)^2 \left[C_i t - \frac{2D_i}{A_i - B_i}\right] C_i e^{-B_i t} + (2\gamma)^2 \left[D_i t - \frac{2C_i}{A_i - B_i}\right] D_i e^{-B_i t}$ $A_i = \frac{1}{2}\left[\beta_i + \left(\beta_i^2 - \frac{8\lambda\gamma}{R}\right)^{1/2}\right]$ $B_i = \frac{1}{2}\left[\beta_i - \left(\beta_i^2 - \frac{8\lambda\gamma}{R}\right)^{1/2}\right]$ $\beta_i = \frac{\lambda}{R}(R+1) + 2\gamma$ $C_i = (A_i - \lambda/R)/(A_i - B_i)$ $D_i = \left(\frac{\lambda}{R} - B_i\right)/(A_i - B_i)$
1c. Pure Delay	well mixed	$h_N(t) = \delta(t - \frac{1}{\gamma})$	$h_D(t) = e^{-\frac{\lambda}{\gamma}}\left[e^{-\frac{\lambda}{R}(t-\frac{1}{\gamma})}\left(\sqrt{\frac{\lambda^2}{R(t-\frac{1}{\gamma})}}\right.\right.$ $\left.\left. I_1\left(2\sqrt{\frac{\lambda^2(t-\frac{1}{\gamma})}{R\gamma}}\right)\right) + \delta\left(t - \frac{1}{\gamma}\right)\right]$
2A. One well mixed compartment	Diffusional	$h_N(t) = \gamma e^{-\gamma t}$	$h_D(t) = 2\gamma\lambda\theta * \sum_{n=1}^{\infty} \frac{\sigma_n^2 e^{-\sigma_n^2 t/\theta}}{\frac{\sigma_n^2}{P}(\lambda+\gamma)\theta - \sigma_n^2)^2 + (P+1)(\gamma\theta - \sigma_n^2)^2 + \lambda\theta(\gamma$ $P = \frac{\lambda\theta}{R}$ $\tan\sigma_n = \frac{P}{\sigma_n}\left(\frac{1}{1 + \lambda\theta/(\gamma\theta - \sigma_n}\right.$

Table 2.1 - Continued

Capillary Space	Extravascular Space	Analytic Form For Non-Diffusible Tracer, $h_N(t)$	Analytic Form For Diffusible Tracer, $h_D(t)$
2B. Two unequal well mixed compartments in series	Diffusional	$h_N(t) = \frac{\gamma_1\gamma_2}{\gamma_2-\gamma_1}(e^{-\gamma_1 t} - e^{-\gamma_2 t})$	$h_D(t) = \frac{2\lambda\theta\,\gamma_1\gamma_2}{\gamma_2-\gamma_1} * \left(\sum_{n=1}^{\infty} \frac{\sigma_n^2 e^{-\sigma_n^2 t/\theta}}{\frac{\sigma_n^2}{\rho}\left[(\lambda+\gamma)\theta-\sigma_n^2\right]^2 + (\rho+1)(\gamma\theta-\sigma_n^2)^2 + \lambda\theta(\gamma\theta+\sigma_n^2)} - \sum_{j=1}^{\infty} \frac{\sigma_j^2 e^{-\sigma_j^2 t/\theta}}{\frac{\sigma_j^2}{\rho}\left[(\lambda+\gamma)\theta-\sigma_j^2\right]^2 + (\rho+1)(\gamma\theta-\sigma_j^2)^2 + \lambda\theta(\gamma\theta+\sigma_j^2)}\right)$

$$\rho = \frac{\lambda\theta}{R}$$

$$\tan\sigma_n = \frac{\rho}{\sigma_n}\left(\frac{1}{1+\frac{\lambda\theta}{\gamma_1\theta-\sigma_n^2}}\right)$$

$$\tan\sigma_j = \frac{\rho}{\sigma_j}\left(\frac{1}{1+\frac{\lambda\theta}{\gamma_2\theta-\sigma_j^2}}\right)$$

CHAPTER 3 - BACKGROUND

Tracer experiments can be grouped into two categories: open loop and closed loop. In this thesis we will limit our discussions to the open loop system, or *in vitro* experiments. The open-loop concentration histories, when suitably normalized, represent the residence time distribution of the tracer.

The closed loop experiments (in-vivo experiments) have their concentration histories complicated by recirculation. Naor et al, (41) has shown that indeterminacy exists in the estimation of flowrate and transport functions when recirculation occurs.

As early as the turn of the century, people were actively engaged in trying to find the permeability of substances from the blood to various organs. Christian Bohr in 1909, and later his pupil, August Krogh, performed experiments which attempted to establish the permeability of oxygen from the lung's capillaries. In the 1920's Stewart and Hamilton initiated quantitative indicator dilution studies which were used in calculating needle to needle blood volumes. These

calculations were later validated on a theoretical basis by Stephenson (1948), and by Meier and Zierler (1954) (22).

In an in vitro, or open loop experiment, one can usually measure the blood flowrate independent of the concentration response. The mass of tracer injected, which is also usually available, divided by the flowrate, is the factor which is used to normalize the concentration response, and to make it a proper probability distribution. This is all well and good, if the system we are dealing with is a linear system. By a linear system, we mean a system to which the principle of superposition can be applied and is homogeneous of degree one.

Linearity precludes non reversible adsorption of tracer particles by the capillary walls. In addition, the tracer particles must simulate the substance whose behavior one is trying to monitor; e.g. tritiated water must have the same properties of water, or radioactive Na_{24}^{+} must have the same properties as regular Na_{23}^{+}.

Substances used as tracers, real tracers, which are not

present in certain parts of the body and are not in equilibrium, and may interact with capillary walls, do not represent linear tracers. However, these tracers have linear ranges, even if side processes occur. For example, at very low concentrations, a reversible absorption which might occur does not, because of the low driving force. On the other hand, in a nonreversible process, the quantity tracer required to saturate the capillary wall may be small, say 1 gram; therefore, if one injects 100 grams, the system will appear linear because if one would inject say 200 grams of tracers, one will measure essentially double the 100 gram response. In these regions of linearity, the tracers are usuable for volume and permeability estimates.

In this context we should mention one other facet of this problem which has been discussed by Perl, that is, the distinction between hold up and volume. Katz and Krambeck (44) have shown that a holdup can always be obtained from the mean of h(t). The best way to distinguish hold up from volume is by example: Let us travel along with a diffusible tracer particle making a trip into a variety of extravascular spaces

and see what one measures.

In the first example, the diffusible tracer particle jumps from the capillary space into the extravascular space, and diffuses to the ends of the extravascular space, perpendicular to the flow pattern. The tracer particle returns to the capillary wall, jumps back to the same point in capillary space from which it left, encountering the same type of resistance as it had when it left. The tracer particle may be washed out of the system after zero, one, two or more trips to the extravascular space. In this case, one measures an accessible volume. It should be noted in places, such as stagnancies, where tracer particles do not reach, they are obviously not measured and are therefore not included in the term volume.

Let us go back to the beginning and follow our diffusible tracer particle on a second trip through the system with a different kind of extravascular space. The tracer particle travels in the capillary space and jumps into the extravascular space. In the extravascular space, it moves perpendicular to the flow direction and is captured by a cell and released

after a time τ jumps back to the same point in the capillary space from which it left and is washed out. The tracer washout occurs after zero, one, two or more trips to the extravascular space. In this case one can no longer measure a volume, rather one measures a hold up, because a portion of the residence time that the tracer particle spent in the extravascular space was due to adsorption by the cell and this portion cannot be counted as part of the extravascular volume.

In addition to containing volume information, multiple tracer experiments contain information about permeability coefficients. Many experimentors have developed a multitude of methods for estimating permeability. Four major classes of measurements which have been used to estimate permeability are:

1. Osmotic pressure
2. Unit Step Concentration Profile
3. Tissue Clearance
4. Unit Impulse Concentration Profile.

A brief summary of the first three methods and some of the shortcomings presented by Crone (48) at the Alfred Benzon Symposium are presented.

A model which uses osmotic pressure measurements and is based on diffusion through cylindrical pores for the transfer of mass from the capillaries into the extravascular space, was developed by Pappenheimer, in the early 1950's. The basic equations used by Pappenheimer (24,. 25) are:

$$\Delta C = \Delta \pi / RT \qquad (3.1)$$

$$P = \frac{F(C_a - C_v)\, RT}{A\, \Delta \pi} \qquad (3.2)$$

where F = Blood Flow Rate, C = Concentration, $\Delta\pi$ = Δ Osmotic Pressure, A = Area, a = Artery, v = vein, p = permeability

Van't Hoff's Law, equation 3.1, is used to find the effective concentration difference. There has been some criticism (48) of the use of Van't Hoff's law in a system where the membrane is not ideally semipermeable.

A second method for finding the permeability of diffusible substances was proposed by Renkin and his co-workers, Crone (45) and Garlick and others. Instead of using a single injection, (a unit impulse), one would continuously infuse a traced material (a unit step). The basic equation is PS = -F ln(1-E), but E has a slightly different meaning, because it refers to the steady state extraction, as obtained in the usual way, where concentrations in arterial and venous blood are related.

Many experimenters have shown the transfer function for a unit step experiment, H(t), is related to the transfer function of a unit impulse experiment, h(t), by:

$$h(t) = \frac{-dH(t)}{dt} \qquad (3.3)$$

A third class of methods, developed independently by Lassen, (1967) (38), Strandell and Shepherd, (1968)(39) and Gosselin, (1967)(43), is known as tissue clearance and tissue uptake methods. In a small extravascular tissue depot, e.g. a muscle, they inject γ - emitting small hydrophilic ions and a dissolved inert gas. Under ideal circumstances, both substances will disappear with a single rate constant, which for the lipophilic gas, gives the flow in the capillaries and for the hydrophilic ions, give the extraction from the tissue. The basic equation is: $K \cdot V = F \cdot E$. The disadvantage of these methods is that inhomogeneity of the tissue perfusion relative to the volume of the perfused compartments, and the occurrence of tissue gradients, could give similar results. (48)

The fourth and major class of experiments, Single Injection (Unit Impulse), is used for finding both volumes and permeability.

This type of experiment is the subject of this thesis.

As early as 1954, Chinard and his associates (8,9),proposed using a single injection which contained multiple tracers in order to determine the size of extravascular volumes, and the permeability of diffusible compounds through organ membranes, in various parts of the body. Numerous investigators have performed multiple tracer experiments, each using his own. variation of the basic method for calculating permeability.

Methods suggested by Crone (11), Martin de Julian, and Yudelevich(40) and Bassingthwaighte(4), estimate the permeability by a relation of the type:

$$\frac{PS}{F} = \ln(1-E) \qquad (3.4)$$

Where the fractional extraction, $E = 1 - \frac{h_D}{h_N}$

The difference in the various methods lies in the procedure used in evaluating E.

Definitions of E and λ are such that equation 3.4 can be written simply in terms of a ratio $h_N(t)$ and $h_D(t)$. We will call this ratio $\Lambda(t)$.

$$\Omega(t) = h_D(t)/h_N(t) \qquad (3.5)$$

Equation 3.4, expressed in terms of λ becomes:

$$\lambda = -\ln \Omega / M_N \qquad (3.6)$$

where Ω is the suitable value of $\Omega(t)$, as defined by the various investigators.

Martin de Julian and Yudilevich suggest that the proper value of $\Omega(t)$ to use in equation 3.4 is $\Omega(t)$ at the appearance time. The MJY method assumes that in the capillaries, there is immediate axial equilibration. Furthermore, any tracer particle, which leaves the capillaries, takes a long time to return to the capillary space, from the extravascular space (no-back diffusion). Crone in 1963 and Levitt in 1970, derive equation 3.4 in their papers. Levitt shows that this method gives an exact solution, if the capillary space acts as a plug flow system. Levitt further shows that for high values of λ, the method fails to be accurate, since back mixing is quite extensive.

A second method for calculating the extraction factor, is the

Bassingthwaighte method. This method takes the maximum value of E(t), or the minimum of value $\Omega(t)$, whether it occurs at the appearance time or later during the cycle. Intuitively, one would say, $\Omega(t)$ should be constant until back diffusion occurs. This is untrue except under very special circumstances. Bassingthwaighte, Knopp, and Hazelrig (46) believe that this maximum value would be a good indication of the maximum value of the coefficient permeability of the system.

Crone and Lassen (47) suggested a method which utilizes the $h_N(t)$ and $h_D(t)$ curves to a greater extent. This method, which we will call the Crone Relative Area (CRA) Method, defines the extraction:

$$E = \frac{\int_0^{t_p} h_N(t) - h_D(t)\ dt}{\int_0^{t_p} h_N(t)\ dt} \qquad (3.7)$$

where t_p can assume any one of three values. These are:

1. t_p=the time at which $\Omega(t)$ is a minimum.
2. t_p=time of the peak for the non-diffusible tracer
3. t_p=the time where $\Omega(t)=1$ (the crossover point).

Due to the difficulty of determining at which time back diffusion has an influence on $h_D(t)$, it is consequently

difficult to say which method is the proper way to choose t_p.

In 1969, Shinnar, Naor and Katz (30), developed a method for relating the sojourn time distribution for diffusible and non diffusible tracers. They presented three different approaches for calculating the permeability in their paper. These are:

1. A probabilistic model, which used Laplace Transforms
2. A modified Extraction Factor Method
3. A method of calculating the various system parameters by model fitting the moments of the diffusible and non-diffusible tracers.

The moment fitting method can be used to give a lower bound on the permeability, even though the the value calculated by assuming an EVS behavior of a well mixed compartment is not valid.

It is assumed, in a multi-tracer experiment, that behavior of the diffusible and non-diffusible tracers in the capillary space is the same, with the exception that, from time to time, diffusible tracer particles jump into the extra-vascular space with a Poissons rate parameter λ jump/time.

The diffusible tracer particle then returns to the capillary at the same point from which it has left.

Shinnar, Naor, and Katz have shown that $h_D(t)$ can be related to $h_N(t)$ by the following relationship:

$$h_D(t) = \int_0^t h_N(t)\, X(t-\tau \mid \tau)\, d\tau \qquad (3.8)$$

where $X(t-\tau \mid \tau)$ is the conditional probability density of a tracer particle, spending time $t-\tau$ in the extravascular space, having spent time τ in the capillaries. If we now consider the physically useful situation, where the times t_1 and t_2, spent in the extravascular space for two non-overlapping time periods τ_1 and τ_2 spent in the capillaries are independent of each other. We then find that X(s/t), the Laplace transform of the conditional probability can be given by:

$$X(s \mid \tau) = e^{-\tau p(s)} \qquad (3.9)$$

where $X(s/\tau)$ is the Laplace transform of $X(t/\tau)$ and p(s) is a suitable function with the following properties:

a. $p(o)=0$

b. $p(s) \geq 0 \quad s > 0$

c. $(-1)^n \dfrac{d^n p(s)}{ds^n} \leq 0 \;,\; s > 0 \;;\; n = 1, 2, 3 \ldots n$

If we further consider a function, $\Phi(t)$, the probability density that a tracer particle which enters the extravascular space spends time t there, p(s) can be related to ϕ (s) in the following manner:

$$p(s) = \lambda(1 - \phi(s)) \qquad (3.10)$$

Further,

$$\lim_{s \to \infty} p(s) = \lambda \qquad (3.10A)$$

Substituting the result of equation 3.9 into equation 3.8, and taking the Laplace transform, we get that

$$h_D(s) = h_N(s + p(s)) \qquad (3.11)$$

$$\text{or } h_D(s) = h_N(s + \lambda(1 - \phi(s))) \qquad (3.11A)$$

Equation 3.10A defines a method of finding λ simply from the transforms of $h_D(t)$ and $h_N(t)$. This limiting method will be called the SNK limit method. A graphical procedure is described in the paper of Shinnar et al; in addition, a numerical procedure suitable for computer calculations is outlined in the appendix of this thesis.

MODIFIED EXTRACTION FACTOR METHOD (MEF)

Equation 3.11 requires that the outlet concentration profiles be converted into the Laplace Transform domain. However, one can calculate λ directly in the time domain. It can be shown that:

$$\lim_{t \to t_a} \frac{-1}{t} \ln \Omega = \lambda \qquad (3.12)$$

where t_a=appearance time.

All of the standard methods which use the extraction as the basis for calculating λ , do not use limiting technique.

MOMENT METHOD

If the transfer function of the extravascular space is known, the unfiltered moments of $h_N(t)$ and $h_D(t)$ can be used to calculate various parameters of the system.

Let us consider the case where the extravascular space is a well mixed compartment without any longitudinal dispersion. Shinnar, et al, have shown that in general, the moments of h_D

and h_N are related in the following manner:

$$M_D = M_N(1 + \lambda M_\phi) \qquad (3.13)$$

$$V_D = V_N(1 + \lambda M_\phi)^2 + \lambda M_N M_{2\phi} \qquad (3.14)$$

where M=mean or 1st moment

M_2=second moment

V=variance

Subscripts D=diffusible tracer

N=non-diffusible tracer

ϕ=sojourn time distribution of the extravascular space.

If the extravascular space is a well mixed compartment, the Laplace transform of the sojourn time distribution is given by:

$$\phi(S) = \frac{1}{\frac{R}{\lambda}S + 1} \qquad (3.15)$$

where R = the ratio of the extravascular volume to the capillary volume

and λ is the permeability coefficient.

The moments of ϕ are

$$M_\phi = R/\lambda \;,\; M_{2\phi} = 2R^2/\lambda^2 \qquad (3.16)$$

Substituting these values into equations 3.13 and 3.14, we get

$$M_D = M_N(1+R) \qquad (3.17)$$

$$V_D = V_N(1+R)^2 + 2M_N R^2/\lambda \qquad (3.18)$$

If we now solve equations 3.17 and 3.18 for λ and R, we find:

$$R = (M_D - M_N)/M_N \qquad (3.19)$$

$$\lambda = \frac{2M_N R^2}{V_D - V_N(1+R)^2} \qquad (3.20)$$

Substituting the results of equations 3.17 into 3.18, λ can be expressed solely as a function of the moments.

$$\lambda = \frac{2M_N(M_D - M_N)^2}{V_D M_N^2 - V_N M_D^2} \qquad (3.21)$$

Equation 3.20 defines an exact way of determining λ when the EVS has the transfer function of a well-mixed compartment. However, equation 3.20 is not exact for any other models which might be ascribed to the EVS. From a physiological viewpoint, all processes that occur in the extravascular

space are diffusional, and a well mixed compartment is just a degenerate case of a reflected diffusor. The coefficient of variation for diffusional processes is equal to or greater than one; the equality holding for a well mixed compartment. Consequently, equation 3.21 can be used as a lower bound on λ, even if the exact model for the EVS is not known.

A summary of the conventional methods and the methods we proposed is given in Tables 3.1 and 3.2.

Table3.1 APPROXIMATE METHODS

Method	Introduced By	Abbreviated Name	Governing Equation	Remarks
1. Convention Extraction Factor	Martin de Julian and Yudilevich C. Crone	MJY	$\Omega = \frac{h_D(t_a)}{h_N(t_a)}$	t_a = appearance time
2. Minimum Extraction Factor	Bassingthwaighte et al	MINΩ	$\Omega = \text{MIN}\ \frac{h_D(t)}{h_N(t)}$	
3. Relative Area Method	Crone	CRA	$\Omega = \frac{\int_o^{t_p} h_D(t)dt}{\int_o^{t_p} h_N(t)dt}$	where t_p can be defined in any one of the following three ways: (A) Peak Time (B) MINΩ Time (C) Crossover Time

Table 3.2 METHODS WE HAVE PROPOSED

Method	Abbreviated Name	Governing Equations	Remarks
1. Modified Extraction Factor	MEF	$\lambda = \lim_{t \to t_a} \frac{1}{t} \ln \Omega(t)$	t_a = appearance time
2. Maximum $\rho(s)$	MAXρ	$h_D(s) = h_N(s + \rho(s))$ $\lambda = \lim_{s \to \infty} \rho(s)$	
3. Moment Method	Moment Method	$R = (M_D - M_N)/M_n$ $\lambda = (2M_N R^2)/(V_D - V_N(1+R)^2)$	M = Mean V = Variance
4. Straight Line $1/\rho(s)$	SLRρ	$\frac{1}{\rho(s)} = \frac{1}{RS} + \frac{1}{\lambda}$	

CHAPTER 4 = VOLUME ESTIMATES

In this chapter, we will establish which volumes and relative volumes are measurable and which volumes are inaccessible. In this context when we use the term volumes, we recognize, as discussed earlier, that we may be dealing with holdup. We are limiting our discussions to the effects of having an imprecise $h_N(t)$ and $h_D(t)$, due either because of measurement error or because of truncation error, on volume estimates. Filter distorted $h_N(t)$ and $h_D(t)$ are considered in Chapter 6.

In a linear system, where the residence time distribution of tracer particles in the extravascular space is independent of time spent in the capillary space, one can apply the principle of superposition, and R, the volume of the extra vascular space, relative to the volume of the capillary space can be found by the following relationship:

$$R = M_d/M_n - 1 \qquad (4.1.1)$$

M_d and M_n are the mean sojourn times of the diffusible and non-diffusible tracers respectively. In in-vitro experiments,

where one has independent knowledge of the amount of tracer injected, and the blood flowrate, the outflow concentration of the various tracers can be suitably normalized, as to yield an $h_N(t)$ and $h_D(t)$, representing the probability distribution that a tracer particle spends t in system. The mean of h_D and h_N are given by:

$$M_d = \int_{t_a}^{\infty} t\, h_D(t)\, dt \qquad (4.1.2)$$

$$M_n = \int_{t_a}^{\infty} t\, h_N(t)\, dt \qquad (4.1.3)$$

where t_a = appearance time.

Equations 4.1.2 and 4.1.3 involve an integration from the appearance time t_a, to infinity. Unfortunately, the sensitivity of the measuring instrument does not allow us to measure h_N and h_D over the entire range of values that the concentration may assume, between the appearance time and infinity. Normally the instrument's sensitivity, limits us to two or three decades of concentration values. Furthermore, even if an infinitely sensitive measuring device were available, infinite time could not be expended to measure the tail. One

is therefore forced into a position where the concentration curve is only available to the investigator for a finite time range which we shall call $t_a \leq t \leq t_m$ for the non-diffusible tracer, and $t_a \leq t \leq t_g$ for the diffusible tracer. There are two approaches that we can explore:

1. Estimates of R available from the truncated functions
2. Use of extrapolating functions which would simulate the tail behavior.

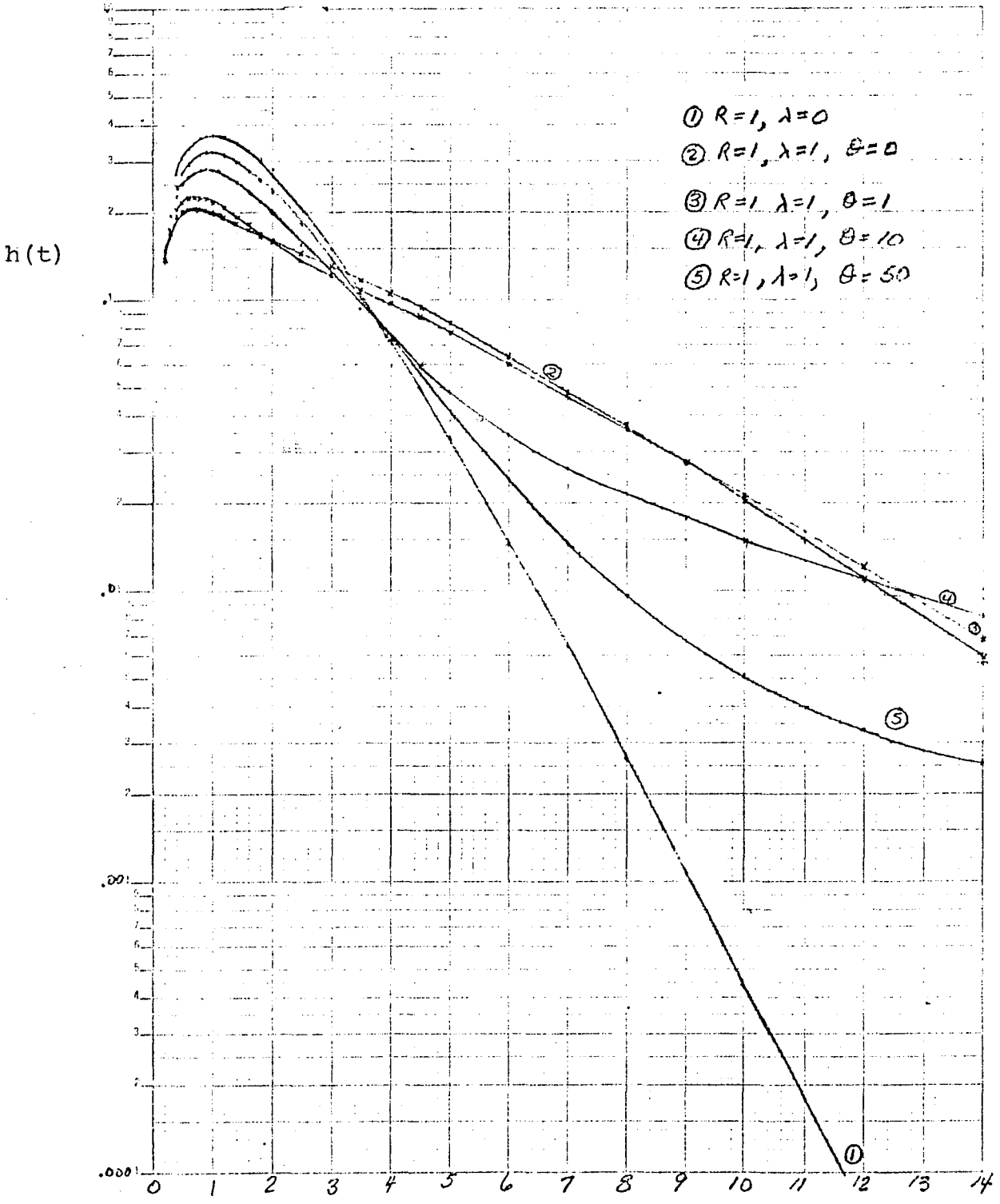
When using extrapolating functions, we must be certain that we are dealing with a portion of the curve, sufficiently removed from the peak, so that the slope of $h_D(t)$ or $h_N(t)$ is near the terminal slope. If, however, the experimental data does not extend for sufficiently long times, extrapolations should not be used. A complete discussion of extrapolation methods will follow in section 4.

Figure 4.1 shows representative curves of a multiple tracer experiment for various values of R, λ , Θ (where $\Theta = L^2/D$; L = diffusion length, D = Diffusity). The curves were derived for two well mixed compartments in series, with a

FIGURE 4.1 - SIMULATED TRACER RESPONSE OF

CAPILLARY SPACE: TWO WELL MIXED COMPARTMENTS IN SERIES

EXTRAVASCULAR SPACE: REFLECTED DIFFUSOR



reflected diffusor extravascular space.

4.2 Minimum Accessible Values of R

Shinnar (30) has shown that all values of R should be attainable from ideal multiple tracer experiments, in the absence of of experimental and truncation errors. However, because of experimental error, small volumes or values of R are not measureable, because the transfer function of the diffusible tracer $h_D(t)$ is indistinguishable from the transfer function of the non-diffusible tracer, $h_N(t)$.

The concentration behavior of a diffusible tracer with a small R approaches the concentration behavior of a diffusible tracer with an infinite λ . For discussion purposes, we will use a capillary space behavior of two well mixed compartments in series and an extravascular space behavior of a well mixed compartment with an infinite λ ; the corresponding transfer functions have the form:

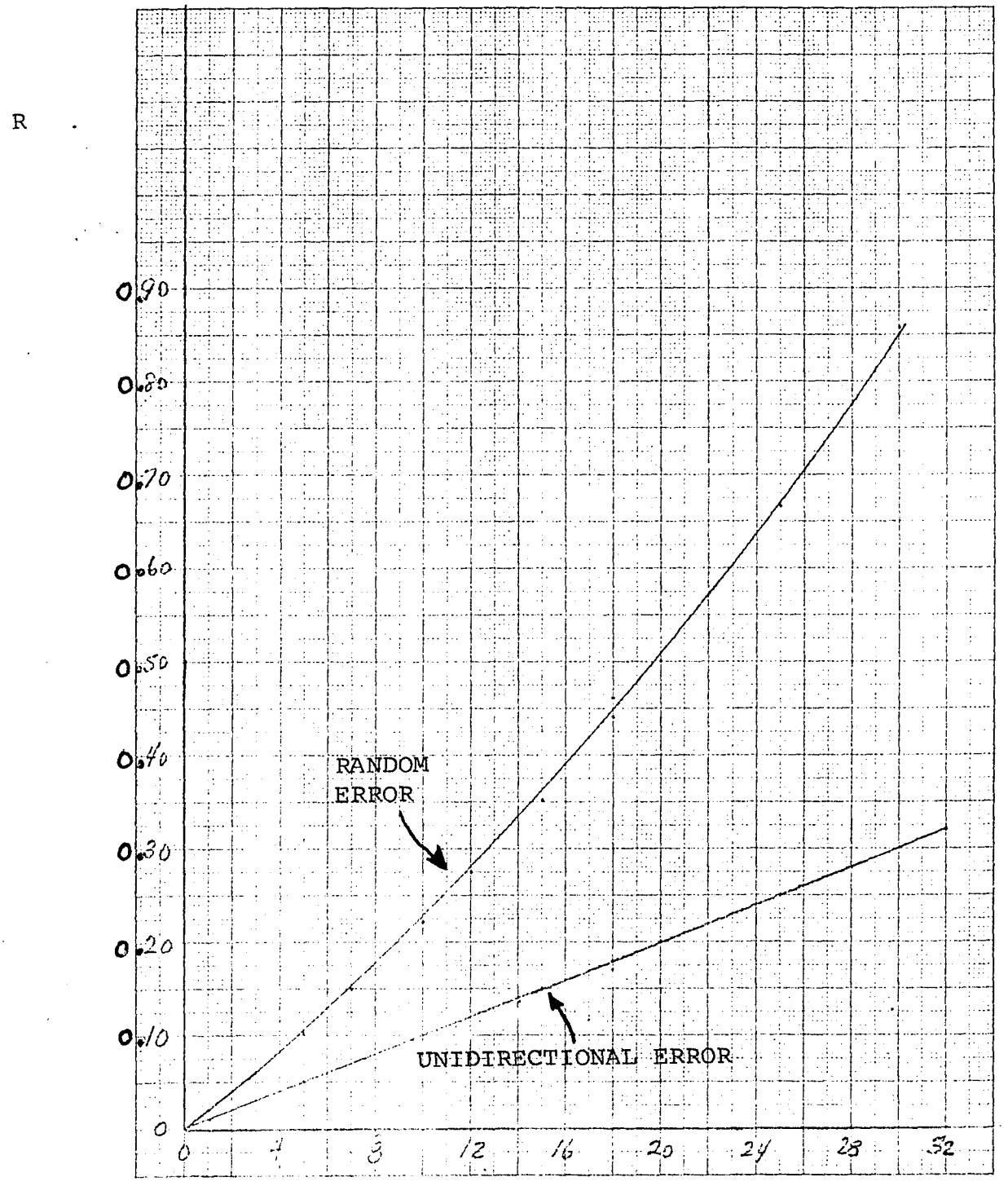
$$h_N(t) = \gamma^2 t e^{-\gamma t} \qquad (4.2.1)$$

$$h_D(t) = \left(\frac{\gamma}{1+R}\right)^2 t e^{-\frac{\gamma}{1+R}t} \qquad (4.2.2)$$

If the maximum experimental error in measuring $h_N(t)$ and $h_D(t)$ is E_r, then, in order to be certain that R can be measured, R must be greater than $2E_r$. That R must be greater than $2E_r$, assumes that the experimental error is random in nature, and overestimates of $h_N(t)$ and $h_D(t)$ result with the same frequency as underestimates of $h_D(t)$ and $h_N(t)$. However, if the error is unidirectional, R must only be greater than E_r. A graphical representation of this result is shown in Figure 4.2.

In order to distinguish between a random error and unidirectional error, let us look at two examples. In the first case, the measurement instrument reads 0.100 for concentrations between 0.099 and 0.101. The experimenter records 0.100 as the appropriate concentration. It is equally likely that the time concentration lies below 0.100 as above 0.100. Therefore a random error of mean zero has been introduced into the h(t) curve. In the second case let us assume that the concentrations 0.099, 0.100 and 0.101 are all measurable. The experimenter records 0.100 for all concentrations greater than 0.099 but less than or equal to 0.100 and similarly records 0.101 as the appropriate concentration for all concentrations greater than 0.100 but less than or equal to 0.101. This is an example of a unidirectional which leads to overestimates in the mean of h(t).

FIGURE 4.2 - MINIMUM VALUE OF MEASURABLE R AS A FUNCTION OF EXPERIMENTAL ERROR



As proof of this result ($R > 2E_r$), we offer the following: R is evaluated from the first moments of $h_D(t)$ and $h_N(t)$ by an equation of the form:

$$R = \frac{M_D - M_N}{M_N} \qquad (4.2.3)$$

The worst situation arises when $h_N(t)$ has a unidirectional error which overestimates $h_N(t)$ and $h_D(t)$ has a unidirectional error which underestimates $h_D(t)$.

Multiplying equations 4.2.1 and 4.2.2 by t and integrating from zero to infinity, the mean sojourn time of the diffusible and non-diffusible tracer is given by:

$$M_N = \int_0^\infty \gamma^2 t^2 e^{-\gamma t} (1+E_R)\, dt = \frac{2}{\gamma}(1+E_R) \qquad (4.2.4)$$

$$M_D = \int_0^\infty \left(\frac{\gamma}{1+R}\right)^2 t^2 e^{-\frac{\gamma t}{1+R}} (1-E_R)\, dt = \frac{2}{\gamma}(1+R)(1-E_R) \qquad (4.2.5)$$

In order to guarantee that an R exists, M_D must exceed M_N. Therefore:

$$1 + R > \frac{1 + E_r}{1 - E_r} \qquad (4.2.6)$$

which for small E reduces to:

$$R > 2E_r \qquad (4.2.7)$$

The fact that R is greater than $2E_r$ merely can be used as an indication of the existence of R, but error in the magnitude of R can be quite large. At $R = 4E_r$, one can

underestimate R by 50%. At $R = 8E_r$, the underestimate can be as large as 25%. The minimum error in any estimate of R is $2E_r/1-E_r$.

Another error which results in the inaccurate estimate of R is that $h_D(t)$ and $h_N(t)$ are measurable only over a finite range, say two or three decades. In our example, the truncation time of $h_N(t)$, given by equation 4.2.1, will almost be identical with the truncation time of $h_D(t)$, given by equation 4.2.2 for small values of R. Therefore, the recoveries will be identical, and will not be a source of error. In addition, the mean M_D will have the same error as the mean M_N, thereby giving a good estimate of R.

4.3 Maximum Values of R

The large values of R are susceptible to different types of errors. Their value could be accurately measured to within $2E_r$, if the entire $h_D(t)$ curve, from t_a to infinity were available. In contrast to the small values of R, the measurability of the tail of $h_D(t)$ is a more serious problem. Let us again use the situation where λ is infinite and the outer phase is well-mixed, so that $h_N(t)$ and $h_D(t)$ are given by equation 4.2.1 and 4.2.2

In many instances, the same instrument is used to measure

$h_N(t)$ and $h_D(t)$ and the range of the instrument is limited to two or three decades. $h_N(t)$ and $h_D(t)$ have maximum values of γ/e and $\gamma/(1+R)e$ respectively. Therefore, in order to measure any response for $h_D(t)$:

$$\frac{\gamma}{(1+R)e} > \frac{\gamma}{e} 10^{-N} \qquad (4.3.1)$$

where N is the number of decades the instrument can measure. Therefore:

$$R < 10^{-N} - 1 \qquad (4.3.2)$$

Even though $h_D(t)$ becomes visible for $R < 99$, the volume calculated from equation 4.1.1 may fall far below the actual volume. 75% of the actual volume can be measured for an R of 20, 80% when R = 15, 85% when R = 10 and 90% for R =6.

4.4 Measurable R and Extrapolation Techniques

We have shown earlier that two types of error arise in the measurement of $h_D(t)$ which bear heavily

in the estimation of R. In the range where R is measurable, errors that arise from inaccuracies in the measurement of h(t), can not be separated from the means of h_D and h_N and are found in all experiments. We will therefore, not concern ourselves further with this type of experimental error.

The second type of error is the truncation error, which can be remedied if one could properly extrapolate $h_N(t)$ and $h_D(t)$ to infinity, so that the moments of h can be evaluated accurately. The problem arises as to what is the proper way to extrapolate $h_N(t)$ and $h_D(t)$. Truncated h(t) curves cannot calculate tracer recoveries equal to 100%; from the knowledge of the flowrate and quantity tracer injected the unrecovered fraction can be calculated. Many experimenters believe that the concentration profile declines exponentially for large times. These two properties can be used in formulating methods for extrapolation.

Yerushalmi et al have proposed a rectangular extrapolation, as a means to putting a lower bound on the volume. They propose using the terminal measured value, $h_D(t_g)$ and extending $h_D(t)$ at the constant value to a new value of time t_g^*, so that:

$$t_g^* = t_g + \mathcal{E}_d / h(t_g) \qquad (4.4.1)$$

where E_d is one minus the recovery. The mean of $h_D(t)$ is then given by:

$$M_d = \int_{t_a}^{t_g} t\, h_D(t)\, dt + E_D t_g + \frac{E_D^2}{2h_D(t_g)} \qquad (4.4.2)$$

Yerushalmi et al have shown that if t_g is greater than three times the mean residence time, the volume calculated from equation 4.4.2 is a minimum.

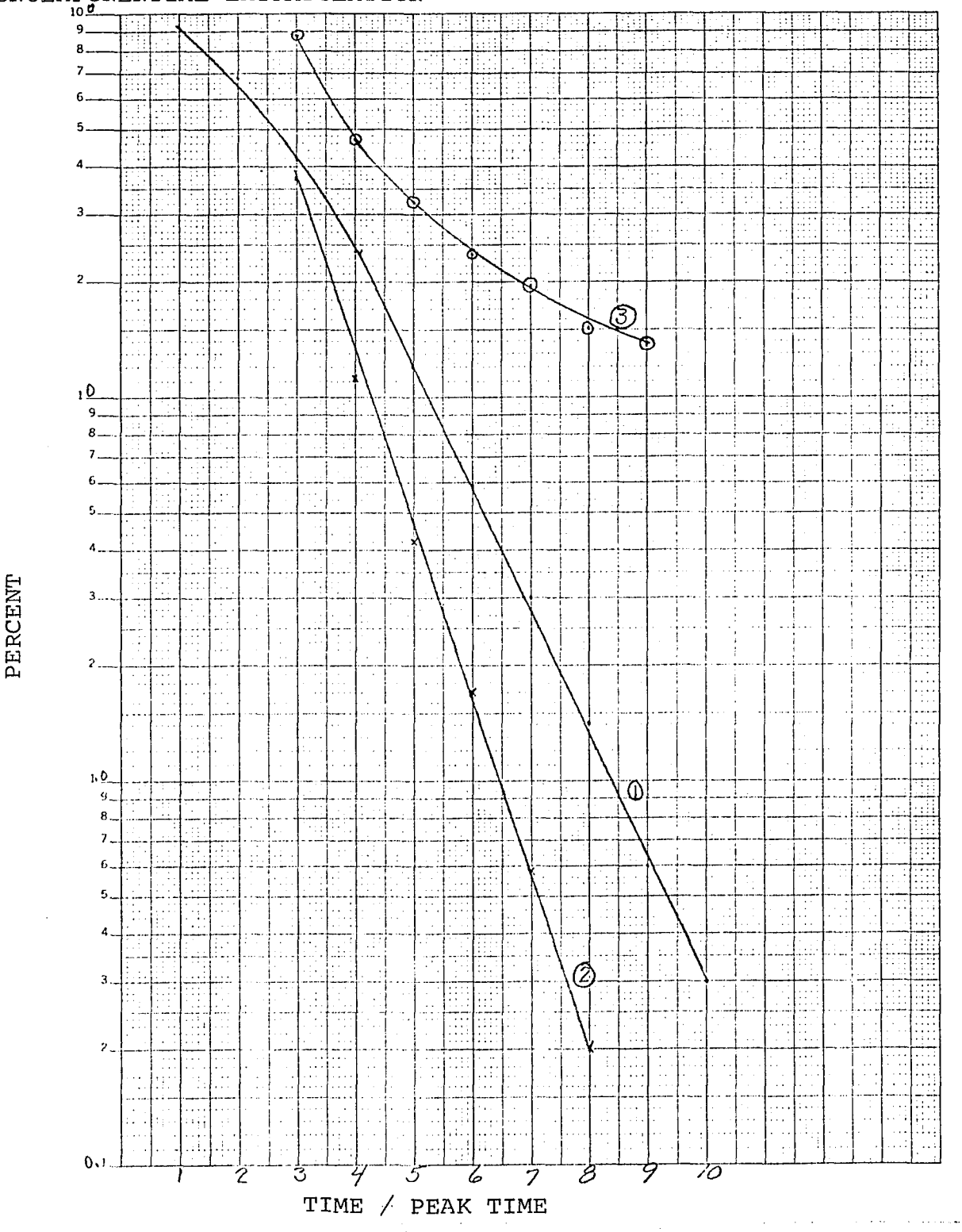
A commonly used extrapolation method which employs the second technique, that of matching the slope at the terminal time, is monoexponential extrapolation; that is, h(t) is assumed to have the form

$$h(t) = h(t_m)\, e^{-\beta(t-t_m)} \quad t > t_m \qquad (4.4.3)$$

$$\beta = \frac{1}{t_{m-1}-t} \ln \frac{h(t_m)}{h(t_{m-1})} \qquad (4.4.4)$$

For many systems this extrapolation gives excellent results, provided that t_m is chosen sufficiently large. Figure 4.4 shows the percentage that one overestimates the incremental and total capillary volumes, if $h_N(t)$ is given by equation 4.2.1, and the extrapolation technique of equation 4.4.3 is employed. Also shown on figure 4.4 is the

FIGURE 4.4 PERCENT OF VOLUME CONTAINED IN THE TAIL AND TOTAL VOLUME AND INCREMENTAL VOLUME ERRORS USING MONOEXPONENTIAL EXTRAPOLATION



CURVE 1: PERCENT VOLUME CONTAINED IN TAIL
2: PERCENT ERROR IN TOTAL VOLUME
3: PERCENT ERROR IN INCREMENT VOLUME

percentage of the volume measured, when integrating $t*h_N(t)$ from various t_M to infinity. It is apparent that at three times the peak, extrapolation error is less than 10% and at five times the peak time, the error is less than 1%. For systems given by equation 4.2.1, it can be shown that 1% of the peak value occurs at 7.64 times the peak time, and 0.1% of peak value occurs at 10.23 times the peak time. Therefore, monoexponential extrapolation with two or three decades measurability, should provide good estimates of the capillary volume.

The problem with monoexponential extrapolation arises with diffusible tracers where a serious underestimate of the total volume may occur. Figure 4.1 shows typical tracer curves for diffusible tracers that one might expect to obtain if the capillary space were two well mixed compartments in series and if the extravascular space had the behavior of a reflected diffusor. The compartments have slightly different residence times. Unequal times were chosen because they were easier to handle numerically. $h_D(t)$ has the analytic form of:

$$h_D(t) = 2\lambda\theta\gamma_1\gamma_2/(\gamma_2-\gamma_1) * \left(\sum_{n=1}^{\infty} \frac{\sigma_n^2 e^{-\sigma_n^2 t/\theta}}{\frac{\sigma_n^2}{\rho}((\lambda+\gamma)\theta-\sigma_n^2)+(\rho+1)(\gamma\theta-\sigma_n^2)^2+\lambda\theta(\gamma\theta+\sigma_n^2)} - \sum_{j=1}^{\infty} \frac{\sigma_j^2 e^{-\sigma_j^2 t/\theta}}{\frac{\sigma_j^2}{\rho}((\lambda+\gamma)\theta-\sigma_j^2)^2+(\rho+1)(\gamma\theta-\sigma_j^2)^2+\lambda\theta(\gamma\theta+\sigma_j^2)} \right) \quad (4.4.5)$$

where $\tan\sigma_n = \frac{\rho}{\sigma_n}\left(\frac{\gamma_1\theta-\sigma_n^2}{\lambda\theta+\gamma_1\theta-\sigma_n^2}\right)$

$$\tan\sigma_j = \frac{\rho}{\sigma_j}\left(\frac{\gamma_2\theta-\sigma_j^2}{\lambda\theta+\gamma_2\theta-\sigma_j^2}\right)$$

Curve 1 of figure 4.1 represents a non-diffusible tracer profile because λ =0, and curve 2 represents a well-mixed outer phase profile θ = 0. It becomes clear that for θ =10 and θ =50, one can severely underestimate the volume by truncation of $h_D(t)$, for example at t=8, where $h_N(t)$ disappears.

From Figure 4.1, one can deduce that for θ values of less than ten, monoexponential extrapolation can be used to estimate the volume if two decade measurability is attainable. This limit (θ = 10) gives excellent volume estimates for values of λ between 0.1 and 10. For values of θ =50 the monoexponential extrapolation seriously underestimates the volume. It can be

seen from figure 4.1 that for θ =50 and t=12, $h_D(t)$ =.0036, which is two decades below the maximum value of $h_N(t)$, the slope of the $h_D(t)$ is becoming flat, and the extrapolating slope is higher than the terminal slope. The higher value of β, solved for by equation 4.4.4, results in a lower incremental volume. The incremental volume is given by:

$$\int_{t_m}^{\infty} t\, h(t)\, dt = \frac{h(t_m)(\beta t_m + 1)}{\beta^2} \qquad (4.4.6)$$

Experimental data which we had obtained from Dr. Bassinthwaighte, (see Chapter 7) showed that monoexponential extrapolation underestimated the extravascular volume. In certain cases it was impossible to measure an extravascular volume, as the mean $h_D(t)$ was less than the mean of $h_N(t)$. Probably the cause is early truncation. Symptomatic of early truncation is that the recovery of the diffusible tracer is considerably less than 1.

One should mention at this point that an extrapolation method using both the slope and the recovery criteria can be formulated. As an example, one could use a double exponential of the form:

$$h(t) = h(t_m)\left((1-a)e^{-\beta_1(t-t_m)} + a\,e^{-\beta_2(t-t_m)}\right) \quad (4.4.7)$$

The constants β_1 and β_2, can be solved from the following equations:

$$\left.\frac{dh}{dt}\right|_{t=t_m} = m = -h(t_m)\left((1-a)\beta_1 + a\beta_2\right) \quad (4.4.8)$$

$$\int_{t_m}^{\infty} h(t)\,dt = E = h(t_m)\left(\frac{1-a}{\beta_1} + \frac{a}{\beta_2}\right) \quad (4.4.9)$$

The system is constrained such that $\beta \geq 0$ and $a \leq 1$. The system is also symmetric so that one needs only to consider $\frac{1}{2} \leq a \leq 1$ or $0 \leq a \leq \frac{1}{2}$. Solving equations 4.4.8 and 4.4.9 for β_1 and β_2, one gets:

$$\beta_1 = \frac{1}{2}\left[-\omega_1 \pm \left(\omega_1^2 + \frac{4M}{\varepsilon}\right)^{1/2}\right]$$

$$\omega_1 = \left(\frac{M}{h(t_m)} - \frac{(1-2a)\,h(t_m)}{\varepsilon}\right)\Big/(1-a) \quad (4.4.10)$$

and $$\beta_2 = \frac{1}{2}\left[-\omega_2 \mp \left(\omega_2^2 + \frac{4M}{\varepsilon}\right)^{1/2}\right]$$

$$\omega_2 = \left(\frac{M}{h(t_m)} + \frac{(1-2a)\,h(t_m)}{\varepsilon}\right)\Big/a \quad (4.4.11)$$

There are however, circumstances where β_1 and β_2 are not real, no matter which value of a is chosen. Thus, this method has only limited applicability. The incremental volume calculated from $\int_{t_g}^{\infty} th(t)dt$ is only loosely dependent on a. Therefore, it may not be a useful method.

4.5 Probablistic Approach

Shinnar (30) have developed a probabilistic approach whose primary purpose is to calculate λ, the permeability coefficient. In the process of obtaining λ, one calculates a function p(s), which is related to a probability distribution of sojourn time spent in the extravascular space. The reciprocal of p(s), as $s \downarrow 0$ or $\frac{1}{s} \uparrow \infty$ has a slope which approaches 1/R. A detailed derivation of this method has been given in Chapter 3.

From the experimental curves $h_N(t)$ and $h_D(t)$, one can numerically compute the transforms and then calculate p(s) from $h_N(s)$ and $h_D(s)$. One can either calculate p(s) numerically or graphically.

Having p(s) in hand, it can be easily shown that

$$\left.\frac{dp(s)}{ds}\right|_{s=0} = R \qquad (4.5.1)$$

$$\left.\frac{d\,1/p(s)}{d\,1/s}\right|_{\frac{1}{s}\to 0} = \frac{1}{R} \qquad (4.5.2)$$

It is more convenient to use equation 4.5.2 as the scale of s near zero is enlarged. Furthermore, Shinnar et al have shown p(s) is just a function of sojourn time distribution, $\phi(s)$, the sojourn time distribution of a tracer particle in the extravascular space. Thus the relationship developed is:

$$\rho(s) = \lambda\,(1-\phi(s)) \qquad (4.5.3)$$

The reciprocal of p(s) has the advantage that diffusional outer phases approach linearity rapidly. For a p(s) calculated for $h_N(t)$ given by equation 4.2.1 and $h_D(t)$, given by equation 4.4.5. , it can be shown that

$$\rho(s) = \frac{\frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}}{1+\frac{R}{\lambda\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}} \qquad (4.5.4)$$

and

$$\frac{1}{\rho(s)} = \frac{1}{\lambda} + \frac{1}{\frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}} \qquad (4.5.5)$$

In the special case where the outer phase is well-mixed, that is, $\theta = 0$, equation 4.5.5 becomes a straight line with an intercept of $\frac{1}{\lambda}$ and slope of $\frac{1}{R}$. Thus,

$$\frac{1}{\rho(s)}\Big|_{s=0} = \frac{1}{\lambda} + \frac{1}{Rs} \qquad (4.5.6)$$

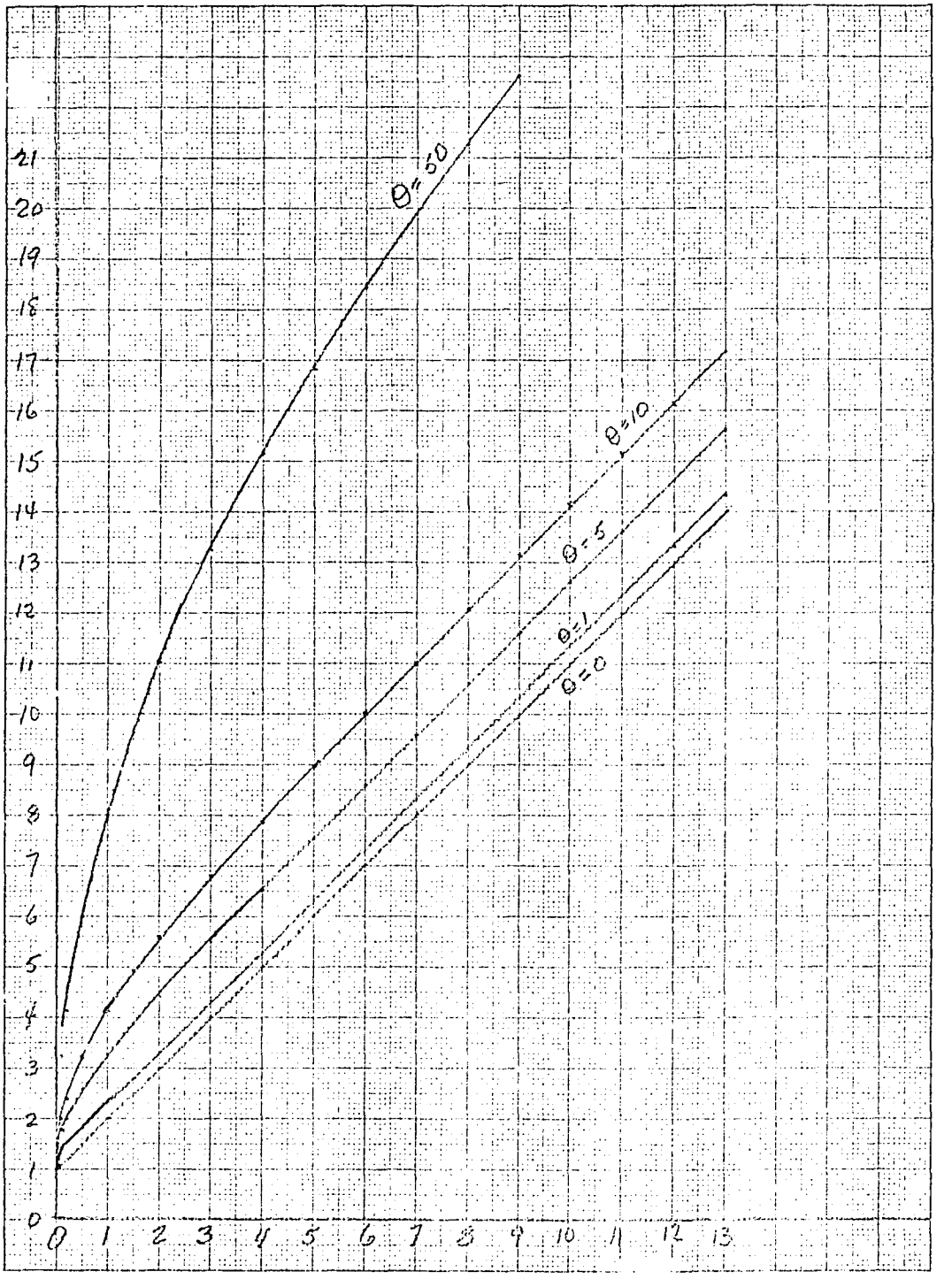
Diffusional outer phase also approaches linearity rapidly. If one takes the derivative of equation 4.5.5 and expands the derivative near s = 0 or $1/s \uparrow \infty$, one finds:

$$\frac{d\,1/\rho(s)}{d\,1/s}\Big|_{\frac{1}{s}\uparrow\infty} = \frac{1}{R}\left(1 + \frac{1}{360}(\theta s)^2\right) \qquad (4.5.7)$$

The slope is only weakly dependent on θ. Therefore for $\theta = 50$ and s=.1, the error in the volume estimate is less than 10%. Figure 4.5 shows $\frac{1}{p(s)}$ versus 1/s as a function of various values of θ.

This method like other methods suffers from the malady of

FIGURE 4.5 - 1/p(s) FOR A REFLECTED DIFFUSOR EVS VERSUS 1/s FOR VARIOUS VALUES OF θ



truncation, however if θ is small, say under ten, relatively large values of s, such as the slope at s=0.5 will give good estimates of R. These medium range values of s are less dependent on the tail and start of h(t) than are the smaller values of s.

4.6 Summary

We have shown that even under the best circumstances where λ is infinite, that is, all of the diffusible tracer has access to the extravascular space, not all values of R can be measured. On the lower end of the scale, for $R \leq 2E_r$, one can not even be sure of the existence of an extravascular space or even calculate its value. On the upper end of the scale the measurement errors are less of a problem, than the tail of $h_D(t)$, and for R greater than six, less than 90% of the extravascular volume is measurable, even for a well-mixed outer phase with an infinite λ . The transform method and monoexponential

extrapolation gave excellent results for R=10. However for large values of R, an R value of ten will be indistinguishable from R of twelve due to experimental error and scatter.

We have also shown that for the range where R is measurable, the nature of the properties of the outer phase, has an effect on the measurability. For example, in a situation where one encounters a small diffusion coefficient, so that θ=50 or higher, one finds R immeasurable, as all of the information about R is contained in the tail of $h_D(t)$, which is immeasurable. Early truncation of $h_D(t)$ has the same effect as large θ. In these cases monoexponential extrapolation is not a useful method.

The reciprocal of p(s) method which we have developed gives the most accurate results. The linear behavior of 1/p(s) for moderate values of s, are not very dependent on the beginning or the tail of h(t). In chapter 7 we will verify this result with experimental data.

CHAPTER 5 - PERMEABILITY ESTIMATES

5.1 General

The methods described above in Table 3.1 & 3.2 have several common assumptions. All methods assume that the system for both the diffusible and non diffusible tracer is linear. By this we mean the principles of Superposition and Homogeneity apply to the system.

Another fundamental assumption of multiple tracer experiments is that the diffusible tracer's behavior in the capillary space is given by the transfer function of the non diffusible tracer. Crone and Lassen (47) have suggested that diffusible properties of the diffusible and non diffusible tracer are not the same. (Taylor diffusion). If this is true, none of the methods discribed in Table 3.1 and 3.2 are valid for estimating permeability (λ).

Another assumption common to all is that tracer response is unfiltered. That is, the concentration profile one uses to calculate λ is not a convolution of transfer functions of the capillary space with any other transfer functions, such as that of the measuring instrument. In many cases of in-vivo and in-vitro experiments, however, there are blood vessels in series with the permeable capillary bed, whose transfer functions are not separable from the overall transfer function, and in

these cases, one cannot measure λ outright. In cases where filters are present, relative λ's however are still measurable. The effect of filters on the measurement of R and λ is discussed in Chapter 6.

The discussions in this chapter will be limited to unit impulses. In mathematical terms, the injection is approximated by a Dirac delta function, injected into an organ's artery. It is also possible to modify the proposed estimating methods for λ to be suitable for step input experiments such as performed by Crone and Garlick(45) . In these experiments the intravascular and extravascular spaces are saturated with diffusible and non-diffusible tracers and the washout of tracers is observed. Many experimenters have shown that the washout-experiment concentration profile is related to the concentration profile, obtained from an impulse experiment by the following relationship:

$$h(t) = \frac{-dH(t)}{dt} \qquad (5.1.3)$$

5.2 Conventional Methods

A. Extraction Factor Methods

In addition to the assumptions mentioned above, the conventional methods, MJY, MIN Ω(t) and CRA, assume that there is no back "diffusion". Back diffusion means that once a tracer particle leaves the capillary space, it does not return. In reality, all systems have back diffusion. However, this assumption is valid for the early part of the concentration profile, where back diffusion may be near zero. In the case of the min Ω(t) and one of the CRA variations, the no back mixing assumption, is extended to the time where min Ω(t) occurs. Back diffusion leads to an underestimate of λ because the value of Ω is artificially higher due to the return of tracer particles.

In addition to assuming no "back diffusing", the conventional methods do not allow for any back mixing. Back mixing is the diffusion of tracer particles, either forward or backward in the direction of the flow. The limiting case of back mixing

is a well mixed compartment; that is, a compartment where the concentration is independent of position; as in the back diffusion cases, the back mixing leads to underestimates of λ .

The conventional extraction factor method (MJY) assumes that the injection of a non-diffusible tracer is not distorted in its passage through the capillaries and the tracer particle leaves the capillaries at some later time $1/\gamma$. Thus, when

$$h_N(t) = \delta\left(t - \frac{1}{\gamma}\right) \qquad (5.2.1)$$

good estimates of λ can be calculated by the MJY method because the mean sojourn time and the appearance time of the non-diffusible tracer are simply the time delay, $1/\gamma$. In many experiments, one finds that the tracer particles of the non-diffusible tracer are dispersed by back mixing and one can appropriately model the capillary space by a number of well mixed compartments in series with a delay.

In order to demonstrate the effect of back mixing on the evaluation of λ , we will for the sake of convenience,

discuss a case where the transfer function of the capillary space is two well mixed compartments in series with a pure delay section.

$$h_N(t) = (2\alpha)^2 (t - \tfrac{1}{\gamma})\, e^{-2\alpha(t-\frac{1}{\gamma})} \qquad (5.2.2)$$

The mean sojourn time of tracer particles in this kind of system is

$$M_N = \frac{1}{\alpha} + \frac{1}{\gamma} \qquad (5.2.3)$$

where α is the blood flowrate divided by the total stirred tank volume and γ is the blood flowrate divided by the volume of the delay. The MJY method, underestimates the true value of by a factor of

$$\frac{\lambda_{ACT}}{\lambda_{CALC}} = \frac{\frac{1}{\gamma} + \frac{1}{\alpha}}{1/\gamma} \qquad (5.2.4)$$

It then becomes quite clear that as $1/\gamma$ approaches zero, the calculated value of λ also approaches zero. For the case, $\frac{1}{\gamma} \downarrow 0$, one can show by L'Hospital's rule that as $\Omega(ta)$ approaches one, and $\ln\Omega$ approaches zero.

In order to avoid some of the shortcomings of the MJY method,

Bassingthwaighte et al have proposed to take min $\mathcal{R}(t)$ regardless of the time in which it occurs. Unfortunately, the minimum $\mathcal{R}$ does not always occur at the same time; that is $\mathcal{R}$ min sometimes occurs before M_N, the mean sojourn time, and sometimes after M_N. This leads to values of λ which either overestimate, underestimate or are equal to the actual values of λ . Consequently, in many instances, one is not sure of the accuracy of the calculated value of λ .

For example in Case 1A, shown in Table 2.1, one finds that the time the minimum $\mathcal{R}$ (t) occurs is given by:

$$t = \frac{1}{A+B} \ln\left(\frac{\gamma - B}{A - \gamma}\right) \qquad (5.2.5)$$

It is evident from equation 5.2.5 that for small values of λ , $(\lambda \downarrow 0)$, the time at which the minimum occurs grows large, $(t \uparrow \infty)$, and one overestimates λ. Similarly as the value of λ grows large, $(\lambda \uparrow \infty)$, the minimum $\mathcal{R}$ time grows small $(t \downarrow 0)$, and one underestimates λ. Under certain fortuitous circumstances, where t is somethat above M_N, the estimate of λ is quite accurate.

The unreliability of predicting λ by the above two methods led us to modify the MJY method. Instead of dividing the $\ln \Omega(ta)$ by the mean residence time, one divides $\ln \Omega(t)$ by the actual time, and takes the limit as $t \downarrow ta$. Thus

$$\lambda = \underset{t \to ta}{\text{Limit}} \ \frac{1}{t} \ln \Omega(t) \qquad (5.2.6)$$

From a theoretical standpoint if $h_N(t)$ and $h_D(t)$ could be measured accurately, then λ could be estimated accurately by equation 5.2.6.

All extraction factor methods use a quotient to evaluate λ, which can lead to serious errors. Let us analyze the situation. $h_N(t)$ and $h_D(t)$ are measured to within some experimental error, E_r, so that in the worse instance:

$$h_{MN}(t) = h_N(t)\,(1-E_r) \qquad (5.2.7)$$

$$h_{DM}(t) = h_D(t)\,(1+E_r) \qquad (5.2.8)$$

$$\Omega_M(t) = \frac{h_D(t)\,(1+E_r)}{h_N(t)\,(1-E_r)} \qquad (5.2.9)$$

Then the MJY method would predict λ to be

$$\lambda_M = \frac{1}{M_N}\left[\ln \Omega(ta) + \frac{\ln(1+E_R)}{1-E_R}\right] \qquad (5.2.10a)$$

for small errors, E_r

$$\lambda_M = \lambda_{MJY} + \frac{2E_r}{M_N} \qquad (5.2.10b)$$

In those cases where $h_N(t)$ is the transfer function of a well mixed compartment, λ_{MJY} equals zero, thus the permeability coefficient, one measures is $\lambda_M = 2E_r/M_N$, a function only of the experimental error.

In the minimum $\Omega(t)$ method, the effect of experimental error is not readily determined. In addition to the error introduced in the evaluation of λ, the experimental error could also alter the time at which the minimum Ω occurred; thus destroying any true relationship between the theoretical and actual λ. The effect of experimental error on the MEF method would yield the following:

$$\lambda_m = \lambda_{ACT} + \lim_{t \downarrow t_a} \frac{2E_R}{t} \qquad (5.2.11)$$

As long as the appearance time is far enough removed from zero, λ is measurable and contains only a small error. However as $t_a \downarrow 0$, the measurement error produces an enormous overestimate, and λ becomes inestimable. Figure 5.2 shows the range in which λ is measurable as a function of appearance time. The solid lines showing the maximum

anticipated experimental error were calculated at the point where $\lambda = 2E_r$.

The extraction factor methods have another difficulty. Just as low values of λ are inestimable due to measurement errors, large values of λ are not measurable as the experiments are not sensitive enough. The instruments used to measure the outflow concentrations have a limited range of two or three decades. Thus the maximum value of Ω that one can attain is 100 or 1,000. In general terms, for an N decade range instrument, the maximum possible Ω is 10^N. Thus the maximum value of λ would be given by

$$\lambda_{MAX} = \frac{2.303N}{ta} \qquad (5.2.12)$$

Generally, at the time $h_D(t)$ becomes measurable, $h_N(t)$ is not at its maximum value and more likely, at least one decade removed from it. Practically then, the maximum value of would more properly be given by

$$\lambda_{MAX} = \frac{2.303(N-1)}{ta} \qquad (5.2.13)$$

Figure 5.2 shows the maximum values of λ, measurable as a function of appearance time, and sensitivity of the measuring instrument. The maximum measurable λ shown on the plots is given by equation 5.2.13

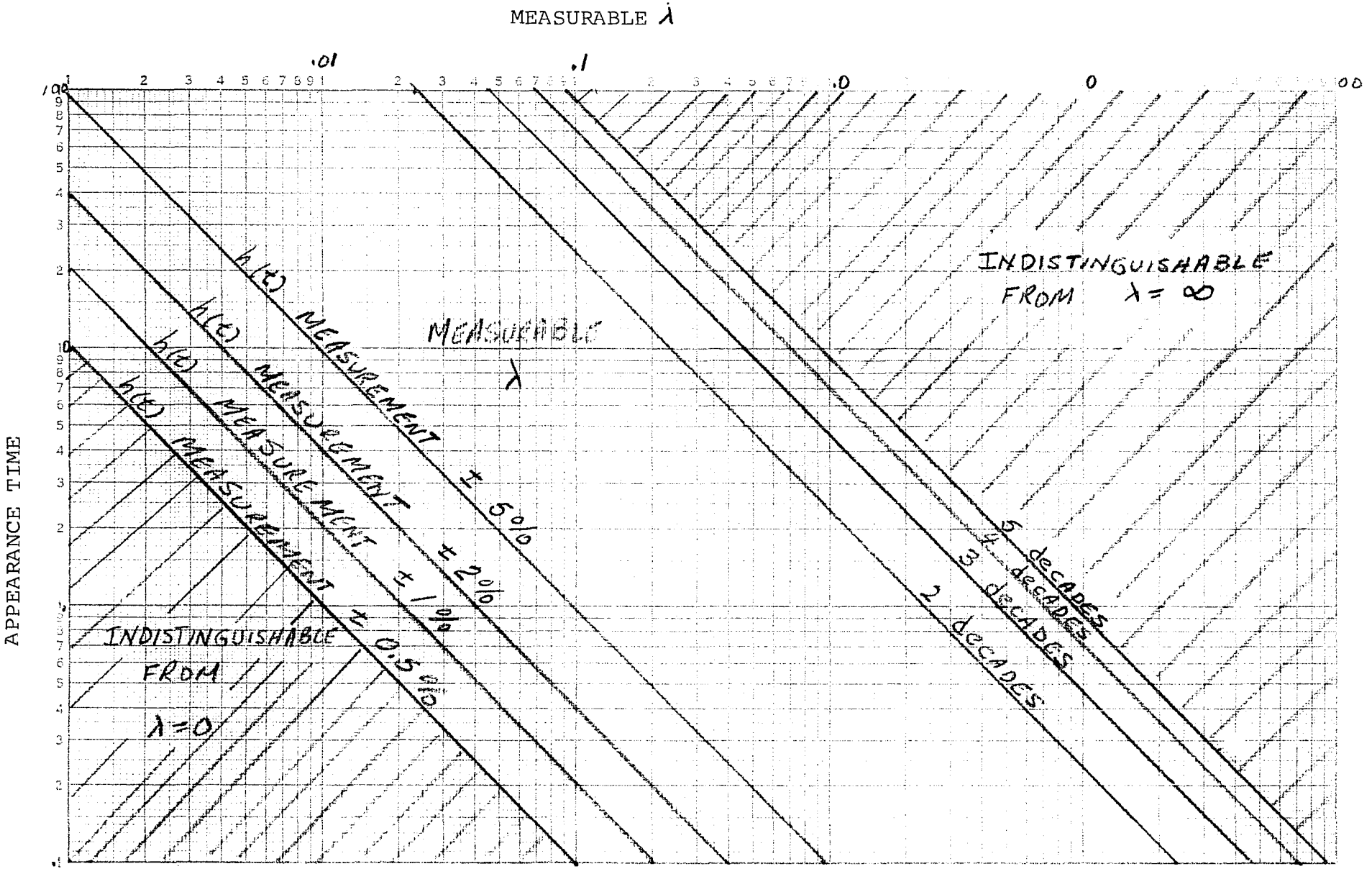


FIGURE 5.2 - MEASURABLE λ AS A FUNCTION OF APPEARANCE TIME

It should be noted that we use the word immeasurable in a very loose manner. It would be more proper, for lower values of λ, to use indistinguishable from zero λ, and for the higher values of λ, it would be more proper to use indistinguishable from an infinite λ.

The bounds on the measurability of λ that we have derived here through the use of the extraction factor methods, are equally applicable to the values of λ derived from transform methods, discussed later.

5.3 Crone Relative Area (CRA)

The CRA method like the conventional extraction factor method, is correct for a capillary space which has the transfer function of a pure delay, where $h_N(t)$ is given by a Dirac delta function. At the appearance time, which also corresponds to the peak time, minimum Ω time and crossover time, $h_N(t)$ has an integral of one. Analogously, $h_D(t)$ has an integral of $e^{-\lambda ta}$. Therefore λ calculated by equation 2.1 gives the appropriate value of the λ. On the other extreme where $h_N(t)$ is a well mixed compartment (large amount of back diffusion), the recovery of the non-diffusible tracer at

the peak time is only 26%. The diffusible tracer's recovery is $1-\frac{1}{\lambda}e^{-\lambda\gamma}+\mu$ where μ results from integration of a second term involving a convolution of $h_N(t)$ and a first order Bessel function. μ in general is small, therefore, as a first approximation, one may consider the recovery of diffusible tracer $1-\frac{1}{\lambda}e^{-\lambda\delta}$. It would be fortuitous if

$$1-\frac{1}{\lambda}e^{-\frac{\lambda}{\delta}} = .26\, e^{-M_N\lambda} \qquad (5.3.1)$$

in which case one would calculate the true value of λ. However, in general, equation 5.3.1 is not true, and underestimates of λ result.

5.4 Moment Method

Shinnar et al have developed a probabilistic model which relates $h_N(t)$ and $h_D(t)$ to the sojourn time distribution of the extravascular space $\phi(t)$. They also developed moment relationships, where the various transfer functions for the extravascular space are:

Well Mixed Outer Phase

$$R = M_D/M_N - 1 \qquad (5.4.1)$$

$$\lambda = \frac{2R^2 M}{V_D - V_N(1+R)^2} \qquad (5.4.2)$$

Reflected Diffusor

R is given by equation 5.4.1

$$\theta = \left(\frac{15}{2R}\left(\eta_3 - \frac{\eta_2^2(1.5)}{R}\right)\right)^{1/2} \qquad (5.4.3)$$

$$\lambda = \frac{2R^2}{\eta_2 - \frac{2}{3}R\theta} \qquad (5.4.4)$$

where $\eta_2 = \frac{V_D - V_N(1+R)^2}{M_N}$

$$\eta_3 = \frac{S_D - (1+R)^3 S_N - 3(1+R) \quad V_N}{M_N}$$

S = Skewness, V = Variance, and M = Mean.

Ideally one could use equations 5.4.1 through 5.4.4 to evaluate R, λ, θ. However, the mean, variance and skewness are not calculable to the accuracy required to allow one to use moments to compute the various parameters. We have shown in Chapter 4 that for a simple task such as evaluating the extra-

vascular volume, many problems arise. Aside from the problem of experimental error, which is inherent in every experiment, truncation of both $h_D(t)$ and $h_N(t)$ concentrations, causes even more serious difficulties.

Truncation is a more serious problem in the evaluation of λ , and makes the evaluation of θ impossible from the moments. Table 7.1. and 7.2 show an example of a truncated transfer function for a capillary space, behaving like two well mixed compartments in series, with each having an extravascular space, which has the behavior of a well mixed compartment. For a non-diffusible tracer concentration profile, which was truncated at the time the recovery was 95%, the second moment was 69% of the actual value, and the third moment was 51% of the actual value. Even at 99%, the third moment was only 79% of its actual value. For the diffusible tracer, the situation is analagous. Although the percentage difference may be relatively small, like 21%, the numerical difference is important. For alternate A, Case B of Table 2.1, the difference between the truncated second moment of $h_D(t)$, and the true moment of $h_D(t)$, is 20, which is almost five times the value of the λ one

is trying to compute.

In general, the recovery of the diffusible tracer is less than the recovery of the non-diffusible tracer, as more of the tracer particles are found in the immeasurable tail. As an example, Table 7.2B shows the errors that result in estimating R and λ, if the recovery of the diffusible tracer is 95%, and the recovery of the non-diffusible tracer is 99%. It is evident that in these cases, the moments do not produce valid results.

Bearing in mind the problem experienced in evaluation of the first two moments and the parameters of a two parameter model, it becomes impossible to evaluate the three parameters, θ, λ, R, for a reflected diffusor. The tails of $h_N(t)$ and $h_D(t)$, even without experimental error, are not measured accurately enough to evaluate the three parameters.

5.5 Probabilistic Approach

Shinnar et al have developed the theory that relates $h_D(t)$ and $h_N(t)$ by a conditional probability, $X(t/\tau)$. The

relationships developed are not in the time domain but use Laplace transforms. They are:

$$\text{(5.5.1)}$$

$$h_D(s) = h_N(s+p(s)) \qquad \text{(5.5.2)}$$

$$p(s) = h_N^{-1}(h_D(s)) - S \qquad \text{(5.5.2b)}$$

$$\lim_{s\to\infty} p(s) = \lambda \qquad \text{(5.5.3)}$$

In addition to containing information about λ, (Eq5.5.3) p(s) contains information about R, and in the diffusional case, the value of θ may also be extracted. In the diffusional case p(s) is given

$$p(s) = \frac{\frac{R\sqrt{\theta s}}{\theta}\tanh\sqrt{\theta s}}{1 + \frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}} \qquad \text{(5.5.4)}$$

and its reciprocal is given by

$$\frac{1}{p(s)} = \frac{1}{\lambda} + \frac{1}{\frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}} \qquad \text{(5.5.5)}$$

If one were able to accurately calculate the Laplace transforms of $h_N(t)$ and $h_D(t)$ and numerically or graphically evaluate p(s) (the numerical procedure is described in Appendix A), the three parameters R, λ, θ, could be evaluated. As shown earlier

$$\underset{\frac{1}{s}\uparrow\infty}{\text{Limit}} \frac{d\frac{1}{p(s)}}{d\frac{1}{s}} = \frac{1}{R} \qquad (5.5.5a)$$

λ can be evaluated by equation 5.5.3 and θ can be evaluated from the knowledge of R and λ and asymptotic form of p(s).

$$\frac{1}{p(s)}\Big|_{s\uparrow\infty} = \frac{1}{Rs} + \frac{1}{\lambda} + \frac{\theta}{3R} \qquad (5.5.6)$$

Unfortunately, truncation and experimental error, which make obtaining measurements near the appearance time difficult, do not allow one to calculate p(s) accurately enough over the entire range to calculate the three parameters. The best that one can do is to assume the outer phase is well mixed, that is θ =0, therefore equation 5.5.6 becomes

$$\frac{1}{p(s)} = \frac{1}{Rs} + \frac{1}{\lambda} \qquad (5.5.7)$$

whose linearity is independent of the value of s. Thus the medium values of 1/s can be used to evaluate p(s) for all values of s, since at these points the transform is not strongly dependent on the tail, or the beginning of the $h_D(t)$ and $h_N(t)$ curves, which are not accurately known.

The SLRρmethod uses the above assumption as its basis, the slope and intercept of the best straight line drawn through 1/p(s) versus 1/s is used to evaluate λ and R.

The sensitivity of max p(s) method (Eq. 5.5.3) is taken to be the same as the MEF method as the theoretical basis is the same for both. From a numerical viewpoint infinite λ may be more distinguishable in terms of the transform method as p(s)=Rs, then by the MEF method where ta must approach zero and $\Omega \neq 1$.

5.6 Relative λ's

Under many circumstances one is not interested in obtaining the absolute value of λ , but is only interested in obtaining relative λ's, for example, the λ of sucrose, divided by the λ of glucose. In cases where $h_N(t)$ and $h_D(t)$ are distorted by filters, that is all one can hope to obtain. (A complete discussion of the filter problem is given in Chapter 6.) The errors in relative λ's can be more severe than the errors in the absolute value of λ , since one can overestimate λ_1 , and in the other case one can underestimate λ_2 . Figure 5.6

shows the maximum error in relative λ's as a function of E_r

$$\Phi_{MAX} = \frac{\lambda_1 (1+E_R)}{\lambda_2 (1-E_R)} \qquad (5.6.1)$$

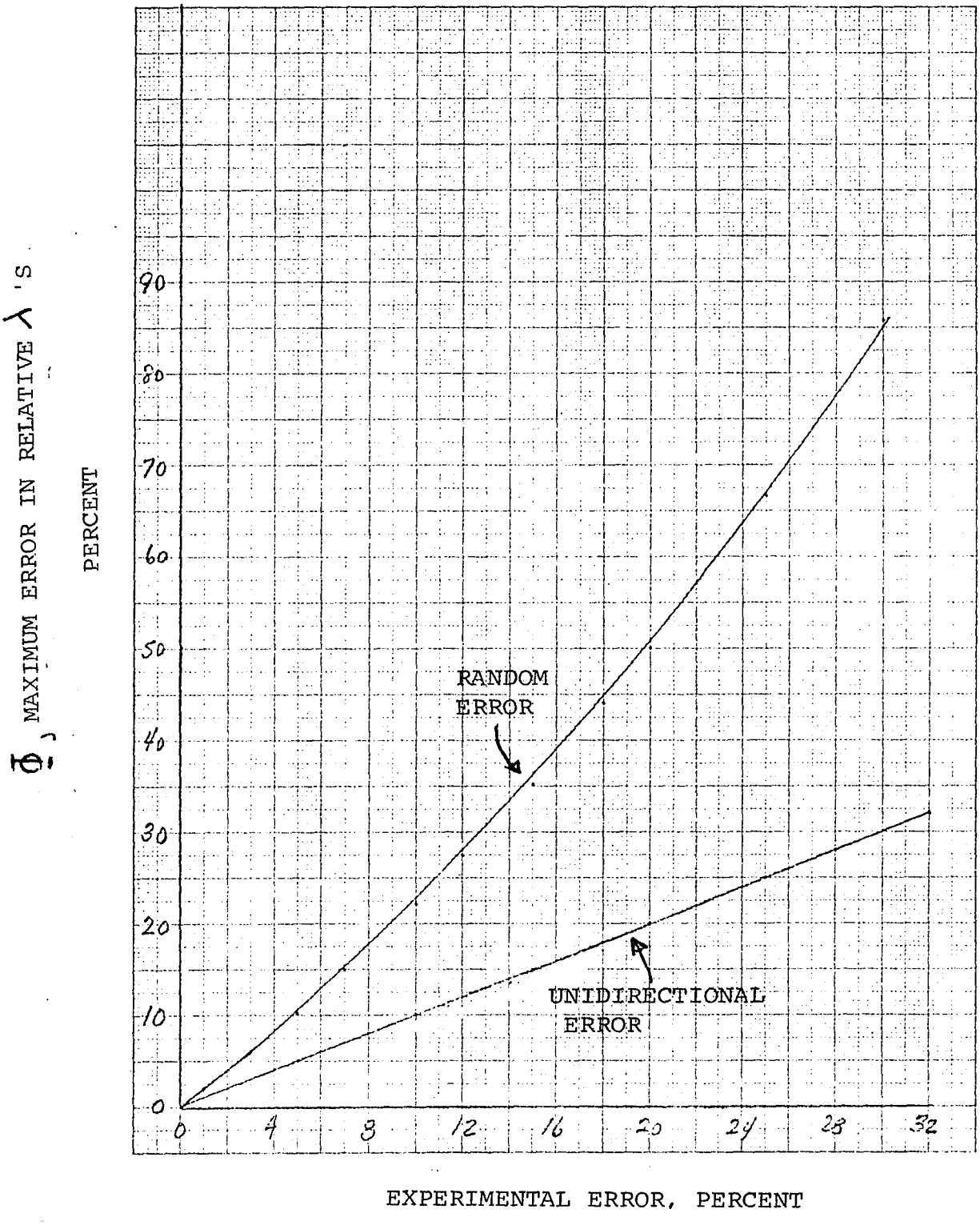
If one were certain that the errors were unidirectional then equation 5.6.1 would reduce to

$$\Phi_{MAX} = \frac{\lambda_1}{\lambda_2}(1+E_R) \qquad (5.6.2)$$

5.7 Summary

In this chapter we have shown the effects of back mixing and back diffusion are not accounted for by the conventional methods. The methods we proposed, however, are equally applicable for systems with or without back diffusion. In addition, figure 5.2.1 establishes values of λ which are measurable. The lower values of λ are very susceptible to measurement errors. However, large values of λ are indistinguishable from infinite λ because of our inability to measure more than a few decades.

FIGURE 5.6 - MAXIMUM ERROR IN RELATIVE λ 'S AS A FUNCTION OF EXPERIMENTAL ERROR



CHAPTER 6 - THE EFFECT OF FILTERS ON VOLUME AND PERMEABILITY ESTIMATES

6.1 INTRODUCTION

We have previously discussed (Chapter 4 and 5) the effect that truncation and experimental error have on the estimates of R(relative volume) and λ (permeability coefficient). In this chapter we will deal with the effect that filters have on estimates of R and λ . The methods we will use in our discussions are the SLRp(s), MEF, Maximum p(s) and the Moment Methods. The governing equations for these methods are given in Tables 3.1 and 3.2. We have chosen these methods because they predict the correct values of R and λ without a filter.

In biological systems, tracer particles, diffusible and non-diffusible, are injected into the main artery of an organ and the concentration is monitored at the main vein of the organ. On its path through the organ, the tracer passes through arteries, arterioles, veinules and veins which do not permit significant permeation in the extra-vascular space.

In between the arterioles and veinules, we find a capillary bed where diffusion occurs. A typical flow path is shown in Figure 1.1. Each blood vessel segment has its own transfer function and therefore distorts the transfer functions that one is seeking, namely those of the capillary bed, $h_N(t)$ and $h_D(t)$. It is impossible to separate the transfer functions' non-diffusing portions from the overall transfer function of the flow path. For the sake of convenience, we will call the transfer function for the non-diffusing segment of the blood vessel $h_b(t)$, recognizing that it is made up of a convolution of functions

$$h_b(t) = h_{ARTERY}(t) * h_{ARTERIOLE}(t) * h_{VEINULE}(t) * h_{VEIN}(t) \qquad (6.1.1)$$

or in Laplace transforms, a simple product

$$h_b(s) = h_{ARTERY}(s) \cdot h_{ARTERIOLE}(s) \cdot h_{VEINULE}(s) \cdot h_{VEIN}(s) \qquad (6.1.2)$$

There is a second kind of filter which is also present in series with the flow path, $h_i(t)$ which is the transfer function of the measuring instrument. Its transfer function, $h_i(t)$, can be convoluted with the blood vessel transfer function, giving the overall transfer function for all the filters of

$$h_f(t) = h_b(t) * h_i(t) \qquad (6.1.3)$$

We will deal with a single flow path and evaluate the effect the filter has on the estimates of R and λ. We will also show that relative R's and λ's are maintained for two or more diffusible tracers.

It should be pointed out that the actual volume of the extravascular space is given by

$$V_E = F(M_{DM}-M_{NM}) \qquad (6.1.4)$$

and is independent of the mean sojourn time of the filter. The accuracy of this volume estimate has been discussed earlier in Chapter 4.

We will discuss effects of filters on R and λ in terms of three simple transfer functions for the extravascular space. These are:

1. A stretch

$$p(s) = Rs \qquad (6.1.5)$$

2. A well mixed outer phase

$$p(s) = \frac{\lambda s}{s + \lambda/R} \qquad (6.1.6)$$

3. A diffusional outer phase

$$p(s) = \frac{\frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}}{1 + \frac{R}{\lambda\theta}\tanh\sqrt{\theta s}} \qquad (6.1.7)$$

Although these three transfer functions may not exactly simulate the behavior of the extravascular space, the effect of a filter on estimating R and λ for these transfer functions is extrapolatable to more complicated behavior.

6.2 INDETERMANCY FOR PURE DELAYS

Some experimenters feel that the transfer function of the capillary space is close to a pure delay, that is

$$h_N(s) = e^{-\mu s} \qquad (6.2.1)$$

and the dispersion that one measures is largely a result of filters. Thus the transfer function that one measures is given by

$$h_{MN}(s) = h_f(s)\, e^{-\mu s} \qquad (6.2.2)$$

The diffusible tracer transfer function $h_{MD}(s)$ is given by

$$h_{MD}(s) = h_f(s)\, e^{-\mu(s+p(s))} \qquad (6.2.3)$$

based on the Shinnar model. Shinnar et al have also shown

that

$$p(s) = \lambda (1 - \phi(s)) \qquad (6.2.4)$$

where ϕ(s) is the Laplace transform of the sojourn time distribution of the extravascular space.

Having only the measured transfer functions for the diffusible and non-diffusible tracers, $h_{MD}(s)$ and $h_{MN}(s)$ in hand, it is impossible for one to calculate the value μ. As a result, one can assume the residence time distribution in the capillary space has a different value for the delay, μ^*, and still produces the same measured transfer function for both diffusible and non-diffusible tracers, making the system indeterminate.

As a proof, we offer the following: Let

$$h_N^*(s) = e^{-\mu^* s} \qquad (6.2.5)$$

In order to obtain the same measured transfer function for the non diffusible tracer, the filter's transfer function is given by:

$$h_f(s) = h_f(s)\, e^{-(\mu - \mu^*)s} \qquad (6.2.6)$$

Similarly, the transfer function for the diffusible tracer must be independent of assumed transfer function for the capillary space. Thus

$$h_f(s)\, e^{-\mu(s+\lambda(1-\phi(s)))} = h_f^*(s)\, e^{-(\mu-\mu^*)s}\, e^{-\mu^*(s+\lambda^*(1+\phi(s)))} \qquad (6.2.7)$$

Solving equation 6.2.7, one finds

$$\lambda^* = \frac{\mu\lambda}{\mu^*} \qquad (6.2.8)$$

and since λ^* is an arbitrary constant, we can choose it so that it satisfies equation 6.2.8. The transfer function for the diffusible tracer is also reproduced by use of a different function, therefore, the system is indeterminate.

6.3 DOUBLE SOLUTIONS FOR STRETCHES

We have just shown that an indeterminacy exists in the estimation of R and λ, if the transfer function of the capillary space is a pure delay, a more limited indeterminacy, namely multiple roots are found, if the capillary space

transfer function is not a pure delay. These multiple solutions however, are limited to cases where the extravascular space behaves like a stretch ($p(s)=Rs$).

In order that multiple roots exist, both transfer functions (diffusible and non-diffusible) must be reproduced from two or more different transfer functions. We will use a "*" to denote a contrived or false transfer function. Thus:

$$h_{MN}(s)=h_f(s)h_N(s)=h_f^*(s)\ h_N^*(s) \qquad (6.3.1)$$

and

$$h_{MD}(s)=h_f(s)\ h_D(s)=h_f^*(s)\ h_D^*(s) \qquad (6.3.2)$$

or

$$h_{MD}(s)=h_f(s)\ h_N(s+p(s)) = h_f^*(s)\ h_N^*(s+p^*(s)) \qquad (6.3.2a)$$

If we assume equations 6.3.2a to be true, then

$$\frac{h_f(s)}{h_f^*(s)} = \frac{h_N(s+p^*(s))}{h_N(s+p(s))} \qquad (6.3.3)$$

For the moment, since $h_f(s)$ is unknown, we can take the liberty to choose $h_f(s)$ as the convolution of two filters, one

of which has the transfer functional form of $h_N(s)$, but has free constants which we can choose in any manner we wish. To illustrate this point we will choose the capillary space transfer function to be a well mixed compartment with a delay, that is

$$h_N(s) = \frac{e^{-\mu s}}{1+\tau s} \qquad (6.3.4)$$

The transfer function for the diffusible tracer of an extravascular space, being a stretch, is then given by

$$h_D(s) = \frac{e^{-\mu s(R+1)}}{1+\tau s(R+1)} \qquad (6.3.5)$$

and the measured transfer functions will be given by:

$$h_{MN}(s) = h_f(s)\, e^{-\mu s} / 1+\tau s \qquad (6.3.6)$$

$$h_{MD}(s) = h_f(s)\, e^{-\mu s(R+1)} / 1+\tau s(1+R) \qquad (6.3.7)$$

We now assume, since we have no knowledge of the nature of $h_f(s)$, that $h_f(s)$ has the form:

$$h_f(s) = h(s)\ e^{-as}/1+bs \qquad (6.3.8)$$

Furthermore, contrived transfer functions, given by equations 6.3.9 and 6.3.10, can be constructed for $h_N^*(s)$ and $h_D^*(s)$.

$$h_N^*(s) = \frac{e^{-s(\mu+a)}}{(1+\tau s)(1+bs)} \qquad (6.3.9)$$

$$h_D^*(s) = \frac{e^{-(\mu+a)s(R^*+1)}}{(1+\tau s(R^*+1))(1+bs(R^*+1))} \qquad (6.3.10)$$

Since the measured transfer functions, $h_{MN}(s)$ and $h_{MD}(s)$, must be the same for both sets of transfer functions, one can solve for the constants a, b, and R* in terms of R, μ, and τ easily shown, by equating coefficients, that the solutions of the constants is given by

$$R^* = (R+1)^{1/2} - 1 \qquad (6.3.12)$$

$$a = \mu(R+1)^{1/2} \qquad (6.3.13)$$

$$b = \tau(R+1)^{1/2} \qquad (6.3.14)$$

One should note that even though the transfer function of the capillary space had a delay portion, the system was not indeterminate. The well mixed phase portion of the transfer

function, $1/1+\tau s$ constrained the value of R*, to be that given by equation 6.3.12, and the same R* must be used for both transfer function segments, thus no general indeterminacy.

Equations 6.3.12, 6.3.13 and 6.3.14 are valid for any number of stirred tanks with delays in series. The value of μ is simply the sum of all the delays and the values of b correspond to each value of τ . In general one would write

$$b_n = \tau_n (R+1)^{1/2} \quad n = 1, 2, \ldots N \qquad (6.3.14a)$$

and

$$\mu = \sum_{i=1}^{N} \mu_i \qquad (6.3.15)$$

A general rule which can be developed, that multiple roots can be found, if one chooses, a portion of the filter to have the same transfer function as the capillary space transfer function. This rule must be slightly modified if the capillary space transfer function is one or more well mixed compartments in series. A series of multiple roots can be found if the filter's transfer function, $h_f(s)$, can be given by a greater number of well mixed compartments in series than are present in the capillary space. In the case of one well mixed compart-

ment, one can take $h_f(s)$ to have the form

$$h_f(s) = h(s) \prod_{i=1}^{n} \frac{1}{1+a_i s} \qquad (6.3.16)$$

Then the corressponding R*, and a_M are given by

$$R^* = (R+1)^{1/n+1} - 1 \qquad (6.3.17)$$

$$a_m = \tau (R+1)^{\frac{M}{n+1}} \quad M = 1, 2, 3 \qquad (6.3.18)$$

A second special case arises when the capillary space is two well mixed compartments in series. Under the special circumstance where τ_1, the sojourn time of the smaller compartment is related to τ_2 by

$$\tau_1 = \tau_2 / (1+R)^{1/3} \qquad (6.3.19)$$

one has the freedom to choose $h_f(s)$ to be

$$h_f(s) = h(s) \frac{1}{1+as} \qquad (6.3.20)$$

a single well mixed compartment. In this instance

$$R^* = (R+1)^{2/3} - 1 \qquad (6.3.21)$$

and

$$a = \frac{(\tau_1 + \tau_2)(R+1)^{2/3}}{(R+1)^{1/3} + 1} \qquad (6.3.22)$$

If we now substitute the relationship of τ_1 to τ_2, given by equation 6.3.19, into equation 6.3.22, we find

$$a = \tau_2 (R+1)^{1/3} \qquad (6.3.23)$$

We have now discussed the necessary conditions under which multiple roots are found. Let us look at an experimental situation and see under what conditions one would encounter these circumstances. It is highly unlikely to find stirred tanks with coefficients related by equation 6.3.14. On the other hand it is quite possible to have delays which are greater than the value of b, given in equation 6.3.14. It is impossible for any experimental data to distinguish between a delay $e^{-\mu s}$, and a large number of well mixed compartments in series $1/(1+\frac{\mu}{n}s)^n$, which makes it more likely to produce the multiple solution.

6.4 NO MULTIPLE SOLUTIONS FOR REFLECTED DIFFUSORS

In the last section, we have shown that multiple solutions exist for stretches, which are a degenerate case of extravascular space with infinite λ . If we take the analogous transfer function for the reflected diffusor, with an infinite λ , the multiple roots do not exist. For this case p(s) is given by:

$$P(s) = \frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s} \qquad (6.4.1)$$

In order to demonstrate this fact we will use a capillary space transfer function of a single well mixed compartment and a filter transfer function of h(s)/1+as. The measured transfer function for a non-diffusible tracer gives us no information about values of a, R*, or θ^*. The false $h_N(s)$ is given by

$$h_N^*(s) = \frac{1}{1+as}\cdot\frac{1}{1+s} \qquad (6.4.2)$$

Then the false $h_D(s)$ will be expressed as:

$$h_D^*(s) = \frac{1}{(1+as(\frac{R^*}{\theta^*}\sqrt{\theta^* s}\tanh\sqrt{\theta^* s}))(1+\mu s(\frac{R^*}{\theta^*}\sqrt{\theta^* s}\tanh\sqrt{\theta^* s}))} \qquad (6.4.3)$$

The information about the parameters is contained in the equality of the diffusible tracer's transfer function. If we set $h_{MD}(s)$ calculated both from the false $h_D^*(s)$ and that calculated from $h_D(s)$, equal to each other, the constraints on the parameters are determined. Thus:

$$(1+as)(1+\tau s) + (1+as)\tau\frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s} =$$

$$(1+as)(1+\tau s) + (a+\tau)\frac{R^*}{\theta^*}\sqrt{\theta^* s}\tanh\sqrt{\theta^* s} + 2\frac{R^*}{\theta}\tau as\sqrt{\theta^* s}\tanh\sqrt{\theta^* s} +$$

$$\tau a\frac{R^{*2}}{\theta^*}s\tanh^2\sqrt{\theta^* s} \qquad (6.4.4)$$

Since equation 6.4.4 must be true for all values of s, we can equate coefficients. From coefficients of $\sqrt{\theta s}\tanh\sqrt{\theta s}$, it is apparent that

$$\theta^* = \theta \qquad (6.4.5)$$

and

$$R^* = \tau R/a+\tau \qquad (6.4.6)$$

Equating the higher order coefficients we get:

$$\frac{R}{\theta}\tau as\sqrt{\theta s}\tanh\sqrt{\theta s} = 2a\tau s\frac{R^*}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s} +$$

$$\frac{a\tau}{\theta}R^{*2}s\tanh^2\sqrt{\theta s} \qquad (6.4.7)$$

Rearranging terms, and substituting 6.4.6, we get,

$$\frac{(a-\tau)(a+\tau)}{\tau^2 R} = \frac{\tanh\sqrt{\theta s}}{\sqrt{\theta s}} \qquad (6.4.8)$$

Since a, τ, R, the left side of equation 6.4.8, is independent of s, the right side must also be independent of s. Therefore $\Theta = 0$. Then this case reduces to a stretch:

$$p(s) = Rs \qquad (6.4.9)$$

6.5 MEASURABILITY OF R AND RELATIVE R's

We have in the previous two sections (6.3 and 6.4), shown that for an extravascular space transfer function of a stretch with an unknown filter, there are cases when the system has multiple roots. However, if the system is a reflected diffusor, it does not have multiple roots. We have now completed an experiment with a filter, before and after the diffusing bed. For the moment, let us assume a perfect experiment having $h_{MN}(t)$ and $h_{MD}(t)$ without any truncation or any experimental errors, and of course, the corresponding $h_{MN}(s)$ and $h_{MD}(s)$ are in hand. We will now try to determine what estimates of R are available.

1. For A Stretch

In the case of a stretch $p(s)=Rs$, estimates of R are

available from the cumulants of $h_{MN}(t)$ and $h_{MD}(t)$. These estimates are inherently underestimates of R because the cumulants of the filter are not separable from the cumulants of $h_N(t)$ and $h_D(t)$.

The estimates one gets from the first two cumulants are given by:

$$M_{MN}=M_N+M_F \qquad (6.5.1)$$

and

$$M_{MD} = M_N(1+R) + M_F \qquad (6.5.2)$$

Thus:

$$R = \frac{M_{MD} - M_{MN}}{M_N} \geq \frac{M_{MD} - M_{MN}}{M_{MN}} \qquad (6.5.3)$$

Similarly in terms of Variances:

$$V_{MN} = V_F + V_N \qquad (6.5.4)$$

$$V_{MD} = V_F + V_N(1+R)^2 \qquad (6.5.5)$$

Thus:

$$R = \left(\frac{V_{MD} - V_{MN}}{V_N} + 1\right)^{\frac{1}{2}} - 1 \qquad (6.5.6)$$

The only variance available to us in V_{MN}, thus:

$$R \geq \left(\frac{V_{MD} - V_{MN}}{V_{MN}} + 1 \right)^{1/2} - 1 \qquad (6.5.7)$$

Or

$$R \geq \left(\frac{V_{MD}}{V_{MN}} \right)^{\frac{1}{2}} - 1 \qquad (6.5.8)$$

In equation 6.5.3, the equality is only true if there is no filter or a good estimate of R is achieved if M_F is small. However, 6.5.8 can give the exact estimate of R even though M_F is large. This is the case where the filter is a pure delay, M_F takes the value of the delay and V_F is zero.

It would be wrong to assume that for all cases, equation 6.5.8 gives a better estimate of R than does equation 6.5.3. When the delay is in the transfer function of the capillary space, the estimate by equation 6.5.3 may be better.

To illustrate what estimates of R we get from equations 6.5.3 and 6.5.8 for various transfer functions, let us consider two cases:

Example 1

$$h_F(s) = e^{-s}, \quad h_N(s) = \frac{1}{1+s}, \quad h_{MN}(s) = \frac{e^{-s}}{1+s},$$

$h_D(s) = \frac{1}{1+2s}$, $h_{MD}(s) = \frac{e^{-s}}{1+2s}$ and R=1

This case does not have multiple roots, as the transfer function of the filter does not have the same form as $h_N(s)$. The cumulants of the measured transfer functions are:

$M_{MN}=2$, $M_F = 1$, $M_N = 1$, $M_{MD} =3$, $M_D = 2$, $V_{MN} = 1$

$V_F = 0$, $V_N = 1$, $V_{MD} = 4$, $V_D =1$

Estimate of R from equation 6.5.3

$$R \geq \frac{M_{MD}-M_{MN}}{M_{MN}} = \frac{3-2}{2} = 0.5$$

From equation 6.5.8

$$R \geq \left(\frac{V_{MD}}{V_{MN}}\right)^{\frac{1}{2}} - 1 = \left(\frac{4}{1}\right)^{\frac{1}{2}} - 1 = 1$$

Example 2

$h_F(s) = \frac{1}{1+2s}$, $h_N(s) = \frac{1}{1+s}$. $h_{MN} = \frac{1}{(1+2s)(1+s)}$, $h_D = \frac{1}{1+4s}$,

$h_{MD}(s) = \frac{1}{(1+2s)(1+4s)}$, and R =3

This case has multiple roots. An $R^* = 1$ would produce the same $h_{MD}(s)$ if one had assumed that $h_N(s) = \frac{1}{(1+2s)(1+s)}$ without any filter.

The cumulants of $h_{MN}(t)$ and $h_{MD}(t)$ are:

$$M_F = 2,\ M_N = 1,\ M_{MN} = 3,\ M_D = 4,\ M_{MD} = 6,\ V_F = 4,\ V_N = 1,$$

$$V_{MN} = 5,\ V_D = 16,\ V_{MD} = 20$$

Estimate of R from 6.5.3

$$R \geq \frac{6-3}{3} = 1$$

Estimate of R from 6.5.8

$$R \geq \left(\frac{20}{5}\right)^{\frac{1}{2}} - 1 = 1$$

In this case both equations gave the same estimate of R, namely that of R^*. We do not mean to imply that for all cases where there are multiple roots that one can use the moments to find their values. If the filter had the transfer function, $h_f = 1/(1+3s)(1+2s)$ instead of $1/1+2s$, the system would still have multiple roots, yet the estimates of R from equations 6.5.3 and 6.5.8 would be 0.5 and 0.44 respectively.

This third example is a case where the means give the better estimates of R rather than the variances.

We could generalize by saying that the best estimate of R is obtained by the largest value of computed from equations 6.5.3 and/or 6.5.8.

It is impossible for one to establish an upper bound in R without having firm knowledge about the transfer function of the capillary space. In many cases, it may also be necessary to have information about the filter's transfer function. If we consider the transfer function of well mixed compartments, the variance of the capillary space is related to its mean by:

$$\frac{M_1^2}{V_n} \leq n \qquad (6.5.9)$$

where n is the number of the well mixed compartments in series. Equation 6.5.9 is only usable if n=1, where the inequality turns into an equality and the locus of R values can be established by choosing various values of V_n between

zero and V_{MN} For other values of n, the equality is only true if the compartments are of equal size.

It should be noted that even if n has an integer value, M, we are not justified in saying that this value M is the number of well mixed compartments in series, if M exceeds unity.

To illustrate how one could establish the unfiltered value of R, knowing that the capillary space transfer function is one well mixed compartment, we will use example 2. The calculated values of R for various values of V_N are tabulated in example 3.

In example 3, knowing that $M_N 2/V_N = 1$ and that estimates of R given by both equations 6.5.3 and 6.5.8 must be equal, we can proceed to find the second root, namely R=3. With multiple unequal tanks in series this would not have been possible unless the value of M_N^2/V_N is known.

Example 3

Given:	$V_{MN} = 5$	$M_{MN} = 3$		$V_{MD} = 20$	$M_{MD} = 6$
Assume V_N	$M_N = \sqrt{V_N}$	V_F	M_F	6.5.3 R	6.5.8 R
5	$\sqrt{5}$	0	$3-\sqrt{5}$	$.6\sqrt{5}$	1
4	2	1	1	1.5	$.5\sqrt{19} - 1$
3	$\sqrt{3}$	2	$3-\sqrt{3}$	3	$\sqrt{6} - 1$
2	$\sqrt{2}$	3	$3-\sqrt{2}$	$1.5\sqrt{2}$	$0.5\sqrt{34} - 1$
1	1	4	2	3	3
3/4	$\sqrt{3}/2$	$4\frac{1}{4}$	$3-\sqrt{3}/2$	$2\sqrt{3}$	$\sqrt{21} - 1$
$\frac{1}{2}$	$\sqrt{2}/2$	$4\frac{1}{2}$	$3-\sqrt{2}/2$	$3\sqrt{2}$	$\sqrt{31} - 1$
$\frac{1}{4}$	$\frac{1}{2}$	43/4	$2\frac{1}{2}$	6	$\sqrt{61} - 1$

If one would have used the pseudo p(s) method on Example 3, the false R*=1 would have been found since the pseudo p(s) would be equal to s. Similarly, for example 1, the slope of the pseudo 1/p(s) vs. 1/s would be 0.5 for large values of 1/s. With the knowledge that p(s) has the form of a stretch (Rs), one could immediately see from the shape of the pseudo p(s) curve, that the system is filtered.

2. R Estimates For Reflected Diffusor And Well Mixed Compartments

In the cases where the extravascular space has a transfer function, other than a stretch, such as a reflected diffusor or a well mixed compartment, one has to settle for equation 6.5.3 as the best lower bound of R. Equation 6.5.8 cannot be used to estimate R because the variances are functions not only of R but also of λ and θ . Without a filter, one has an upper bound on R, but with a filter one can no longer be certain that the value of R obtained from equation 6.5.8 is an upper bound, particularly if the filter has a large residence time.

3. The Effect Of Filters On The Accuracy of R Estimates

In addition to the inherent bias of filters in giving underestimates of R, the filter can also diminish the accuracy of the estimate of R. The term accuracy is used in the context of chapter 4, where truncation error and experimental errors are discussed. Filters have no effect on experimental errors (mismeasurements), namely inaccurate weighings and indistinguishability between two concentrations. However, certain

types of filters will affect the truncation point.

A well mixed compartment which adds to the dispersion of the system, lowers the measurable range of the transfer functions $h_{MN}(t)$ and $h_{MD}(t)$, leading to an extra error in the moments. Therefore, lower estimates of R result than in an unfiltered experiment. On the other hand, pure delays do not have any effect on truncation error, and merely cause underestimates due to their presence.

6.6 THE EFFECT OF FILTERS ON THE ESTIMATES OF PERMEABILITY

As was the case with the estimates of R, we treat the measured transfer function as if it were the transfer function for the capillary space. Thus, for the MEF method λ is estimated by an equation of the form:

$$\lambda_m = \lim_{t \downarrow t_a} \frac{1}{t} \ln \mathcal{R}(t) \qquad (6.6.1)$$

where $\mathcal{R}(t) = h_{MN}(t) / h_{MO}(t)$

$$h_{MD}(s) = h_{MN}(s+p(s)) \qquad (6.6.2)$$

and

$$\lambda_m = \lim_{s \uparrow \infty} p_m(s) \qquad (6.6.3)$$

However, unlike the case of R, the estimate of λ is affected by the nature of the transfer function of the filter and the transfer function of the capillary space. In order to treat results in a systematic manner, we must treat the system by various cases. We will first consider a capillary space which has a transfer function of a pure delay, $h_N(s) = e^{-\mu s}$, and the filter having the transfer function of a delay, and the second case where the filter's transfer function is a well mixed compartment. We will then proceed to consider the effect of the same two filters on a capillary space whose transfer function is that of a well mixed compartment.

A. Capillary Space Of A Pure Delay - Filter Pure Delay

The capillary space transfer is given by

$$h_N(s) = e^{-\tau s} \tag{6.6.4}$$

and the filter transfer function

$$h_f(s) = e^{-\mu s} \tag{6.6.5}$$

Therefore, the measured transfer function is

$$h_{MN}(s) = e^{-(\tau + \mu)s} \tag{6.6.6}$$

and for the diffusible tracer

$$h_{MD}(s) = e^{-\mu s}\, e^{-\tau (s+p(s))} \tag{6.6.7}$$

If we consider the estimate of λ by the MEF method, we find that instead of an appearance time of τ, we measure an appearance time of $\tau+\mu$. However, $\mathcal{R}(t+\mu)=\mathcal{R}(t)$, the $\mathcal{R}$ for the unfiltered case. Therefore

$$\lambda_m = \lambda\tau/(\tau+\mu) \tag{6.6.8}$$

The same result is obtained by using transforms. This can be shown using equations 6.6.2, 6.6.3 and 6.6.7. Thus,

$$e^{-(\tau+\mu)(s+\rho_m(s))} = e^{-\mu s} e^{-\tau(s+\rho(s))} \tag{6.6.9}$$

Solving for p_m (s) we obtain

$$\rho_m(s) = \tau/\tau+\mu\ \rho(s) \tag{6.6.10}$$

and

$$\lim_{s\to\infty} \rho_m(s) = \frac{\tau\lambda}{\tau+\mu} \tag{6.6.11}$$

Two significant results are obtained from equations 6.6.8, 6.6.11 and 6.6.10. First relative λ's are sustained. That is, in a three tracer experiment, where capillary space transfer function is the same for both diffusible tracers, namely $e^{-\mu s}$, the relative λ's (λ_1/λ_2) are maintained because the scale factor $\tau/\tau+\mu$ cancels out. Second, the shape of

p(s) is not distorted. That is, if for example, the extravascular space transfer function is that of a well mixed compartment, $1/P_M(s)$ remains linear with respect to 1/s. The same will hold true for a reflected diffusor. Thus, the nature of the behavior of the extravascular space in principle, if not in practice, can be obtained.

B. Capillary Space - Pure Delay, Filter-Well Mixed Compartment

In this case, the capillary space transfer function is given by equation 6.6.4 and the filter transfer function is given by

$$h_f = \frac{1}{1+\mu s} \qquad (6.6.12)$$

The MEF method gives the correct estimate of λ because $\mathcal{L}(ta)$ is unaffected by a well mixed compartment.

$$h_{MN}(ta) = 1/\mu \qquad (6.6.13)$$

$$h_{MD}(ta) = 1/\mu\, e^{-\lambda t_a} \qquad (6.6.14)$$

at the appearance time, ta, $\mathcal{L}(ta) = e^{-t_a\lambda}$. Thus, the MEF method predicts the correct value of λ .

If we try to estimate λ from the Laplace transform, we find

$$\frac{e^{-\tau(s+\rho_M(s))}}{1+\mu(s+\rho_M(s))} = \frac{e^{-\tau(s+\rho(s))}}{1+\mu s} \qquad (6.6.15)$$

If we rearrange equation 6.6.15 and take the limit as approaches infinity, we find

$$\lim_{s\uparrow\infty} e^{-\tau(\rho_M(s)-\rho(s))} = \frac{1+\mu(s+\rho_M(s))}{1+\mu s} \qquad (6.6.16)$$

If we assume $p_M(s)$ approaches a finite limit as $s \uparrow \infty$, then the left hand side of equation 6.6.16 approaches unity. Therefore, the right hand side also approaches unity and $p_M(s)$ approaches $p(s)$, namely λ.

It should be noted here that although the value of λ is conserved and of course relative λ's, $p_M(s)$ no longer has the same general shape as does $p(s)$ and the information contained in $p(s)$ about the extravascular space is lost.

C. General Information About Delays

When one takes the Laplace transform of the measured

transfer function for the diffusible and non-diffusible tracers, a convolution which is present in the time domain is transformed into a simple product. Therefore, the quotient of the non-diffusible tracer's transfer function divided by the diffusible trader's transfer function:

$$\frac{h_{MN}(s)}{h_{MD}(s)} = \frac{h_N(s)}{h_D(s)} \qquad (6.6.17)$$

and is independent of the filter's transfer function. For the case, where the capillary space is pure delay

$$\ln\left(h_{MN}(s)/h_{MD}(s)\right) = \tau\rho(s) \qquad (6.6.18)$$

and

$$\underset{s\uparrow\infty}{\text{LIMIT}} \ln\left(h_{MN}(s)/h_{MD}(s)\right) = \tau\lambda \qquad (6.6.19)$$

Two things are again evident, relative λ's and the shape of p(s) are maintained. However, unlike our previous approach of finding a pseudo p(s), $p_M(s)$, where in certain instances, the $p_M(s)$'s did not have the same shape as p(s), equation 6.6.18 is independent of the filter's transfer function and so the shape of p(s) is maintained. On the other hand, the estimate of R and λ, obtained from the use of equations 6.6.18 and

6.6.19 may be poorer than those obtained from the pseudo p(s), except for the case where τ is near unity.

D. Capillary Space Behavior Of A Well Mixed Compartment, Filter A Pure Delay

The Capillary space transfer function is given by

$$h_N(s) = 1/1+\tau s \qquad (6.6.20)$$

and the filter's transfer function is given by

$$h_f(s) = e^{-\mu s} \qquad (6.6.21)$$

Both MEF and pseudo p(s) methods predict $\lambda = 0$. The MEF method predicts $\lambda = 0$ because $\mathcal{R}(t)$ is equal to unity at the appearance time. The delay of the filter changes the appearance time from zero to μ and λ_M is given by

$$\lambda_M = \lim_{t \uparrow \mu} \frac{1}{t} \ln \mathcal{R}(t) = \frac{1}{\mu} \ln(1) = 0 \qquad (6.6.22)$$

Analagously, the pseudo p(s) method also predicts zero. The governing equation is given by

$$e^{-\mu \rho_M(s)} = \frac{1+\tau(s+\rho_M(s))}{1+\tau(s+\rho(s))} \qquad (6.6.23)$$

If we assume that $p_M(s)$ has a finite limit, $e^{-\mu \rho_M(s)}$ must approach unity as $s \uparrow \infty$ and since $\mu \neq 0$, $p_M(s)$ must approach zero. Although we have derived the result that $p_M(s)$ approaches zero as $s \uparrow \infty$, using the transfer function for a single well mixed compartment, the same result would have been obtained if the capillary space transfer function were N well mixed compartments in series. It is immediately evident that one has better estimates of λ than zero. We will discuss later what quantitative estimates of λ are available.

Relative λ 's as before are still maintained

$$\frac{\lambda_{1M}}{\lambda_{2M}} = \lim_{t \uparrow \mu} \frac{\ln \Omega_1(t)}{\ln \Omega_2(t)} \qquad (6.6.24)$$

which is indeterminate as $\Omega(t)$ approaches unity as $t \uparrow \mu$. Applying L'Hospitals rule one gets

$$\frac{\lambda_{1M}}{\lambda_{2M}} = \frac{d\Omega_1(t)}{dt} \Big/ \frac{d\Omega_2(t)}{dt} \Big|_{t \uparrow \mu} \qquad (6.6.25)$$

which in reality is λ_1/λ_2. Similarly, since $p_M(s)$ approaches zero

$$\frac{\lambda_{1M}}{\lambda_{2M}} = \frac{d\rho_{M_1}(s)}{ds} \Big/ \frac{d\rho_M(s)}{ds} \Big|_{s \uparrow \infty} = \frac{\lambda_1}{\lambda_2} \qquad (6.6.26)$$

Some typical values are tabulated in Table 6.1 for a case where $\mu = \tau = 1$. Relative λ's calculated are within 10% of the actual value. It should be noted that the information contained in the shape of p(s) curve has been lost.

E. Capillary Space - - Well Mixed Compartment Filter--Well Mixed Compartment

The corresponding transfer functions for this case are:

$$h_N(s) = 1/1 + \tau s \qquad (6.6.27)$$

$$h_f(s) = 1/1 + \mu s \qquad (6.6.28)$$

The MEF method predicts $\lambda_m = \lambda$, while the pseudo p(s) method predicts $\lambda/2$. We will now proceed to demonstrate these results.

In the time domain

$$h_{MN}(t) = \frac{e^{-\frac{1}{\mu}t} - e^{-\frac{1}{\tau}t}}{\mu - \tau} \qquad \mu \neq \tau \qquad (6.6.29)$$

and

$$h_{MD}(t) = \frac{1}{\mu\tau}\left[\frac{C e^{-At}}{\frac{1}{\mu} - A} + \frac{D e^{-Bt}}{\frac{1}{\tau} - B} - e^{-\frac{1}{\mu}t}\left(\frac{C}{\frac{1}{\mu} - A} + \frac{D}{1/\mu + B}\right)\right] \qquad (6.6.30)$$

TABLE 6.1 ESTIMATES OF RELATIVE λ 'S

λ	Value $1/P_M(s)$	Slope $1/P_M(s)$	Estimate of Relative λ 's
.5	23.549	1.264	0.501
1	12.445	.634	1.0
2	6.894	.313	2.03
10	2.455	.0572	11.08

The constants A,B,C,D are defined in Table 2.1. Both $h_{MN}(t)$ and $h_{MD}(t)$ are equal to zero at the appearance time, t = 0, therefore, their first derivative is required to evaluate $\Omega(0)$, which is equal to unity. The second derivatives are then required to evaluate λ . Thus

$$\lambda_m = \left. \frac{\frac{d^2 h_{MN}(t)}{dt^2} - \frac{d^2 h_{MD}(t)}{dt^2}}{\frac{d h_{MD}(t)}{dt}} \right|_{t=0} \qquad (6.6.31)$$

Taking the second derivative of equations 6.6.29 and 6.6.30 and substituting the results into equation 6.6.31, one gets

$$\lambda_m = \frac{-\frac{1}{\mu^2\tau} - \frac{1}{\mu\tau^2} + \frac{1}{\mu^2\tau} + \frac{CA + DB}{\mu\tau}}{\mu\tau} = \lambda \qquad (6.6.32)$$

If we now look at the results using pseudo $p(s)$, we find that

$$(1+\mu(s+\rho_M(s))(1+\tau(s+\rho_M(s)) = (1+\mu s)(1+\tau(s+\rho(s)) \qquad (6.6.33)$$

or

$$\rho_M^2(s) + \left(\frac{1}{\mu} + \frac{1}{\tau} + 2s\right)\rho_M(s) = \left(\frac{1}{\mu} + s\right)\rho(s) \qquad (6.6.33a)$$

Solving for $\rho_M(s)$ one finds:

$$\rho_M(s) = \frac{1}{2}\left[-\left(\frac{1}{\mu} + \frac{1}{\tau} + 2s\right) + \left[\left(\frac{1}{\mu} + \frac{1}{\tau} + 2s\right)^2 + 4\left(\frac{1}{\mu} + s\right)\rho(s)\right]^{1/2}\right] \qquad (6.6.34)$$

If we take the bionomial expansion of the square root of the discriminant and consider only large values of s, we find

$$\rho_M(S) \approx \frac{1}{2}\rho(S) = \frac{1}{2}\lambda \qquad (6.6.34A)$$

It is apparent from equation 6.6.34, that the shape of p(s) curves is not maintained; however, relative λ 's are maintained.

The limit that $p_M(s)$ approaches $\frac{1}{2}p(s)$ is particular to this case. In general, however

$$\lim_{S \uparrow \infty} \rho_M(S) = \frac{i\lambda}{i+j} \qquad (6.6.35)$$

where i is the number of well-mixed compartments which simulate the capillary space behavior and j is the number of well mixed compartments contained in the filter. To demonstrate this result, we will choose a case where the filter and capillary space have the same residence time. The governing equation becomes

$$(1+\tau(S+\rho_M(S)))^{i+j} = (1+\tau S)^j (1+\tau(S+\rho(S)))^i \qquad (6.6.36)$$

or

$$(1+\tau(s+\rho_M(s)))^{\mu+j} = (1+\tau s)^{\mu+j}\left(1+\frac{\tau\rho(s)}{1+\tau s}\right)^{\mu} \qquad (6.6.36A)$$

If we take μ+j, the root of equation 6.6.36A and expand it for large values of s, we find:

$$\rho_M(s) \approx \frac{\mu}{\mu+j}\rho(s) = \frac{\mu}{\mu+j}\lambda \qquad (6.6.37)$$

F. General Properties Of Well-Mixed Compartments

As we noted earlier, $h_{MN}(s)/h_{MD}(s)$ is independent of the filter's transfer function. If we take a general case

$$\frac{h_{MN}(s)}{h_{MD}(s)} = \prod_{j=1}^{n}\frac{1+\tau_j(s+\rho(s))}{1+\tau_j s} = \prod_{j=1}^{n}\left(1+\frac{\tau_j\rho(s)}{1+\tau_j s}\right) \qquad (6.6.38)$$

$$\ln\frac{h_{MN}(s)}{h_{MD}(s)} = \sum_{j=1}^{n}\ln\left(1+\frac{\tau_j\rho(s)}{1+\tau_j s}\right) \qquad (6.6.39)$$

and if p(s) approaches a finite limit, then

$$\underset{S\uparrow\infty}{\text{LIMIT}} \ln\left(\frac{h_{MN}(S)}{h_{MD}(S)}\right) = \sum_{j=1}^{n} \frac{\rho(S)}{S} =$$

$$\frac{n\rho(S)}{S} = \frac{n\lambda}{S} \qquad (6.6.40)$$

Therefore,

$$\underset{S\uparrow\infty}{\text{LIMIT}} \; S \ln\left(\frac{h_{MN}(S)}{h_{MD}(S)}\right) = n\lambda \qquad (6.6.41)$$

Unlike, the case of a pure delay, where the shape of the p(s) curve is maintained, the case of a well mixed compartment does not maintain the shape of the p(s) curve; however, relative λ's are maintained.

6.7 SUMMARY

In this chapter we have shown the effect of filters on the estimates of R and λ, and have also shown that when the transfer function of the capillary space is a pure delay, the system is indeterminate. However, the nature of the behavior in the extravascular space can be extracted from the quotient of $h_N(t)/h_D(t)$. On the other hand if the transfer function

of the capillary space is a well mixed compartment with or without a delay, this indetermancy no longer exists. In this case, multiple solutions exist for the special case of a stretch ($\rho(s)=Rs$) but not for the cases where the extravascular space has a transfer function of a well mixed compartment or a reflected diffusor. In the case of a capillary space, whose transfer function is a well mixed compartment, the nature of the diffusion process in the extravascular space cannot be extracted from the quotient of $h_N(t)/h_D(t)$. Finally for all systems where the filter is the same, relative R's and λ's are maintained. This is true for electrolytes such as Na ion and K ion. However in the case of radioactive gases where diffusion occurs in the arteries and veins, the filter's transfer function is no longer the same, and the comparison becomes invalid.

CHAPTER 7. EXPERIMENTAL DATA

7.1 Introduction

In this chapter, we will apply the theories discussed in the previous chapters to multiple diffusible tracers injected into a heart. These experiments, performed by Doctors Bassingthwaighte and Yipinstoi, were made with and without drugs. The values of R and λ , shown in Tables 7.3A and 7.3 B, correspond to the estimation techniques described in Tables 3.1 and 3.2. The numerical procedures used to calculate each value of R and λ is described in Appendix A.

7.2 Methodology

The heart data supplied to us was in the tabular form. The range of measurability of this data was about three decades. Monoexponential extrapolation was used to calculate the normalized concentrations to values of the order of 10^{-6}, before truncating the data. The data was then entered into our computer program, where various values of R and λ were calculated.

In order to check the validity of our results, we attempted to reconstruct $h_D(t)$ from $h_N(t)$, assuming that extravascular space had the transfer function of a well mixed compartment.

$$P(S) = \frac{RS}{\frac{RS}{\lambda} + 1} \qquad (7.2.1)$$

and therefore:

$$h_D(t) = e^{-\lambda t} + \int_0^t h_N(\tau)\, e^{-\lambda\tau} e^{-\frac{\lambda}{R}(t-\tau)} \sqrt{\frac{\lambda^2\tau}{R(t-\tau)}}\, I_1\left(2\sqrt{\frac{\lambda^2\tau(t-\tau)}{R}}\right) d\tau \qquad (7.2.2)$$

7.3 Discussion

Tables 7.3A and 7.3B summarize the values R and λ calculated by the various methods. In addition to flow conditions and the weight of each heart, the recoveries of the diffusible and non-diffusible tracer is also given. With the exception of experiment 29059 curve 3, the recovery of the non-diffusible tracer exceeded 90%, while the recovery of the diffusible tracer averaged about 80%, with a high of 99.6% and a low of 58.5%.

The Modified Extraction Factor Method, (MEF) and the Straight Line Reciprocal P(S) (SLRP) give the highest values of λ. Of the conventional methods, the min $\mathcal{R}(t)$ method, gave the

TABLE 7.1A

The Capillary Space Behaves As Two Well Mixed Compartments In Series $(h_N(t) = t^{e-t})$

Moments Of Non-Diffusible Tracer

Tracer	Case A	Case B	Case C
Recovery	95%	99%	100%
Mean	1.7041	1.9223	2.0
2nd Moment	4.1830	5.3840	6.0
3rd Moment	12.3236	18.9935	24.0
4th Moment	40.7044	78.0904	120.0
Truncation Time	4.7439	6.6384	∞

TABLE 7.1B

The Capillary Space Behaves As Two Well Mixed Compartments In Series, The Extravascular Space Behaves As A Single Well Mixed Compartment

Moments Of The Diffusible Tracer

$$h_D(t) = \frac{36}{49} e^{-6t}\left(t - \frac{2}{35}\right) + \frac{e^{-t/6}}{49}\left(t + \frac{72}{35}\right)$$

	Case A	Case B	Case C
Tracer Recovery	95%	99%	100%
Mean	8.648	9.885	10.333
2nd Moment	117.699	156.327	176.832
3rd Moment	2132.09	3350.64	4315.08
4th Moment	36409.16	75247.82	122094.36
Truncation Time	26.6569	38.0144	∞

$$h_D(t) = \frac{12100}{12321} e^{-1.1t}\left(t - \frac{2}{111}\right) + \frac{e^{-t/11}}{12321}\left(t + \frac{24200}{111}\right)$$

	Case A	Case B	Case C
Tracer Recovery	95%	99%	100%
Mean	2.431	3.356	3.818
2nd Moment	13.178	35.570	58.231
3rd Moment	117.64	684.78	1882.69
4th Moment	1335.36	16314.47	85714.94
Truncation Time	16.2462	34.7498	∞

highest estimates of λ which were 50% to 100% lower than the values of λ calculated by SLRp. The Crone Relative Area Methods (CRA) gave estimates which were somewhat lower than the Min $\mathcal{R}$ (t) method. In many cases, the estimates of λ , were independent of tp. As expected, the conventional Extraction Factor Method, (MJY) gave the lowest values of λ.

The use of the first two moments $h_N(t)$ and $h_D(t)$ to calculate R and λ by equations 3.19 and 3.21, was impossible. The major contributing factor to the uselessness of moments, was that the recoveries of the diffusible and non-diffusible tracer were different. Table 7.1A shows the first four moments of a theoretical non-diffusible, at 95%, 99% and 100% tracer recovery. Similarly Table 7.1B shows these moments for the two theoretical diffusible tracer cases. It is quite evident that for small values of R and λ , the truncation of the diffusible tracer curves, gives serious underestimates of the moments. Table 7.2 shows the effect of using two different tracer recoveries to calculate parameters. For small values of R and λ , the underestimates of these parameters is by a factor of two to three; while for the larger values of R and λ, the underestimates were of the order of 20% to 50%.

TABLE 7.2 - Stirred Tank Parameters Calculated From Truncated Moments

Capillary Space No. of Well Mixed Compartments In Series	2	2
Actual R	.909	4.167
Actual	.0909	4.167
Non-Diffusible Tracer Recovery	99%	99%
Diffusible Tracer Recovery	95%	95%
Calculate R	0.265	3.498
%Error	243.	20.0
Calculated	0.0307	2.805
% Error	196	48.5

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Reconstructions of $h_D(t)$, assuming a two parameter model, for the extravascular space (Equations 7.2.1 and 7.2.2) is shown in Tables 7.4 through 7.6. The reconstructions of $h_D(t)$, were made, using the Min Ω (highest conventional estimate of λ) and the SLRP method. The reconstruction of $h_D(t)$, using the higher values of ,predicted by the SLRP method, were significantly better. The value of R predicted by the SLRP method was used in both reconstructions as the Min Ω method does not predict any values of R.

Reconstruction of the water data was feasible. From the raw data, it is obvious that shunting had occurred, since some values of $h_D(t)$ exceeded the values of $h_N(t)$. In these cases, none of the models discussed is valid, and the predicted values of R and λ have no real significance. Therefore, the reconstruction is also meaningless.

The drug used in the experiments was not supposed to affect the permeability of the various substances. The values of λ calculated confirmed this result.

7.4 Conclusion

In this chapter, the various estimation techniques were applied to experimental data. The methods that we have developed give significantly higher values for λ than do the conventional methods. (See Tables 3.1 and 3.2- Chapter 3.) As discussed previously, this result is not unexpected. A summary of the calculated values for radioactive sodium chloride, water, glucose and potassium chloride are given in Tables 7.3A and 7.3B. Tables 7.4 - 7.6 show reconstructions of $h_D(t)$, assuming that the extravascular space transfer function, was a well mixed compartment. (Equations 7.2.1 and 7.2.2).

The highest estimates of λ were obtained using the Max ρ and the SLRP methods. The SLRP method also gives an estimate of R. These values of R and λ were used in the reconstruction of $h_D(t)$. In spite of the fact that all real data is filtered, the reconstruction of the measured $h_D(t)$ was excellent. The best method was found to be the SLRP method, where the error was less than 20% for the unextroplated data.

Another significant conclusion of this analysis is that moment methods may be virtually useless. The data supplied had been truncated and large errors and inconsistancies resulted.

The data for radioactive water was not reproducible. Significant shunting was measured, as many of the values of $h_D(t)$ exceeded those measured for $h_N(t)$. For all cases where shunting is significant, none of the models presented in this thesis are valid.

It is evident from the excellent results obtained in the reconstruction of $h_D(t)$, using the λ's and R's calculated by the SLRp method we developed, that some extra effort is justified in calculating these better values.

ABLE 7.3A-λAND R CALCULATED BY THE METHODS WE HAVE DEVELOPED - NON DIFFUSIBLE TRACER IS I - ALBUMIN

e Tracer	EXP #	Curve #	Drugs	Flow ML/MIN	Heart Wt,GM	$\int_0^\infty h_N(t)dt$	$\int_0^\infty h_D(t)dt$	MAX ρ λ	SLR ρ λ	R	MEF λ	MOMENTS λ	R	M_N
	30037	6	No	310.1	102	0.978	0.862	0.0733	0.0593	0.651	0.0603	0.0049	0.101	10.69
	7047	3	No	144.8	71	1.005	0.996	0.1080	0.0729	0.597	0.0970	0.0595	1.027	11.00
	7059	1	No	38	56.9	0.909	0.879	0.0637	0.0589	0.492	0.0562	0.0330	0.329	24.89
	7059	3	Yes	97.5	59.4	0.952	0.784	0.0606	0.0547	0.374	0.0547	--	--	17.82
	29059	1	No	105	47.2	0.923	0.779	0.3660	0.3520	1.179	0.4610	0.603	0.774	11.49
	29059	3	No	79	55.4	0.530	0.506	0.2290	0.2490	1.024	0.2850	0.123	1.019	10.24
	29059	9	Yes	142	60.4	0.936	0.915	0.4270	0.4650	1.781	0.6210	0.265	1.958	8.22
se	29059	1	No	105	47.2	0.923	0.834	0.0770	0.0522	0.391	0.0677	0.0035	0.078	11.49
se	29059	3	No	79	55.4	0.530	0.533	0.0407	0.0366	0.316	0.0457	0.0523	0.336	10.24
se	29059	9	Yes	142	60.4	0.936	0.846	0.0670	0.0487	0.819	0.0494	0.0148	0.306	8.22
se	7059	1	No	38	56.9	0.909	0.876	0.0476	0.0395	0.426	0.0353	0.0223	0.340	24.89
se	7059	3	Yes	97.5	59.4	0.952	0.767	0.0486	0.0409	0.365	0.0491	--	--	17.82
se	7059	1	No	38	56.9	0.909	0.895	0.0318	0.0266	0.257	0.0255	0.0196	0.221	24.89
se	7059	3	Yes	97.5	59.4	0.952	0.806	0.0325	0.0257	0.268	0.0286	--	--	17.82
ride	29059	1	No	105	47.2	0.923	0.647	0.1790	0.1200	0.761	0.222	--	--	11.49
ride	29059	3	No	79	55.4	0.530	0.300	0.0960	0.0923	1.199	0.180	--	--	10.24
ride	29059	9	Yes	142	60.4	0.936	0.585	0.1070	0.0980	3.369	0.122	--	--	8.22
ride	7059	1	No	38	56.9	0.909	0.781	0.0683	0.0614	0.630	0.0619	0.0079	0.251	24.89
ride	7059	3	Yes	97.5	59.4	0.952	0.789	0.0653	0.0591	0.413	0.0692	--	--	17.82

le 7.3B-λ's FOR APPROXIMATE METHODS--NON-DIFFUSIBLE TRACER IS I-ALBUMIN

fusible cer	Exp No.	Curve No.	Drugs	MJY	MinΩ(t)	CRONE RELATIVE AREA t_p=peak time	t_p=MinΩ(t) time	t_p=crossover time
Sodium	30037	6	No	0.0314	0.0451	0.0371	0.0371	0.0312
Sodium	7047	3	No	0.0265	0.0341	0.0304	0.0304	0.0265
Sodium	7059	1	No	0.0195	0.0345	0.0295	0.0310	0.0214
Sodium	7059	3	Yes	0.0206	0.0256	0.0233	0.0245	0.0158
Water	29059	1	No	0.244	0.244	0.143	0.0577	0.083
Water	29059	3	No	0.234	0.234	0.176	0.076	0.097
Water	29059	9	Yes	0.459	0.459	0.288	0.094	0.199
4 Glucose	29059	1	No	0.0236	0.0323	0.0277	0.0277	0.0245
4 Glucose	29059	3	No	0.0268	0.0352	0.0287	0.0306	0.0284
4 Glucose	29059	9	Yes	0.0346	0.0422	0.0319	0.0313	0.0341
Glucose	7059	1	No	0.0148	0.0219	0.0209	0.0197	0.0165
Glucose	7059	3	Yes	0.0183	0.0200	0.0182	0.0182	0.0137
4 Sucrose	7059	1	No	0.0083	0.0162	0.0136	0.0140	0.0111
4 Sucrose	7059	3	Yes	0.0107	0.0161	0.0125	0.0123	0.0096
2 Chloride	29059	1	No	0.0773	0.0820	0.0571	0.0675	0.0385
2 Chloride	29059	3	No	0.105	0.122	0.0817	0.0882	0.0556
2 Chloride	29059	9	Yes	0.0310	0.0871	0.0805	0.0805	0.0751
2 Chloride	7059	1	No	0.0237	0.0359	0.0318	0.0299	0.0238
2 Chloride	7059	3	Yes	0.1056	0.122	0.0817	0.0881	0.0555

CASE 7059-CURVE 1-NO DRUGS
NON DIFFUSIBLE TRACER IS I-131 ALBUMIN
DIFFUSIBLE TRACER IS HYDROGEN-3 GLUCOSE

THE VALUE OF LAMBDA IS 0.021900
THE VALUE OF R IS 0.426000

TIME	G CALC	G ACT	PCT ERROR
11.4000	0.0	0.0	0.0
12.0000	0.001713	0.001620	5.725
13.0000	0.007299	0.006080	20.052
14.0000	0.010777	0.008990	19.875
15.0000	0.017280	0.014080	22.729
16.0000	0.021091	0.017620	19.700
17.0000	0.025039	0.020190	24.019
18.0000	0.026767	0.021970	21.835
19.0000	0.029906	0.024750	20.832
20.0000	0.031866	0.026480	20.341
21.0000	0.032364	0.027220	18.900
22.0000	0.032653	0.028070	16.326
23.0000	0.032576	0.028100	15.928
24.0000	0.031299	0.027630	13.280
25.0000	0.030629	0.027550	11.177
26.0000	0.030231	0.027300	10.737
27.0000	0.028284	0.025740	9.885
28.0000	0.026367	0.024840	6.146
29.0000	0.024313	0.023700	2.586
30.0000	0.020706	0.022550	8.907
31.0000	0.020728	0.021700	4.689
32.0000	0.019105	0.020280	6.151
33.0000	0.017782	0.019530	9.828
34.0000	0.016760	0.018750	11.876
35.0000	0.015831	0.017780	12.308
36.0000	0.014930	0.017700	18.552
37.0000	0.013888	0.016430	18.301
38.0000	0.013312	0.015540	16.733
39.0000	0.012380	0.014850	19.953
40.0000	0.011741	0.014200	20.948
41.0000	0.011154	0.013550	21.481
42.0000	0.010613	0.012930	21.827
43.0000	0.010110	0.012340	22.058
44.0000	0.009648	0.011780	22.101
45.0000	0.009212	0.011240	22.016
46.0000	0.008808	0.010730	21.819
47.0000	0.008433	0.010240	21.424
48.0000	0.008081	0.009770	20.907
49.0000	0.007744	0.009320	20.356
50.0000	0.007433	0.008900	19.743
51.0000	0.007140	0.008490	18.910
52.0000	0.006858	0.008110	18.250
53.0000	0.006592	0.007740	17.420
54.0000	0.006342	0.007380	16.372
55.0000	0.006103	0.007050	15.526
56.0000	0.005876	0.006720	14.369
57.0000	0.005658	0.006420	13.458
58.0000	0.005451	0.006130	12.447
59.0000	0.005253	0.005850	11.362
60.0000	0.005063	0.005580	10.203

63.0000	0.004541	0.004850	6.812
64.0000	0.004385	0.004630	5.590
65.0000	0.004228	0.004420	4.552
66.0000	0.004080	0.004220	3.437
67.0000	0.003938	0.004020	2.077
68.0000	0.003799	0.003840	1.066
69.0000	0.003668	0.003660	0.212
70.0000	0.003542	0.003500	1.208
71.0000	0.003419	0.003340	2.360
72.0000	0.003301	0.003180	3.792
73.0000	0.003186	0.003040	4.806
74.0000	0.003078	0.002900	6.124
75.0000	0.002969	0.002770	7.187
76.0000	0.002869	0.002640	8.690
77.0000	0.002768	0.002520	9.843
78.0000	0.002674	0.002410	10.952
79.0000	0.002582	0.002300	12.263
80.0000	0.002492	0.002190	13.785
81.0000	0.002409	0.002090	15.253
82.0000	0.002323	0.002000	16.161
83.0000	0.002245	0.001910	17.548
84.0000	0.002168	0.001820	19.094
85.0000	0.002091	0.001740	20.176
86.0000	0.002007	0.001660	20.886
87.0000	0.001938	0.001580	22.685
88.0000	0.001871	0.001510	23.881
89.0000	0.001807	0.001440	25.469
90.0000	0.001743	0.001370	27.223
91.0000	0.001682	0.001310	28.407
92.0000	0.001624	0.001250	29.881
93.0000	0.001567	0.001190	31.666
94.0000	0.001513	0.001140	32.702
95.0000	0.001461	0.001090	34.029
96.0000	0.001410	0.001040	35.563
97.0000	0.001360	0.000990	37.346
98.0000	0.001312	0.000950	38.061
99.0000	0.001266	0.000900	40.623
100.0000	0.001221	0.000860	41.997
101.0000	0.001179	0.000820	43.724
102.0000	0.001137	0.000780	45.824
103.0000	0.001099	0.000750	46.515
104.0000	0.001060	0.000710	49.356
105.0000	0.001021	0.000680	50.203
106.0000	0.000985	0.000650	51.566
107.0000	0.000950	0.000620	53.291
108.0000	0.000917	0.000590	55.452
109.0000	0.000885	0.000570	55.185
110.0000	0.000853	0.000540	57.996
111.0000	0.000823	0.000520	58.323
112.0000	0.000794	0.000490	62.043
113.0000	0.000766	0.000470	63.077
114.0000	0.000741	0.000450	64.588
115.0000	0.000713	0.000430	65.877
116.0000	0.000687	0.000410	67.680
117.0000	0.000663	0.000390	70.019
118.0000	0.000639	0.000370	72.747
119.0000	0.000617	0.000350	76.203
120.0000	0.000595	0.000340	74.948
121.0000	0.000573	0.000320	78.987

124.0000	0.000515	0.000280	83.796
125.0000	0.000497	0.000270	83.995
126.0000	0.000479	0.000260	84.370
127.0000	0.000463	0.000240	92.715
128.0000	0.000445	0.000230	93.603
129.0000	0.000429	0.000220	94.837
130.0000	0.000413	0.000210	96.566
131.0000	0.000398	0.000202	96.985
132.0000	0.000383	0.000193	98.599
133.0000	0.000370	0.000184	100.938
134.0000	0.000357	0.000176	102.608
135.0000	0.000344	0.000168	104.763
136.0000	0.000332	0.000160	107.188
137.0000	0.000320	0.000153	108.913
138.0000	0.000308	0.000146	111.078
139.0000	0.000297	0.000139	113.983
140.0000	0.000287	0.000133	115.571
141.0000	0.000277	0.000127	117.721
142.0000	0.000266	0.000121	120.139
143.0000	0.000257	0.000115	123.315
144.0000	0.000248	0.000110	125.001
145.0000	0.000238	0.000105	126.880
146.0000	0.000230	0.000100	129.563
147.0000	0.000221	0.000096	130.356
148.0000	0.000213	0.0	100.000
149.0000	0.000205	0.0	100.000
150.0000	0.000198	0.0	100.000
151.0000	0.000190	0.0	100.000

CASE 7059-CURVE 1-NO DRUGS
NON DIFFUSIBLE TRACER IS I-131 ALBUMIN
DIFFUSIBLE TRACER IS HYDROGEN-3 GLUCOSE

THE VALUE OF LAMBDA IS 0.039500
THE VALUE OF R IS 0.426000

TIME	G CALC	G ACT	PCT ERROR
11.4000	0.0	0.0	0.0
12.0000	0.001397	0.001620	15.946
13.0000	0.005918	0.006080	2.738
14.0000	0.008749	0.008990	2.757
15.0000	0.013937	0.014080	1.028
16.0000	0.017064	0.017620	3.257
17.0000	0.020295	0.020190	0.521
18.0000	0.021879	0.021970	0.418
19.0000	0.024486	0.024750	1.078
20.0000	0.026261	0.026480	0.832
21.0000	0.027001	0.027220	0.811
22.0000	0.027576	0.028070	1.793
23.0000	0.027883	0.028100	0.780
24.0000	0.027361	0.027630	0.984
25.0000	0.027200	0.027550	1.287
26.0000	0.027194	0.027300	0.391
27.0000	0.026180	0.025740	1.710
28.0000	0.025131	0.024840	1.172
29.0000	0.023953	0.023700	1.066
30.0000	0.021774	0.022550	3.565
31.0000	0.021674	0.021700	0.121
32.0000	0.020627	0.020280	1.710
33.0000	0.019722	0.019530	0.985
34.0000	0.018964	0.018750	1.144
35.0000	0.018241	0.017780	2.594
36.0000	0.017525	0.017700	1.001
37.0000	0.016719	0.016430	1.757
38.0000	0.016148	0.015540	3.915
39.0000	0.015390	0.014850	3.637
40.0000	0.014770	0.014200	4.017
41.0000	0.014176	0.013550	4.623
42.0000	0.013605	0.012930	5.220
43.0000	0.013053	0.012340	5.776
44.0000	0.012525	0.011780	6.325
45.0000	0.012012	0.011240	6.872
46.0000	0.011521	0.010730	7.371
47.0000	0.011050	0.010240	7.914
48.0000	0.010596	0.009770	8.460
49.0000	0.010153	0.009320	8.941
50.0000	0.009733	0.008900	9.362
51.0000	0.009329	0.008490	9.883
52.0000	0.008935	0.008110	10.176
53.0000	0.008556	0.007740	10.548
54.0000	0.008196	0.007380	11.052
55.0000	0.007845	0.007050	11.276
56.0000	0.007510	0.006720	11.752
57.0000	0.007186	0.006420	11.939
58.0000	0.006876	0.006130	12.163
59.0000	0.006578	0.005850	12.447
60.0000	0.006291	0.005580	12.741

63.0000	0.005496	0.004850	13.318
64.0000	0.005258	0.004630	13.556
65.0000	0.005019	0.004420	13.557
66.0000	0.004797	0.004220	13.664
67.0000	0.004583	0.004020	14.016
68.0000	0.004375	0.003840	13.944
69.0000	0.004178	0.003660	14.141
70.0000	0.003990	0.003500	14.012
71.0000	0.003809	0.003340	14.049
72.0000	0.003636	0.003180	14.325
73.0000	0.003468	0.003040	14.079
74.0000	0.003312	0.002900	14.206
75.0000	0.003157	0.002770	13.982
76.0000	0.003014	0.002640	14.153
77.0000	0.002873	0.002520	14.000
78.0000	0.002741	0.002410	13.714
79.0000	0.002614	0.002300	13.632
80.0000	0.002491	0.002190	13.734
81.0000	0.002376	0.002090	13.702
82.0000	0.002264	0.002000	13.178
83.0000	0.002159	0.001910	13.030
84.0000	0.002057	0.001820	13.024
85.0000	0.001959	0.001740	12.589
86.0000	0.001864	0.001660	12.319
87.0000	0.001776	0.001580	12.433
88.0000	0.001691	0.001510	11.983
89.0000	0.001611	0.001440	11.858
90.0000	0.001533	0.001370	11.867
91.0000	0.001459	0.001310	11.348
92.0000	0.001388	0.001250	11.053
93.0000	0.001321	0.001190	10.999
94.0000	0.001257	0.001140	10.269
95.0000	0.001197	0.001090	9.794
96.0000	0.001138	0.001040	9.459
97.0000	0.001082	0.000990	9.338
98.0000	0.001029	0.000950	8.311
99.0000	0.000979	0.000900	8.725
100.0000	0.000930	0.000860	8.167
101.0000	0.000885	0.000820	7.901
102.0000	0.000841	0.000780	7.845
103.0000	0.000800	0.000750	6.715
104.0000	0.000761	0.000710	7.170
105.0000	0.000722	0.000680	6.195
106.0000	0.000686	0.000650	5.599
107.0000	0.000652	0.000620	5.157
108.0000	0.000620	0.000590	5.003
109.0000	0.000589	0.000570	3.251
110.0000	0.000559	0.000540	3.510
111.0000	0.000531	0.000520	2.163
112.0000	0.000504	0.000490	2.893
113.0000	0.000479	0.000470	1.893
114.0000	0.000456	0.000450	1.292
115.0000	0.000432	0.000430	0.458
116.0000	0.000410	0.000410	0.016
117.0000	0.000390	0.000390	0.106
118.0000	0.000369	0.000370	0.223
119.0000	0.000351	0.000350	0.144
120.0000	0.000333	0.000340	2.118
121.0000	0.000315	0.000320	1.507

124.0000	0.000270	0.000280	3.805
125.0000	0.000256	0.000270	5.497
126.0000	0.000243	0.000260	7.072
127.0000	0.000231	0.000240	3.957
128.0000	0.000218	0.000230	5.336
129.0000	0.000207	0.000220	6.385
130.0000	0.000196	0.000210	7.106
131.0000	0.000186	0.000202	8.563
132.0000	0.000176	0.000193	9.632
133.0000	0.000167	0.000184	10.230
134.0000	0.000158	0.000176	11.115
135.0000	0.000150	0.000168	11.751
136.0000	0.000142	0.000160	12.519
137.0000	0.000135	0.000153	13.477
138.0000	0.000128	0.000146	14.271
139.0000	0.000121	0.000139	14.578
140.0000	0.000115	0.000133	15.949
141.0000	0.000109	0.000127	16.855
142.0000	0.000103	0.000121	17.547
143.0000	0.000098	0.000115	17.727
144.0000	0.000093	0.000110	18.813
145.0000	0.000087	0.000105	20.219
146.0000	0.000083	0.000100	20.880
147.0000	0.000078	0.000096	22.527
148.0000	0.000074	0.0	100.000
149.0000	0.000070	0.0	100.000
150.0000	0.000066	0.0	100.000
151.0000	0.000063	0.0	100.000

CASE 7059-CURVE 1-NO DRUGS
NON DIFFUSIBLE TRACER IS I-131 ALBUMIN
DIFFUSIBLE TRACER IS CARBON-14 SUCROSE

THE VALUE OF LAMBDA IS 0.016200
THE VALUE OF R IS 0.257000

TIME	G CALC	G ACT	PCT ERROR
11.4000	0.0	0.0	0.0
12.0000	0.001833	0.001790	2.424
13.0000	0.007854	0.007820	0.432
14.0000	0.011651	0.010400	12.025
15.0000	0.018776	0.016000	17.350
16.0000	0.023019	0.020790	10.720
17.0000	0.027448	0.024630	11.443
18.0000	0.029456	0.025680	14.703
19.0000	0.033045	0.028270	16.892
20.0000	0.035341	0.031080	13.709
21.0000	0.036002	0.033390	7.824
22.0000	0.036424	0.035640	2.199
23.0000	0.036425	0.035930	1.379
24.0000	0.035045	0.034920	0.358
25.0000	0.034344	0.033900	1.311
26.0000	0.033946	0.032000	6.082
27.0000	0.031734	0.029810	6.456
28.0000	0.029534	0.027910	5.818
29.0000	0.027151	0.026340	3.080
30.0000	0.022907	0.024760	8.090
31.0000	0.022954	0.023500	2.381
32.0000	0.021028	0.022010	4.672
33.0000	0.019449	0.020840	7.150
34.0000	0.018224	0.019830	8.815
35.0000	0.017106	0.018910	10.548
36.0000	0.016014	0.017650	10.215
37.0000	0.014743	0.016510	11.982
38.0000	0.014044	0.015800	12.507
39.0000	0.012897	0.014870	15.301
40.0000	0.012113	0.014000	15.579
41.0000	0.011393	0.013210	15.950
42.0000	0.010729	0.012450	16.037
43.0000	0.010112	0.011750	16.199
44.0000	0.009546	0.011080	16.069
45.0000	0.009014	0.010450	15.927
46.0000	0.008523	0.009860	15.685
47.0000	0.008068	0.009300	15.267
48.0000	0.007643	0.008770	14.751
49.0000	0.007239	0.008270	14.241
50.0000	0.006868	0.007800	13.578
51.0000	0.006520	0.007360	12.879
52.0000	0.006190	0.006940	12.116
53.0000	0.005879	0.006550	11.407
54.0000	0.005590	0.006170	10.383
55.0000	0.005316	0.005820	9.474
56.0000	0.005059	0.005490	8.526
57.0000	0.004815	0.005180	7.586
58.0000	0.004585	0.004890	6.663
59.0000	0.004365	0.004610	5.607
60.0000	0.004158	0.004350	4.608

63.0000	0.003601	0.003650	1.365
64.0000	0.003437	0.003440	0.080
65.0000	0.003277	0.003240	1.133
66.0000	0.003125	0.003060	2.127
67.0000	0.002982	0.002890	3.173
68.0000	0.002844	0.002720	4.565
69.0000	0.002715	0.002570	5.653
70.0000	0.002592	0.002420	7.128
71.0000	0.002473	0.002280	8.467
72.0000	0.002361	0.002150	9.796
73.0000	0.002254	0.002030	11.050
74.0000	0.002152	0.001920	12.078
75.0000	0.002053	0.001860	10.368
76.0000	0.001963	0.001710	14.782
77.0000	0.001872	0.001610	16.252
78.0000	0.001788	0.001520	17.635
79.0000	0.001707	0.001430	19.402
80.0000	0.001629	0.001350	20.682
81.0000	0.001558	0.001270	22.698
82.0000	0.001486	0.001200	23.804
83.0000	0.001420	0.001130	25.687
84.0000	0.001356	0.001070	26.767
85.0000	0.001294	0.001040	24.422
86.0000	0.001215	0.000950	27.842
87.0000	0.001160	0.000900	28.901
88.0000	0.001107	0.000850	30.244
89.0000	0.001057	0.000800	32.146
90.0000	0.001009	0.000750	34.472
91.0000	0.000963	0.000710	35.568
92.0000	0.000919	0.000670	37.091
93.0000	0.000877	0.000630	39.149
94.0000	0.000837	0.000600	39.447
95.0000	0.000799	0.000560	42.683
96.0000	0.000762	0.000530	43.831
97.0000	0.000727	0.000500	45.439
98.0000	0.000693	0.000470	47.545
99.0000	0.000662	0.000440	50.410
100.0000	0.000631	0.000420	50.284
101.0000	0.000602	0.000400	50.615
102.0000	0.000575	0.000370	55.357
103.0000	0.000549	0.000350	56.859
104.0000	0.000524	0.000330	58.754
105.0000	0.000499	0.000310	60.962
106.0000	0.000476	0.000300	58.710
107.0000	0.000454	0.000280	62.129
108.0000	0.000433	0.000260	66.561
109.0000	0.000413	0.000250	65.239
110.0000	0.000394	0.000230	71.267
111.0000	0.000376	0.000220	70.898
112.0000	0.000358	0.000210	70.653
113.0000	0.000342	0.000196	74.468
114.0000	0.000327	0.000185	76.706
115.0000	0.000311	0.000174	78.810
116.0000	0.000297	0.000164	80.824
117.0000	0.000283	0.000155	82.615
118.0000	0.000270	0.000146	84.608
119.0000	0.000257	0.000138	86.314
120.0000	0.000245	0.000130	88.730
121.0000	0.000233	0.000123	89.804

124.0000	0.000203	0.000103	96.850
125.0000	0.000193	0.000097	99.415
126.0000	0.000185	0.0	100.000
127.0000	0.000176	0.0	100.000
128.0000	0.000168	0.0	100.000
129.0000	0.000160	0.0	100.000
130.0000	0.000152	0.0	100.000
131.0000	0.000145	0.0	100.000
132.0000	0.000138	0.0	100.000
133.0000	0.000132	0.0	100.000
134.0000	0.000125	0.0	100.000
135.0000	0.000120	0.0	100.000
136.0000	0.000114	0.0	100.000
137.0000	0.000109	0.0	100.000
138.0000	0.000104	0.0	100.000
139.0000	0.000099	0.0	100.000
140.0000	0.000094	0.0	100.000
141.0000	0.000090	0.0	100.000
142.0000	0.000086	0.0	100.000
143.0000	0.000082	0.0	100.000
144.0000	0.000078	0.0	100.000
145.0000	0.000074	0.0	100.000
146.0000	0.000070	0.0	100.000
147.0000	0.000067	0.0	100.000
148.0000	0.000064	0.0	100.000
149.0000	0.000061	0.0	100.000
150.0000	0.000058	0.0	100.000
151.0000	0.000055	0.0	100.000

CASE 7059-CURVE 1-NO DRUGS
NON DIFFUSIBLE TRACER IS I-131 ALBUMIN
DIFFUSIBLE TRACER IS CARBON-14 SUCROSE

THE VALUE OF LAMBDA IS 0.026600
THE VALUE OF R IS 0.257000

TIME	G CALC	G ACT	PCT ERROR
11.4000	0.0	0.0	0.0
12.0000	0.001627	0.001790	10.043
13.0000	0.006949	0.007820	12.532
14.0000	0.010330	0.010400	0.673
15.0000	0.016594	0.016000	3.711
16.0000	0.020407	0.020790	1.878
17.0000	0.024382	0.024630	1.017
18.0000	0.026327	0.025680	2.520
19.0000	0.029581	0.028270	4.636
20.0000	0.031781	0.031080	2.257
21.0000	0.032645	0.033390	2.283
22.0000	0.033291	0.035640	7.055
23.0000	0.033581	0.035930	6.994
24.0000	0.032751	0.034920	6.623
25.0000	0.032411	0.033900	4.593
26.0000	0.032283	0.032000	0.885
27.0000	0.030742	0.029810	3.127
28.0000	0.029155	0.027910	4.460
29.0000	0.027384	0.026340	3.965
30.0000	0.024168	0.024760	2.448
31.0000	0.024036	0.023500	2.279
32.0000	0.022486	0.022010	2.163
33.0000	0.021161	0.020840	1.542
34.0000	0.020070	0.019830	1.212
35.0000	0.019044	0.018910	0.707
36.0000	0.018032	0.017650	2.166
37.0000	0.016889	0.016510	2.296
38.0000	0.016131	0.015800	2.098
39.0000	0.015074	0.014870	1.373
40.0000	0.014254	0.014000	1.813
41.0000	0.013481	0.013210	2.048
42.0000	0.012750	0.012450	2.406
43.0000	0.012056	0.011750	2.605
44.0000	0.011404	0.011080	2.927
45.0000	0.010782	0.010450	3.182
46.0000	0.010196	0.009860	3.412
47.0000	0.009644	0.009300	3.703
48.0000	0.009121	0.008770	4.001
49.0000	0.008619	0.008270	4.225
50.0000	0.008151	0.007800	4.496
51.0000	0.007707	0.007360	4.713
52.0000	0.007283	0.006940	4.943
53.0000	0.006881	0.006550	5.060
54.0000	0.006504	0.006170	5.411
55.0000	0.006145	0.005820	5.582
56.0000	0.005806	0.005490	5.750
57.0000	0.005484	0.005180	5.873
58.0000	0.005180	0.004890	5.928
59.0000	0.004892	0.004610	6.108
60.0000	0.004619	0.004350	6.175

63.0000	0.003885	0.003650	6.430
64.0000	0.003670	0.003440	6.678
65.0000	0.003461	0.003240	6.819
66.0000	0.003266	0.003060	6.730
67.0000	0.003082	0.002890	6.640
68.0000	0.002906	0.002720	6.853
69.0000	0.002742	0.002570	6.686
70.0000	0.002587	0.002420	6.898
71.0000	0.002439	0.002280	6.974
72.0000	0.002300	0.002150	6.966
73.0000	0.002168	0.002030	6.815
74.0000	0.002045	0.001920	6.506
75.0000	0.001926	0.001860	3.568
76.0000	0.001817	0.001710	6.274
77.0000	0.001712	0.001610	6.308
78.0000	0.001613	0.001520	6.144
79.0000	0.001520	0.001430	6.328
80.0000	0.001432	0.001350	6.065
81.0000	0.001350	0.001270	6.325
82.0000	0.001271	0.001200	5.911
83.0000	0.001198	0.001130	6.024
84.0000	0.001129	0.001070	5.468
85.0000	0.001062	0.001040	2.138
86.0000	0.000992	0.000950	4.398
87.0000	0.000933	0.000900	3.660
88.0000	0.000877	0.000850	3.153
89.0000	0.000824	0.000800	3.029
90.0000	0.000774	0.000750	3.237
91.0000	0.000728	0.000710	2.468
92.0000	0.000683	0.000670	1.983
93.0000	0.000642	0.000630	1.901
94.0000	0.000603	0.000600	0.446
95.0000	0.000566	0.000560	1.146
96.0000	0.000531	0.000530	0.277
97.0000	0.000499	0.000500	0.177
98.0000	0.000468	0.000470	0.424
99.0000	0.000440	0.000440	0.105
100.0000	0.000412	0.000420	1.939
101.0000	0.000387	0.000400	3.385
102.0000	0.000363	0.000370	1.966
103.0000	0.000341	0.000350	2.765
104.0000	0.000320	0.000330	3.263
105.0000	0.000299	0.000310	3.583
106.0000	0.000281	0.000300	6.747
107.0000	0.000263	0.000280	6.439
108.0000	0.000246	0.000260	5.481
109.0000	0.000231	0.000250	8.121
110.0000	0.000217	0.000230	6.200
111.0000	0.000203	0.000220	8.220
112.0000	0.000190	0.000210	10.446
113.0000	0.000178	0.000196	10.053
114.0000	0.000168	0.000185	10.432
115.0000	0.000156	0.000174	11.300
116.0000	0.000146	0.000164	12.024
117.0000	0.000138	0.000155	12.652
118.0000	0.000128	0.000146	13.819
119.0000	0.000120	0.000138	14.886
120.0000	0.000113	0.000130	15.278
121.0000	0.000105	0.000123	16.976

124.0000	0.000086	0.000103	19.272
125.0000	0.000081	0.000097	20.140
126.0000	0.000076	0.0	100.000
127.0000	0.000071	0.0	100.000
128.0000	0.000066	0.0	100.000
129.0000	0.000062	0.0	100.000
130.0000	0.000058	0.0	100.000
131.0000	0.000054	0.0	100.000
132.0000	0.000050	0.0	100.000
133.0000	0.000047	0.0	100.000
134.0000	0.000044	0.0	100.000
135.0000	0.000041	0.0	100.000
136.0000	0.000038	0.0	100.000
137.0000	0.000036	0.0	100.000
138.0000	0.000034	0.0	100.000
139.0000	0.000032	0.0	100.000
140.0000	0.000029	0.0	100.000
141.0000	0.000027	0.0	100.000
142.0000	0.000026	0.0	100.000
143.0000	0.000024	0.0	100.000
144.0000	0.000022	0.0	100.000
145.0000	0.000021	0.0	100.000
146.0000	0.000019	0.0	100.000
147.0000	0.000018	0.0	100.000
148.0000	0.000017	0.0	100.000
149.0000	0.000016	0.0	100.000
150.0000	0.000015	0.0	100.000
151.0000	0.000014	0.0	100.000

CASE 7059-CURVE 1-NO DRUGS
NON DIFFUSIBLE TRACER IS I-131 ALBUMIN
DIFFUSIBLE TRACER IS SODIUM-24 CHLORIDE

THE VALUE OF LAMBDA IS 0.034500
THE VALUE OF R IS 0.492000

TIME	G CALC	G ACT	PCT ERROR
11.4000	0.0	0.0	0.0
12.0000	0.001478	0.001450	1.940
13.0000	0.006257	0.004600	36.032
14.0000	0.009214	0.007610	21.079
15.0000	0.014676	0.010610	38.324
16.0000	0.017888	0.012440	43.791
17.0000	0.021195	0.014760	43.597
18.0000	0.022700	0.016870	34.557
19.0000	0.025321	0.019380	30.654
20.0000	0.027014	0.021880	23.466
21.0000	0.027568	0.023310	18.268
22.0000	0.027956	0.024730	13.044
23.0000	0.028062	0.024980	12.338
24.0000	0.027264	0.025610	6.460
25.0000	0.026905	0.026240	2.534
26.0000	0.026740	0.025820	3.562
27.0000	0.025453	0.025400	0.209
28.0000	0.024169	0.024930	3.149
29.0000	0.022774	0.024350	6.918
30.0000	0.020277	0.023760	17.177
31.0000	0.020239	0.022970	13.495
32.0000	0.019094	0.022370	17.154
33.0000	0.018140	0.021570	18.909
34.0000	0.017374	0.020780	19.602
35.0000	0.016663	0.019980	19.910
36.0000	0.015965	0.019180	20.137
37.0000	0.015172	0.018370	21.080
38.0000	0.014667	0.017580	19.860
39.0000	0.013939	0.016570	18.875
40.0000	0.013385	0.015950	19.164
41.0000	0.012863	0.015210	18.249
42.0000	0.012367	0.014500	17.248
43.0000	0.011893	0.013820	16.198
44.0000	0.011446	0.013180	15.148
45.0000	0.011014	0.012570	14.125
46.0000	0.010604	0.011980	12.980
47.0000	0.010213	0.011420	11.814
48.0000	0.009838	0.010890	10.689
49.0000	0.009473	0.010380	9.580
50.0000	0.009128	0.009900	8.462
51.0000	0.008796	0.009440	7.316
52.0000	0.008473	0.009000	6.223
53.0000	0.008162	0.008580	5.127
54.0000	0.007865	0.008100	2.983
55.0000	0.007577	0.007800	2.949
56.0000	0.007300	0.007440	1.916
57.0000	0.007032	0.007090	0.819
58.0000	0.006774	0.006760	0.210
59.0000	0.006526	0.006450	1.181
60.0000	0.006286	0.006150	2.205

63.0000	0.005613	0.005320	5.515
64.0000	0.005411	0.005080	6.506
65.0000	0.005205	0.004840	7.533
66.0000	0.005012	0.004620	8.485
67.0000	0.004827	0.004400	9.694
68.0000	0.004644	0.004200	10.564
69.0000	0.004469	0.004000	11.723
70.0000	0.004303	0.003810	12.941
71.0000	0.004141	0.003640	13.756
72.0000	0.003984	0.003470	14.816
73.0000	0.003832	0.003310	15.758
74.0000	0.003689	0.003150	17.118
75.0000	0.003546	0.003010	17.804
76.0000	0.003413	0.002870	18.918
77.0000	0.003280	0.002730	20.160
78.0000	0.003156	0.002600	21.379
79.0000	0.003035	0.002480	22.380
80.0000	0.002917	0.002370	23.079
81.0000	0.002807	0.002250	24.755
82.0000	0.002696	0.002150	25.407
83.0000	0.002594	0.002050	26.527
84.0000	0.002493	0.001960	27.177
85.0000	0.002394	0.001820	31.548
86.0000	0.002298	0.001780	29.081
87.0000	0.002209	0.001700	29.918
88.0000	0.002121	0.001620	30.917
89.0000	0.002038	0.001540	32.367
90.0000	0.001957	0.001470	33.109
91.0000	0.001879	0.001400	34.212
92.0000	0.001804	0.001340	34.651
93.0000	0.001732	0.001270	36.408
94.0000	0.001664	0.001210	37.519
95.0000	0.001599	0.001160	37.808
96.0000	0.001535	0.001100	39.517
97.0000	0.001472	0.001050	40.224
98.0000	0.001413	0.001000	41.266
99.0000	0.001356	0.000960	41.224
100.0000	0.001301	0.000910	42.978
101.0000	0.001249	0.000870	43.552
102.0000	0.001199	0.000830	44.420
103.0000	0.001152	0.000790	45.765
104.0000	0.001105	0.000750	47.347
105.0000	0.001059	0.000720	47.018
106.0000	0.001015	0.000690	47.151
107.0000	0.000974	0.000650	49.836
108.0000	0.000934	0.000620	50.724
109.0000	0.000896	0.000590	51.883
110.0000	0.000859	0.000570	50.763
111.0000	0.000824	0.000540	52.675
112.0000	0.000790	0.000520	51.995
113.0000	0.000758	0.000490	54.763
114.0000	0.000729	0.000470	55.003
115.0000	0.000697	0.000450	54.989
116.0000	0.000668	0.000430	55.411
117.0000	0.000641	0.000410	56.295
118.0000	0.000614	0.000390	57.391
119.0000	0.000589	0.000370	59.090
120.0000	0.000564	0.000351	60.780
121.0000	0.000540	0.000335	61.194

124.0000	0.000476	0.000290	64.210
125.0000	0.000457	0.000277	64.876
126.0000	0.000438	0.000264	65.860
127.0000	0.000420	0.000252	66.659
128.0000	0.000402	0.000247	62.627
129.0000	0.000384	0.000230	67.078
130.0000	0.000368	0.000220	67.187
131.0000	0.000352	0.000210	67.802
132.0000	0.000337	0.000198	70.285
133.0000	0.000323	0.000189	70.957
134.0000	0.000310	0.000180	72.024
135.0000	0.000297	0.000172	72.564
136.0000	0.000284	0.000164	73.191
137.0000	0.000272	0.000156	74.410
138.0000	0.000261	0.000149	74.891
139.0000	0.000250	0.000142	75.968
140.0000	0.000239	0.000135	77.137
141.0000	0.000229	0.000129	77.564
142.0000	0.000219	0.000123	78.215
143.0000	0.000210	0.000117	79.494
144.0000	0.000201	0.000112	79.529
145.0000	0.000192	0.000107	79.532
146.0000	0.000184	0.000102	80.283
147.0000	0.000176	0.000097	81.430
148.0000	0.000169	0.0	100.000
149.0000	0.000161	0.0	100.000
150.0000	0.000154	0.0	100.000
151.0000	0.000147	0.0	100.000

CASE 7059-CURVE 1-NC DRUGS
NON DIFFUSIBLE TRACER IS I-131 ALBUMIN
DIFFUSIBLE TRACER IS SODIUM-24 CHLORIDE

THE VALUE OF LAMBDA IS 0.058900
THE VALUE OF R IS 0.492000

TIME	G CALC	G ACT	PCT ERROR
11.4000	0.0	0.0	0.0
12.0000	0.001118	0.001450	29.667
13.0000	0.004716	0.004600	2.527
14.0000	0.007008	0.007610	8.591
15.0000	0.011107	0.010610	4.687
16.0000	0.013691	0.012440	10.055
17.0000	0.016359	0.014760	10.833
18.0000	0.017843	0.016870	5.769
19.0000	0.020047	0.019380	3.439
20.0000	0.021685	0.021880	0.900
21.0000	0.022608	0.023310	3.106
22.0000	0.023394	0.024730	5.711
23.0000	0.023978	0.024980	4.177
24.0000	0.023995	0.025610	6.730
25.0000	0.024194	0.026240	8.455
26.0000	0.024460	0.025920	5.560
27.0000	0.024083	0.025400	5.469
28.0000	0.023627	0.024930	5.517
29.0000	0.023043	0.024350	5.674
30.0000	0.021819	0.023760	8.898
31.0000	0.021694	0.022970	5.880
32.0000	0.021045	0.022370	6.298
33.0000	0.020439	0.021570	5.535
34.0000	0.019884	0.020780	4.504
35.0000	0.019325	0.019980	3.388
36.0000	0.018756	0.019180	2.258
37.0000	0.018125	0.018370	1.351
38.0000	0.017598	0.017580	0.104
39.0000	0.016971	0.016570	2.423
40.0000	0.016398	0.015950	2.811
41.0000	0.015834	0.015210	4.103
42.0000	0.015276	0.014500	5.355
43.0000	0.014726	0.013820	6.554
44.0000	0.014188	0.013180	7.646
45.0000	0.013656	0.012570	8.642
46.0000	0.013138	0.011980	9.667
47.0000	0.012635	0.011420	10.640
48.0000	0.012144	0.010890	11.512
49.0000	0.011658	0.010380	12.311
50.0000	0.011193	0.009900	13.061
51.0000	0.010741	0.009440	13.787
52.0000	0.010298	0.009000	14.417
53.0000	0.009868	0.008580	15.009
54.0000	0.009456	0.008100	16.741
55.0000	0.009053	0.007800	16.062
56.0000	0.008666	0.007440	16.476
57.0000	0.008291	0.007090	16.938
58.0000	0.007929	0.006760	17.292
59.0000	0.007582	0.006450	17.552
60.0000	0.007246	0.006150	17.817

63.0000	0.006310	0.005320	18.615
64.0000	0.006029	0.005080	18.690
65.0000	0.005747	0.004840	18.750
66.0000	0.005485	0.004620	18.717
67.0000	0.005233	0.004400	18.932
68.0000	0.004987	0.004200	18.738
69.0000	0.004753	0.004000	18.816
70.0000	0.004531	0.003810	18.935
71.0000	0.004317	0.003640	18.612
72.0000	0.004112	0.003470	18.510
73.0000	0.003914	0.003310	18.260
74.0000	0.003731	0.003150	18.437
75.0000	0.003549	0.003010	17.899
76.0000	0.003379	0.002870	17.748
77.0000	0.003214	0.002730	17.740
78.0000	0.003059	0.002600	17.651
79.0000	0.002910	0.002480	17.351
80.0000	0.002767	0.002370	16.744
81.0000	0.002633	0.002250	17.024
82.0000	0.002502	0.002150	16.372
83.0000	0.002380	0.002050	16.103
84.0000	0.002262	0.001960	15.403
85.0000	0.002149	0.001820	18.061
86.0000	0.002042	0.001780	14.731
87.0000	0.001941	0.001700	14.170
88.0000	0.001843	0.001620	13.761
89.0000	0.001751	0.001540	13.717
90.0000	0.001662	0.001470	13.073
91.0000	0.001578	0.001400	12.717
92.0000	0.001498	0.001340	11.793
93.0000	0.001422	0.001270	11.943
94.0000	0.001350	0.001210	11.529
95.0000	0.001281	0.001160	10.462
96.0000	0.001216	0.001100	10.522
97.0000	0.001153	0.001050	9.805
98.0000	0.001093	0.001000	9.316
99.0000	0.001037	0.000960	7.988
100.0000	0.000983	0.000910	8.015
101.0000	0.000932	0.000870	7.167
102.0000	0.000884	0.000830	6.497
103.0000	0.000839	0.000790	6.144
104.0000	0.000795	0.000750	5.989
105.0000	0.000752	0.000720	4.500
106.0000	0.000713	0.000690	3.352
107.0000	0.000675	0.000650	3.912
108.0000	0.000640	0.000620	3.213
109.0000	0.000606	0.000590	2.731
110.0000	0.000574	0.000570	0.691
111.0000	0.000544	0.000540	0.713
112.0000	0.000515	0.000520	1.067
113.0000	0.000487	0.000490	0.593
114.0000	0.000462	0.000470	1.704
115.0000	0.000437	0.000450	3.064
116.0000	0.000413	0.000430	4.096
117.0000	0.000391	0.000410	4.747
118.0000	0.000370	0.000390	5.521
119.0000	0.000350	0.000370	5.804
120.0000	0.000331	0.000351	5.992
121.0000	0.000312	0.000335	7.209

124.0000	0.000265	0.000290	9.629
125.0000	0.000250	0.000277	10.799
126.0000	0.000236	0.000264	11.712
127.0000	0.000224	0.000252	12.501
128.0000	0.000211	0.000247	17.082
129.0000	0.000199	0.000230	15.527
130.0000	0.000188	0.000220	16.929
131.0000	0.000178	0.000210	18.000
132.0000	0.000168	0.000198	18.120
133.0000	0.000158	0.000189	19.400
134.0000	0.000150	0.000180	20.280
135.0000	0.000142	0.000172	21.533
136.0000	0.000133	0.000164	23.121
137.0000	0.000126	0.000156	24.009
138.0000	0.000119	0.000149	25.526
139.0000	0.000112	0.000142	26.452
140.0000	0.000106	0.000135	27.845
141.0000	0.000100	0.000129	29.533
142.0000	0.000094	0.000123	30.925
143.0000	0.000089	0.000117	31.715
144.0000	0.000084	0.000112	33.569
145.0000	0.000079	0.000107	36.090
146.0000	0.000074	0.000102	37.582
147.0000	0.000070	0.000097	38.759
148.0000	0.000066	0.0	100.000
149.0000	0.000062	0.0	100.000
150.0000	0.000058	0.0	100.000
151.0000	0.000055	0.0	100.000

APPENDIX A

Numerical Methods

The analytical forms of $h_N(t)$ and $h_D(t)$ have been developed for five simple models of capillary and extravascular spaces behavior, and the analytical machinery for calculating λ by the various methods. However, in a multiple tracer experiment, neither the analytic form of the capillary space nor the analytic form of the extravascular space is known, the concentration profile at the organ's vein is only monitored. It is therefore, necessary to find suitable numerical procedures to evaluate the Laplace transform, the moments of $h_N(t)$ and $h_D(t)$, the extraction factor at the appearance time, and the integrals employed in the CRA method calculations, directly from the experimental data.

SNK Limit Method

The SNK limit method for finding λ requires the Laplace transform of $h_N(t)$ and $h_D(t)$. Instead of attempting to curve fit $h_N(t)$ or $h_D(t)$ to an n^{th} order polynomial, or to an exponential function and then taking the Laplace transform, the function

is divided into small increments and fitted to a second order polynomial. The Laplace transform can then be taken as a finite sum. In order to illustrate this procedure, we will use the typical $h_N(t)$ or $h_D(t)$ curve versus time found in Figure A.1. The curve is broken up in n points such that $t_1 < t_2 < t_3 \ldots < t_n$. The points are grouped in sets of three and the three quadratic coefficients are found. Thus

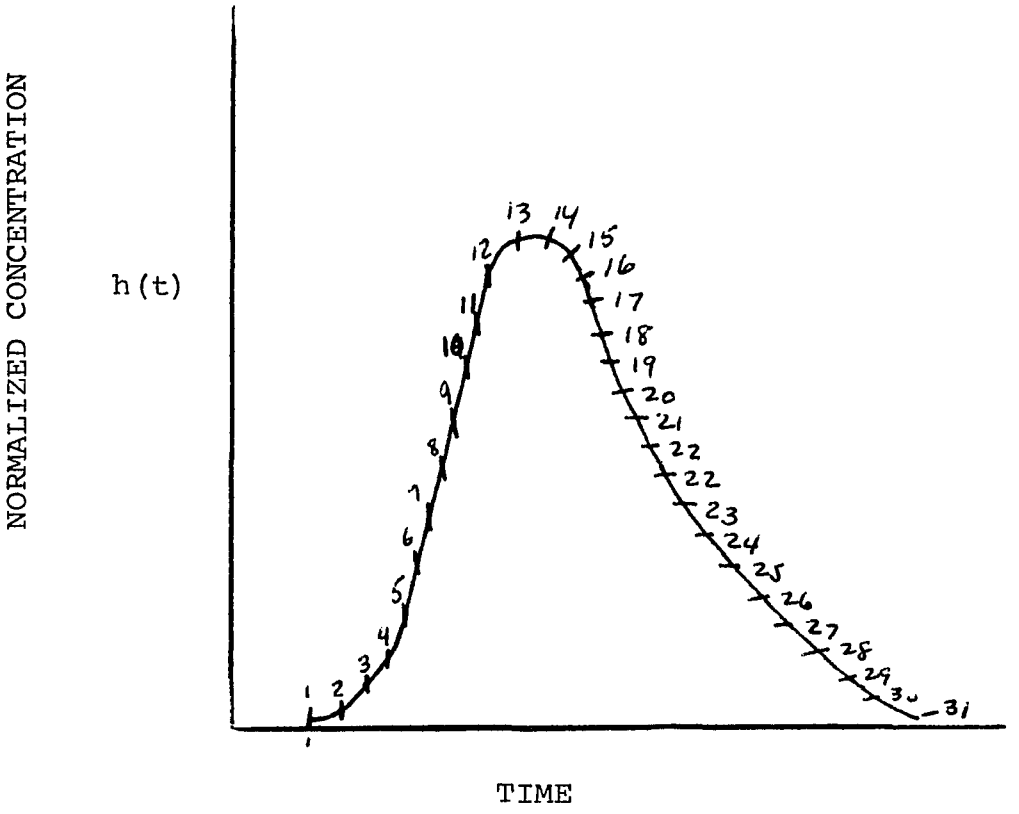
$$\begin{aligned} h_1(t) &= a_1 t^2 + b_1 t + c_1 \qquad & ta = t_1 < t < t_3 \\ h_2(t) &= a_2 t^2 + b_2 t + c_2 \qquad & t_3 < t < t_5 \\ &\vdots \\ h_j(t) &= a_j t^2 + b_j t + c_j \qquad & t_{2j} - 1 < t < t_{2j+1} \end{aligned} \tag{A.1}$$

Therefore, the entire $h_N(t)$ or $h_D(t)$ curves can be represented as

$$h(t) = \sum_{j=1}^{\frac{n-1}{2}} h_j(t) \tag{A.2}$$

Equation A.2 gives an accurate representation $h_N(t)$ or $h_D(t)$ and can easily handle an $h_N(t)$ or $h_D(t)$, represented by discrete points. The Laplace transform of the normalized

FIGURE - A.1 - NORMALIZED CONCENTRATION VERSUS TIME BROKEN DOWN INTO N DISCRETE POINTS



concentration versus time curve can now be computed as finite sum for all real value of s. The Laplace transform of h(t) is defined as

$$h(s) = \int_0^{\infty} h(t)e^{-st}\,dt \qquad (A.3)$$

If h(t) is now represented by equation A.2, the Laplace transform becomes:

$$h(s) = \int_{t_1}^{t_3} h_1(t)e^{-st}dt + \int_{t_3}^{t_5} h_2(t)e^{-st}dt + \ldots \qquad (A.4)$$
$$\int_{t_{2j-1}}^{t_{2j+1}} h_j(t)e^{-st}dt \ldots + \int_{t_{2n-1}}^{\infty} h_n(t)e^{-st}dt$$

If we substitute the quadratic representation of $h_j(t)$ shown in equation A.1 into equation A.4 and choose t_{2n-1} sufficiently large so that $h_n(t)$ is approximately zero, one gets:

$$h(s) = \int_{t_1}^{t_3} (a_1t^2+b_1t+c_1)e^{-st}dt + \ldots.$$
$$\int_{t_{2j-1}}^{t_{2j+1}} (a_jt^2+b_jt+c_j)e^{-st}dt + \ldots \qquad (A.5)$$
$$\int_{t_{2n-1}}^{\infty} (a_nt^2+b_nt+c_n)e^{-st}dt$$

Each term of equation A.5 can be explicitly evaluated and h(s) can be expressed as a finite sum for all real values of s.

$$h(s) \sum_{j=1}^{n} e^{-st_{2j-1}}\left[\left(\frac{a_j t_{2j-1}^2}{s}\right) + (2a_j + b_j s)\frac{t_{2j-1}}{s^2} + \frac{1}{s^3}(2a_j + b_j s + c_j s^2)\right] - e^{-st_{2j+1}}\left[\frac{a_j t_{2j+1}^2}{s} + (2a_j + b_j s)\frac{t_{2j+1}}{s^2} + \frac{1}{s^3}(2a_j + b_j s + c_j s^2)\right] \quad (A.6)$$

In order to determine the accuracy of the procedure described above, the Laplace transform of various functions has been constructed in accordance with equation A.6. The functions which were chosen for this test were: $f(t) = t$, $t^2/2$, $t^3/6$, e^{-t}, te^{-t}, $t^2 e^{-t}/2$. The calculated Laplace transform along with the actual transform are shown in tables A.1 through A.6. In order to numerically simulate the real data, the functions were evaluated at 68 discrete points for $0 \leq t \leq 70$.

The Laplace transform for the polynomials is shown in Tables A.1 through A.3. The maximum error computed was 22.4% for the function $f(t) = t^3/6$ at a value of $s=0.077$. For values of $0.2 \leq s \leq 48$, the maximum error found was less than 0.5%. For the lower order polynomial $f(t)=t$ and $t^2/2$ the maximum error is several orders of magnitude lower.

The Laplace transform computed for the exponential functions (Tables A.4 through A.6) give considerably better results. The

TABLE A1

F(X)=X
THE LAPLACE TRANSFORM OF F(X) IS 1./S**2

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
0.077000	163.746002	168.662689	2.915100
0.083330	141.129456	144.011673	2.001377
0.090900	119.487961	121.024368	1.269502
0.100000	99.270599	100.000092	0.729491
0.111110	80.703766	81.001648	0.367748
0.125000	63.901108	64.000000	0.154519
0.143000	48.877960	48.902176	0.049519
0.166670	35.994644	35.998566	0.010894
0.200000	24.999649	25.000000	0.001404
0.250000	15.999991	16.000000	0.000054
0.300000	11.111119	11.111121	0.000017
0.333300	9.001801	9.001801	0.000011
0.375000	7.111109	7.111111	0.000027
0.400000	6.250001	6.250001	0.0
0.450000	4.938271	4.938272	0.000019
0.500000	3.999997	4.000000	0.000072
0.550000	3.305781	3.305786	0.000144
0.600000	2.777774	2.777778	0.000137
0.650000	2.366859	2.366863	0.000161
0.700000	2.040812	2.040816	0.000234
0.750000	1.777772	1.777778	0.000322
0.800000	1.562495	1.562500	0.000305
0.850000	1.384079	1.384083	0.000276
0.900000	1.234563	1.234568	0.000386
0.950000	1.108030	1.108033	0.000258
1.000000	1.000000	1.000000	0.000030
1.099999	0.826448	0.826448	0.000029
1.200000	0.694445	0.694445	0.000026
1.299999	0.591717	0.591717	0.0
1.400000	0.510204	0.510205	0.000058
1.500000	0.444444	0.444444	0.000054
1.599999	0.390625	0.390625	0.000061
1.700000	0.346021	0.346021	0.000086
1.799999	0.308642	0.308642	0.000097
1.900000	0.277008	0.277008	0.000108
2.000000	0.250000	0.250000	0.000143
2.200000	0.206611	0.206612	0.000202
2.400000	0.173611	0.173611	0.000275
2.599999	0.147929	0.147929	0.000322

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
2.799999	0.127551	0.127551	0.000327
3.000000	0.111111	0.111111	0.000429
3.299999	0.091827	0.091827	0.000325
3.599999	0.077160	0.077160	0.000463
4.000000	0.062500	0.062500	0.000030
4.400000	0.051653	0.051653	0.000022
4.799999	0.043403	0.043403	0.000043
5.000000	0.040000	0.040000	0.000047
5.500000	0.033058	0.033058	0.000045
6.000000	0.027778	0.027778	0.000067
7.000000	0.020408	0.020408	0.000146
8.000000	0.015625	0.015625	0.000191
9.000000	0.012346	0.012346	0.000181
10.000000	0.010000	0.010000	0.000224
11.000000	0.008264	0.008264	0.000316
12.000000	0.006944	0.006944	0.000376
13.000000	0.005917	0.005917	0.000252
14.000000	0.005102	0.005102	0.000438
15.000000	0.004444	0.004444	0.000419
16.000000	0.003906	0.003906	0.000036
17.000000	0.003460	0.003460	0.000034
18.000000	0.003086	0.003086	0.000030
19.000000	0.002770	0.002770	0.000034
20.000000	0.002500	0.002500	0.000047
22.000000	0.002066	0.002066	0.000034
24.000000	0.001736	0.001736	0.000054
26.000000	0.001479	0.001479	0.000031
28.000000	0.001276	0.001276	0.000073
30.000000	0.001111	0.001111	0.000084
32.000000	0.000977	0.000977	0.000095
34.000000	0.000865	0.000865	0.000081
36.000000	0.000772	0.000772	0.000121
38.000000	0.000693	0.000693	0.000134
40.000000	0.000625	0.000625	0.000075
42.000000	0.000567	0.000567	0.000123
44.000000	0.000517	0.000517	0.000180
46.000000	0.000473	0.000473	0.000099
48.000000	0.000434	0.000434	0.000161

THE LAPLACE TRANSFORM OF F(X) IS 1./S**3

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
0.077000	1981.4199	2190.4258	9.541785
0.083330	1607.5056	1728.2102	6.984365
0.090900	1268.0288	1331.4023	4.759908
0.100000	970.3643	1000.0015	2.963716
0.111110	717.1038	729.0222	1.634854
0.125000	508.1033	512.0000	0.761080
0.143000	341.0330	341.9734	0.275001
0.166670	215.8381	215.9873	0.069043
0.200000	124.9883	125.0000	0.009375
0.250000	63.9997	64.0000	0.000477
0.300000	37.0370	37.0371	0.000124
0.333300	27.0081	27.0081	0.000056
0.375000	18.9629	18.9630	0.000161
0.400000	15.6250	15.6250	0.000049
0.450000	10.9739	10.9739	0.000061
0.500000	8.0000	8.0000	0.000167
0.550000	6.0105	6.0105	0.000048
0.600000	4.6296	4.6296	0.000021
0.650000	3.6413	3.6413	0.000079
0.700000	2.9154	2.9155	0.000262
0.750000	2.3704	2.3704	0.000201
0.800000	1.9531	1.9531	0.000391
0.850000	1.6283	1.6283	0.000234
0.900000	1.3717	1.3717	0.000209
0.950000	1.1663	1.1664	0.000245
1.000000	1.0000	1.0000	0.000036
1.059999	0.7513	0.7513	0.000135
1.200000	0.5787	0.5787	0.000165
1.299999	0.4552	0.4552	0.000118
1.400000	0.3644	0.3644	0.000016
1.500000	0.2963	0.2963	0.000080
1.599999	0.2441	0.2441	0.000146
1.700000	0.2035	0.2035	0.000117
1.799999	0.1715	0.1715	0.000070
1.900000	0.1458	0.1458	0.000245
2.000000	0.1250	0.1250	0.000286
2.200000	0.0939	0.0939	0.000444
2.400000	0.0723	0.0723	0.000412
2.599999	0.0569	0.0569	0.0

TABLE A.2 (CONTINUED)

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
2.799999	0.0456	0.0456	0.000114
3.000000	0.03703701	0.03703703	0.000060
3.299999	0.02782648	0.02782650	0.000054
3.599999	0.02143347	0.02143348	0.000070
4.000000	0.01562498	0.01562500	0.000143
4.400000	0.01173927	0.01173930	0.000317
4.799999	0.00904221	0.00904226	0.000536
5.000000	0.00799995	0.00800000	0.000559
5.500000	0.00601050	0.00601052	0.000372
6.000000	0.00462960	0.00462963	0.000563
7.000000	0.00291545	0.00291545	0.000160
8.000000	0.00195312	0.00195313	0.000072
9.000000	0.00137174	0.00137174	0.000051
10.000000	0.00100000	0.00100000	0.000233
11.000000	0.00075131	0.00075131	0.000341
12.000000	0.00057870	0.00057870	0.000201
13.000000	0.00045516	0.00045517	0.000409
14.000000	0.00036443	0.00036443	0.000447
15.000000	0.00029630	0.00029630	0.000157
16.000000	0.00024414	0.00024414	0.000030
17.000000	0.00020354	0.00020354	0.000079
18.000000	0.00017147	0.00017147	0.000144
19.000000	0.00014579	0.00014579	0.000060
20.000000	0.00012500	0.00012500	0.000163
22.000000	0.00009391	0.00009391	0.000062
24.000000	0.00007234	0.00007234	0.000080
26.000000	0.00005690	0.00005690	0.000179
28.000000	0.00004555	0.00004555	0.000160
30.000000	0.00003704	0.00003704	0.000157
32.000000	0.00003052	0.00003052	0.000286
34.000000	0.00002544	0.00002544	0.000286
36.000000	0.00002143	0.00002143	0.000272
38.000000	0.00001822	0.00001822	0.000319
40.000000	0.00001562	0.00001562	0.000093
42.000000	0.00001350	0.00001350	0.000054
44.000000	0.00001174	0.00001174	0.000070
46.000000	0.00001027	0.00001027	0.000035
48.000000	0.00000904	0.00000904	0.000060

THE LAPLACE TRANSFORM OF F(X) IS 1./S**4

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
0.077000	22069.0859	28447.1055	22.420624
0.083330	17056.9297	20739.3633	17.755753
0.090900	12691.2813	14646.8984	13.351744
0.100000	9054.8203	10000.0195	9.451973
0.111110	6151.6719	6561.2695	6.242658
0.125000	3939.0483	4096.0000	3.831827
0.143000	2338.6516	2391.4243	2.206747
0.166670	1279.8347	1295.8977	1.239526
0.200000	620.9104	625.0002	0.654374
0.250000	255.3145	256.0000	0.267762
0.300000	123.3286	123.4570	0.104018
0.333300	80.9894	81.0324	0.053158
0.375000	50.5570	50.5679	0.021454
0.400000	39.0580	39.0625	0.011523
0.450000	24.3861	24.3865	0.001627
0.500000	16.0004	16.0000	0.002575
0.550000	10.9287	10.9282	0.004163
0.600000	7.7164	7.7161	0.004561
0.650000	5.6023	5.6020	0.004477
0.700000	4.1651	4.1649	0.004305
0.750000	3.1606	3.1605	0.004043
0.800000	2.4415	2.4414	0.003516
0.850000	1.9157	1.9157	0.003087
0.900000	1.5242	1.5242	0.002753
0.950000	1.2278	1.2277	0.002330
1.000000	1.0000	1.0000	0.002098
1.099999	0.6830	0.6830	0.001632
1.200000	0.4823	0.4823	0.000927
1.299999	0.3501	0.3501	0.000596
1.400000	0.2603	0.2603	0.000069
1.500000	0.1975	0.1975	0.000091
1.599999	0.1526	0.1526	0.000586
1.700000	0.1197	0.1197	0.000846
1.799999	0.0953	0.0953	0.001189
1.900000	0.0767	0.0767	0.001321
2.000000	0.0625	0.0625	0.001121
2.200000	0.0427	0.0427	0.001440
2.400000	0.0301	0.0301	0.001743
2.599999	0.0219	0.0219	0.001890

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
2.799999	0.0163	0.0163	0.002175
3.000000	0.01234538	0.01234568	0.002354
3.299999	0.00843204	0.00843227	0.002783
3.599999	0.00595355	0.00595374	0.003191
4.000000	0.00390611	0.00390625	0.003648
4.400000	0.00266790	0.00266803	0.004686
4.799999	0.00188370	0.00188380	0.005722
5.000000	0.00159990	0.00160000	0.006141
5.500000	0.00109273	0.00109282	0.008117
6.000000	0.00077152	0.00077160	0.010591
7.000000	0.00041642	0.00041649	0.017050
8.000000	0.00024408	0.00024414	0.025398
9.000000	0.00015236	0.00015242	0.036538
10.000000	0.00009995	0.00010000	0.049942
11.000000	0.00006826	0.00006830	0.065855
12.000000	0.00004818	0.00004823	0.083796
13.000000	0.00003498	0.00003501	0.103987
14.000000	0.00002600	0.00002603	0.125669
15.000000	0.00001972	0.00001975	0.148591
16.000000	0.00001523	0.00001526	0.172168
17.000000	0.00001195	0.00001197	0.196673
18.000000	0.00000950	0.00000953	0.221665
19.000000	0.00000765	0.00000767	0.246333
20.000000	0.00000623	0.00000625	0.270826
22.000000	0.00000426	0.00000427	0.317004
24.000000	0.00000300	0.00000301	0.357874
26.000000	0.00000218	0.00000219	0.391802
28.000000	0.00000162	0.00000163	0.417145
30.000000	0.00000123	0.00000123	0.433322
32.000000	0.00000095	0.00000095	0.439340
34.000000	0.00000075	0.00000075	0.435801
36.000000	0.00000059	0.00000060	0.422792
38.000000	0.00000048	0.00000048	0.400571
40.000000	0.00000039	0.00000039	0.369881
42.000000	0.00000032	0.00000032	0.331313
44.000000	0.00000027	0.00000027	0.285344
46.000000	0.00000022	0.00000022	0.232727
48.000000	0.00000019	0.00000019	0.174139

maximum error calculated was 0.42% for the lowest values, namely 0.077. The maximum error in the range $0.2 \leq s \leq 48$ was was 0.04% and in many instances was less by several order of magnitude. The exponential functions are representative of the non-diffusible tracer response for one, two and three equal stirred tanks in series respectively.

The calculated Laplace Transform showed such good agreement with the actual transform, that is, it was advantageous to see what results could be obtained when fewer points are used to calculate the Laplace Transform. Table A.7 shows the results obtained if one uses 35 points to approximate $h_N(t)$ instead of the original 68, for $0 \leq t \leq 70$. The error in the transform of $x^2\ e^{-x}/2$ goes up by a factor of two. The maximum error calculated was 0.4% and for $0.2 \leq s \leq 48$, the maximum error was 0.1%. The results shown in Tables A.1 through A.7 demonstrate that the Laplace Transform calculated by equation A.6 gives very accurate results even for a small number of points.

Equation A.6 gives a suitable Laplace Transform for $h_N(t)$ and

F(X)=EXP(-X)
THE LAPLACE TRANSFORM OF F(X) IS 1./(S+1)

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
0.077000	0.928495	0.928505	0.001143
0.083330	0.922749	0.923080	0.035928
0.090900	0.917680	0.916675	0.109622
0.100000	0.909557	0.909091	0.051246
0.111110	0.900186	0.900001	0.020557
0.125000	0.889129	0.888889	0.027017
0.143000	0.875246	0.874891	0.040611
0.166670	0.857125	0.857140	0.001829
0.200000	0.833317	0.833333	0.001945
0.250000	0.799990	0.800000	0.001237
0.300000	0.769216	0.769231	0.001960
0.333300	0.750035	0.750019	0.002122
0.375000	0.727263	0.727273	0.001303
0.400000	0.714281	0.714286	0.000684
0.450000	0.689650	0.689655	0.000726
0.500000	0.666664	0.666667	0.000358
0.550000	0.645158	0.645162	0.000610
0.600000	0.625000	0.625000	0.000048
0.650000	0.606059	0.606061	0.000256
0.700000	0.588237	0.588235	0.000284
0.750000	0.571429	0.571429	0.000094
0.800000	0.555555	0.555556	0.000107
0.850000	0.540539	0.540541	0.000320
0.900000	0.526316	0.526316	0.000068
0.950000	0.512821	0.512821	0.000081
1.000000	0.500000	0.500000	0.000012
1.099999	0.476190	0.476191	0.000025
1.200000	0.454545	0.454545	0.000105
1.299999	0.434782	0.434783	0.000082
1.400000	0.416667	0.416667	0.000029
1.500000	0.399999	0.400000	0.000119
1.599999	0.384615	0.384615	0.000108
1.700000	0.370370	0.370370	0.000113
1.799999	0.357143	0.357143	0.000067
1.900000	0.344827	0.344828	0.000086
2.000000	0.333333	0.333333	0.000107
2.200000	0.312500	0.312500	0.000114
2.400000	0.294117	0.294118	0.000061
2.599999	0.277778	0.277778	0.000043

S	TRANSFORM	TRANSFORM	PCT ERROR
2.799999	0.263158	0.263158	0.000136
3.000000	0.250000	0.250000	0.000095
3.299999	0.232558	0.232558	0.000128
3.599999	0.217391	0.217391	0.000219
4.000000	0.200000	0.200000	0.000238
4.400000	0.185185	0.185185	0.000257
4.799999	0.172413	0.172414	0.000242
5.000000	0.166666	0.166667	0.000286
5.500000	0.153846	0.153846	0.000349
6.000000	0.142857	0.142857	0.000334
7.000000	0.125000	0.125000	0.000381
8.000000	0.111111	0.111111	0.000215
9.000000	0.099999	0.100000	0.000477
10.000000	0.090909	0.090909	0.000393
11.000000	0.083333	0.083333	0.000501
12.000000	0.076923	0.076923	0.000542
13.000000	0.071428	0.071429	0.000417
14.000000	0.066666	0.066667	0.000536
15.000000	0.062500	0.062500	0.000060
16.000000	0.058824	0.058824	0.000013
17.000000	0.055556	0.055556	0.000074
18.000000	0.052632	0.052632	0.000057
19.000000	0.050000	0.050000	0.000075
20.000000	0.047619	0.047619	0.000070
22.000000	0.043478	0.043478	0.000043
24.000000	0.040000	0.040000	0.000075
26.000000	0.037037	0.037037	0.000040
28.000000	0.034483	0.034483	0.000043
30.000000	0.032258	0.032258	0.000058
32.000000	0.030303	0.030303	0.000049
34.000000	0.028571	0.028571	0.000052
36.000000	0.027027	0.027027	0.000055
38.000000	0.025641	0.025641	0.000044
40.000000	0.024390	0.024390	0.000046
42.000000	0.023256	0.023256	0.000080
44.000000	0.022222	0.022222	0.000034
46.000000	0.021277	0.021277	0.000053
48.000000	0.020408	0.020408	0.000091

F(X)=X*EXP(-X)

THE LAPLACE TRANSFORM OF F(X) IS 1./(S+1)**2

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
0.077000	0.858488	0.862123	0.421625
0.083330	0.851575	0.852078	0.058949
0.090900	0.839752	0.840293	0.064301
0.100000	0.826767	0.826448	0.038599
0.111110	0.809377	0.810002	0.077111
0.125000	0.789248	0.790123	0.110779
0.143000	0.764566	0.765434	0.113480
0.166670	0.734283	0.734690	0.055354
0.200000	0.694380	0.694445	0.009381
0.250000	0.639911	0.640000	0.013961
0.300000	0.591617	0.591717	0.016923
0.333300	0.562492	0.562529	0.006601
0.375000	0.528883	0.528926	0.008024
0.400000	0.510173	0.510205	0.006227
0.450000	0.475602	0.475624	0.004637
0.500000	0.444436	0.444444	0.001918
0.550000	0.416226	0.416234	0.001704
0.600000	0.390620	0.390625	0.001251
0.650000	0.367304	0.367310	0.001493
0.700000	0.346019	0.346021	0.000689
0.750000	0.326528	0.326531	0.000730
0.800000	0.308639	0.308642	0.001043
0.850000	0.292181	0.292184	0.000979
0.900000	0.277007	0.277008	0.000538
0.950000	0.262982	0.262985	0.001133
1.000000	0.249998	0.250000	0.000668
1.099999	0.226756	0.226757	0.000657
1.200000	0.206610	0.206612	0.000692
1.299999	0.189035	0.189036	0.000725
1.400000	0.173610	0.173611	0.000721
1.500000	0.159999	0.160000	0.000745
1.599999	0.147928	0.147929	0.000846
1.700000	0.137173	0.137174	0.000826
1.799999	0.127550	0.127551	0.000935
1.900000	0.118905	0.118906	0.000952
2.000000	0.111110	0.111111	0.000858
2.200000	0.097655	0.097656	0.000916
2.400000	0.086504	0.086505	0.000965
2.599999	0.077160	0.077160	0.000850

S	TRANSFORM	TRANSFORM	PCT ERROR
2.799999	0.069251	0.069252	0.000947
3.000000	0.062500	0.062500	0.000578
3.299999	0.054083	0.054083	0.000613
3.599999	0.047259	0.047259	0.000733
4.000000	0.040000	0.040000	0.000633
4.400000	0.034293	0.034294	0.000717
4.799999	0.029726	0.029727	0.000777
5.000000	0.027778	0.027778	0.000818
5.500000	0.023668	0.023669	0.000960
6.000000	0.020408	0.020408	0.001077
7.000000	0.015625	0.015625	0.001287
8.000000	0.012346	0.012346	0.001388
9.000000	0.010000	0.010000	0.001602
10.000000	0.008264	0.008264	0.001893
11.000000	0.006944	0.006944	0.001985
12.000000	0.005917	0.005917	0.002015
13.000000	0.005102	0.005102	0.002190
14.000000	0.004444	0.004444	0.002347
15.000000	0.003906	0.003906	0.001973
16.000000	0.003460	0.003460	0.001817
17.000000	0.003086	0.003086	0.001961
18.000000	0.002770	0.002770	0.002009
19.000000	0.002500	0.002500	0.002002
20.000000	0.002268	0.002268	0.001992
22.000000	0.001890	0.001890	0.001983
24.000000	0.001600	0.001600	0.001834
26.000000	0.001372	0.001372	0.001697
28.000000	0.001189	0.001189	0.001566
30.000000	0.001041	0.001041	0.001432
32.000000	0.000918	0.000918	0.001268
34.000000	0.000816	0.000816	0.001198
36.000000	0.000730	0.000730	0.000924
38.000000	0.000657	0.000657	0.000815
40.000000	0.000595	0.000595	0.000744
42.000000	0.000541	0.000541	0.000603
44.000000	0.000494	0.000494	0.000519
46.000000	0.000453	0.000453	0.000411
48.000000	0.000416	0.000416	0.000280

F(X)=X**2*EXP(-X)
THE LAPLACE TRANSFORM OF F(X) IS 2./(S+1)**3

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
0.077000	1.604138	1.600972	0.197767
0.083330	1.573245	1.573072	0.010973
0.090900	1.541631	1.540550	0.070138
0.100000	1.503130	1.502633	0.033065
0.111110	1.457044	1.458006	0.065998
0.125000	1.404302	1.404663	0.025732
0.143000	1.339773	1.339343	0.032113
0.166670	1.259251	1.259465	0.017037
0.200000	1.157281	1.157409	0.011041
0.250000	1.023947	1.023999	0.005122
0.300000	0.910333	0.910334	0.000210
0.333300	0.843814	0.843815	0.000113
0.375000	0.769354	0.769346	0.001023
0.400000	0.728899	0.728864	0.004841
0.450000	0.656018	0.656034	0.002371
0.500000	0.592586	0.592593	0.001116
0.550000	0.537073	0.537076	0.000455
0.600000	0.488279	0.488282	0.000513
0.650000	0.445225	0.445224	0.000134
0.700000	0.407083	0.407084	0.000205
0.750000	0.373177	0.373178	0.000224
0.800000	0.342935	0.342936	0.000417
0.850000	0.315875	0.315875	0.000132
0.900000	0.291589	0.291588	0.000285
0.950000	0.269729	0.269728	0.000221
1.000000	0.250001	0.250000	0.000262
1.099999	0.215962	0.215960	0.001187
1.200000	0.187829	0.187829	0.000063
1.299999	0.164379	0.164379	0.000109
1.400000	0.144676	0.144676	0.0
1.500000	0.128000	0.128000	0.000325
1.599999	0.113791	0.113792	0.000262
1.700000	0.101610	0.101611	0.000235
1.799999	0.091108	0.091108	0.000393
1.900000	0.082004	0.082004	0.000435
2.000000	0.074074	0.074074	0.000080
2.200000	0.061035	0.061035	0.000043
2.400000	0.050886	0.050885	0.000329
2.599999	0.042867	0.042867	0.000921

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
2.799999	0.036449	0.036448	0.000613
3.000000	0.031250	0.031250	0.001204
3.299999	0.025155	0.025155	0.001274
3.599999	0.020548	0.020547	0.001305
4.000000	0.016000	0.016000	0.001932
4.400000	0.012702	0.012701	0.002289
4.799999	0.010251	0.010251	0.002798
5.000000	0.009260	0.009259	0.002977
5.500000	0.007283	0.007283	0.003632
6.000000	0.005831	0.005831	0.004409
7.000000	0.003907	0.003906	0.006676
8.000000	0.002744	0.002743	0.008886
9.000000	0.002000	0.002000	0.011094
10.000000	0.001503	0.001503	0.013635
11.000000	0.001158	0.001157	0.016053
12.000000	0.000911	0.000910	0.018492
13.000000	0.000729	0.000729	0.020892
14.000000	0.000593	0.000593	0.023181
15.000000	0.000488	0.000488	0.025463
16.000000	0.000407	0.000407	0.027339
17.000000	0.000343	0.000343	0.029330
18.000000	0.000292	0.000292	0.030902
19.000000	0.000250	0.000250	0.032317
20.000000	0.000216	0.000216	0.033873
22.000000	0.000164	0.000164	0.035623
24.000000	0.000128	0.000128	0.036414
26.000000	0.000102	0.000102	0.036333
28.000000	0.000082	0.000082	0.035473
30.000000	0.000067	0.000067	0.033901
32.000000	0.000056	0.000056	0.031769
34.000000	0.000047	0.000047	0.029012
36.000000	0.000039	0.000039	0.025983
38.000000	0.000034	0.000034	0.022530
40.000000	0.000029	0.000029	0.018955
42.000000	0.000025	0.000025	0.015330
44.000000	0.000022	0.000022	0.011537
46.000000	0.000019	0.000019	0.007479
48.000000	0.000017	0.000017	0.003681

F(X)=X**2*EXP(-X)
THE LAPLACE TRANSFORM OF F(X) IS 2./(S+1)**3

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
0.077000	1.607234	1.600972	0.391126
0.083330	1.575479	1.573072	0.152957
0.090900	1.543055	1.540550	0.162562
0.100000	1.504775	1.502633	0.142547
0.111110	1.459851	1.458006	0.126567
0.125000	1.406629	1.404663	0.139928
0.143000	1.341246	1.339343	0.142053
0.166670	1.261433	1.259465	0.156211
0.200000	1.158743	1.157409	0.115274
0.250000	1.025087	1.023999	0.106264
0.300000	0.911187	0.910334	0.093682
0.333300	0.844603	0.843815	0.093432
0.375000	0.770021	0.769346	0.087678
0.400000	0.729456	0.728864	0.081230
0.450000	0.656513	0.656034	0.072994
0.500000	0.592964	0.592593	0.062723
0.550000	0.537374	0.537076	0.055590
0.600000	0.488526	0.488282	0.049975
0.650000	0.445422	0.445224	0.044407
0.700000	0.407243	0.407084	0.039284
0.750000	0.373305	0.373178	0.034196
0.800000	0.343037	0.342936	0.029339
0.850000	0.315958	0.315875	0.026172
0.900000	0.291654	0.291588	0.022486
0.950000	0.269779	0.269728	0.018805
1.000000	0.250040	0.250000	0.016117
1.099999	0.215984	0.215960	0.011206
1.200000	0.187843	0.187829	0.007489
1.299999	0.164385	0.164379	0.003336
1.400000	0.144677	0.144676	0.000865
1.500000	0.127998	0.128000	0.001350
1.599999	0.113788	0.113792	0.002986
1.700000	0.101606	0.101611	0.004224
1.799999	0.091103	0.091108	0.005103
1.900000	0.081999	0.082004	0.006251
2.000000	0.074069	0.074074	0.006518
2.200000	0.061031	0.061035	0.007611
2.400000	0.050881	0.050885	0.008448
2.599999	0.042863	0.042867	0.009472

S	CALCULATED TRANSFORM	ACTUAL TRANSFORM	PCT ERROR
2.799999	0.036445	0.036448	0.010262
3.000000	0.031246	0.031250	0.011444
3.299999	0.025152	0.025155	0.013180
3.599999	0.020544	0.020547	0.015229
4.000000	0.015997	0.016000	0.017975
4.400000	0.012699	0.012701	0.021147
4.799999	0.010248	0.010251	0.024386
5.000000	0.009257	0.009259	0.026111
5.500000	0.007280	0.007283	0.030487
6.000000	0.005829	0.005831	0.035203
7.000000	0.003904	0.003906	0.044906
8.000000	0.002742	0.002743	0.054603
9.000000	0.001999	0.002000	0.064273
10.000000	0.001502	0.001503	0.073492
11.000000	0.001156	0.001157	0.081633
12.000000	0.000910	0.000910	0.088571
13.000000	0.000728	0.000729	0.094044
14.000000	0.000592	0.000593	0.097990
15.000000	0.000488	0.000488	0.100231
16.000000	0.000407	0.000407	0.100606
17.000000	0.000343	0.000343	0.099260
18.000000	0.000291	0.000292	0.096458
19.000000	0.000250	0.000250	0.091922
20.000000	0.000216	0.000216	0.086041
22.000000	0.000164	0.000164	0.071370
24.000000	0.000128	0.000128	0.053672
26.000000	0.000102	0.000102	0.034686
28.000000	0.000082	0.000082	0.015740
30.000000	0.000067	0.000067	0.001756
32.000000	0.000056	0.000056	0.016682
34.000000	0.000047	0.000047	0.028388
36.000000	0.000039	0.000039	0.036634
38.000000	0.000034	0.000034	0.040657
40.000000	0.000029	0.000029	0.040920
42.000000	0.000025	0.000025	0.037139
44.000000	0.000022	0.000022	0.029703
46.000000	0.000019	0.000019	0.018734
48.000000	0.000017	0.000017	0.004708

$h_D(t)$. A procedure to find p(s) must now be developed. In order to find p(s), one starts with a value of s and $h_D(s)$, then one searches the set of $h_N(s)$ until reaching a value of $h_N(s)$ which is less than $h_D(s)$. This value of $h_N(s)$, along with preceding and following values of $h_N(s)$ are chosen, and these three values are fitted to a second order polynomial.

$$h_N(s^*) = as^{*2}+bs^*+c \qquad s_{i-1} < s^* < s_{i+1} \qquad (A.7)$$

Where s_i is the first value of s, where $h_N(s) \geq h_D(s)$. Equation A.7 can be solved directly for s* by setting $h_N(s)=h_D(s)$. However,

$$s^*=s+p(s) \qquad \text{Therefore,}$$

$$p(s)=s^*-s=\frac{-2as -b + (b^2-4a(c-h_D(s)))^{1/2}}{2a} \qquad (A.8)$$

This method can only be employed if there are no sharp discontinuities in the value of s at which $h_N(s)$ is evaluated. If a large discontinuity is found, p(s) is evaluated by linear extrapolation with the nearer value of s to the actual s*.

The Moment Method

The second method is the moment method. This method is based on assuming a model for the extravascular space and calculating the various model parameters from the moments. Equations 3.19 and 3.20 can be used to calculate parameters R and λ if we assume that the extravascular space is well mixed. Equations have also been developed to solve the moment relationships for an extravascular space which behaves like a reflected diffusor.

The moments are computed by constructing a pseudo function, g(t), which consists of multiplying h(t) by t^{k-1}, point for point, for the k^{th} moment. Then one must recompute the quadratic coefficients for g(t) and then calculate M_{kh} by equation A.9

$$M_{Kh} = \sum_{j=1}^{n} \frac{a_j}{3}(t_{2j+1}^3 - t_{2j-1}^3) + \frac{b_j}{2}(t_{2j+1}^2 - t_{2j-1}^2) + C(t_{2j+1} - t_{2j-1}) \quad (A.9)$$

The Modified Extraction

The basis of the modified extraction factor method is a limiting process. However, numerically the computer cannot calculate a limit, therefore, if the equation is evaluated near the appearance time, an accurate representation of λ can be obtained. Furthermore, all values of λ, calculated, are lower than the true λ, consequently the highest calculated λ can be used as a measure of the lower bound on λ.

The Martin deJulian, and Yudilevich Method

With the exception of the case where the capillary space acts as a single well mixed compartment, $h_N(ta)$ and $h_D(ta)$ are zero. Therefore, one cannot directly compute $h_D(ta)/h_N(ta)$, and the following extrapolation procedure is to calculate $h_D(ta)/h_N(ta)$. Take points two through six from the $h_N(t)$ and $h_D(t)$ curves and compute $\Omega(t)$. For the five values of $\Omega(t)$ computed, a straight line is fitted using least squares. Then using the least squares straight line, extrapolate $\Omega(t)$ back to the appearance time. Then $E_o=1-\Omega(ta)$ and permeability and λ can be calculated.

The Bassingthwaighte Method For Minimum $h_D(t)/h_N(t)$

The computation of the minimum $h_D(t)/h_N(t)$ was accomplished by first computing $\Omega(t)$ and then checking for the minimum value of $\Omega(t)$. No attempt was made to compute $\Omega(t)$ for values other than those available from the data points. It is felt that this minimum value shall not differ from the true minimum by a maximum of a few percent.

The CRA Methods

1. t_p equals peak time

In order to determine the point at which the integration shall be stopped, $h_N(t)$ was scanned for the first maximum value. This time was taken to be the peak time. The integration technique used was similar to the one described before for calculation of the mean. $h_N(t)$ and $h_D(t)$ are broken up into small segments and fitted to a second order polynomial. Each polynomial can then be integrated explicitly and the

integral can be expressed as a sum. Thus,

$$\int_0^{t_p} h(t) = \sum_{J=1}^{K^*} \frac{a_j}{3}(t_{2j+1}^3 - t_{2j-1}^3) + \frac{b_j}{2}(t_{2j+1}^2 - t_{2j-1}^2) - c_j(t_{2j+1} - t_{2j-1}) \quad \text{(A. 10)}$$

where $k^* = \frac{n-1}{2}$ and n = number of the data point at which $h_N(t)$ has its peak.

2. t_p equals minimum $h_D(t)/h_N(t)$ time

The minimum value of $\Omega(t)$ has been established for the Bassingthwaighte method. Simultaneously, the time t, at which the minimum $\Omega(t)$ has also been determined. The integration is done in accordance to equation A.10 where $k^* = \frac{n-1}{2}$ and n is the number of the data points until Ω is a minimum.

3. t_p equals the crossover time

The crossover time is established by scanning the vector for the first value $\Omega(t)$ that is greater than one. The integration is accomplished by use of equation 4.10 where $k^* = \frac{n-1}{2}$ and n = the number of the data point until $\Omega(t) \geq 1$.

APPENDIX B

REFLECTED DIFFUSOR, WELL MIXED COMPARTMENT, STRETCHES

Throughout our discussions we have dealt with three basic types of EVS transfer functions: (1) Reflected Diffusors (2) Well Mixed compartments, (3) Stretches. The well mixed compartment is a special case of a Reflected Diffusor, namely that of $\theta = 0$. Similarly the stretch is a special case of a well mixed compartment, namely $\lambda = \infty$.

The basic differential equation for the reflected diffusor is:

$$\frac{\partial C(z,t)}{\partial t} = \mathcal{D} \frac{\partial^2 C(z,t)}{\partial z^2} \qquad 0 \leq z \leq L \qquad \text{(B.1)}$$

The differential equation for a one dimensional diffusor.

At the far end of the diffusor, $z = L$, we impose a reflecting boundary condition.

$$\frac{\partial C(L,t)}{\partial t} = 0 \qquad \text{(B.2)}$$

Shinnar (30) has shown for a linear two phase system, p(s) is independent of the capillary space transfer function

and for a reflected diffusor p(s) is given by:

$$\rho(s) = \frac{\frac{R}{\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}}{1 + \frac{R}{\lambda\theta}\sqrt{\theta s}\tanh\sqrt{\theta s}} \qquad \text{(B.3)}$$

where

$$\theta = L^2/\alpha$$

R = EVS volume/capillary volume

λ is defined by Eq 2.1

In the special case where $\theta \downarrow 0$, which gives us the transfer function of a well mixed compartment we get

$$\rho(s) = \frac{Rs}{1 + \frac{Rs}{\lambda}} \qquad \text{(B.4)}$$

If we now go one step further and allow $\lambda \uparrow \infty$, we get the transfer function for a stretch:

$$p(s) = Rs \qquad \text{(B.5)}$$

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