APPLICATION AND ANALYSIS OF A MICROSCOPE DIFFUSION CHAMBER

by

Keith D. Sherban

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Mechanical Engineering

1987

ABSTRACT

THE APPLICATION AND ANALYSIS OF A
MICROSCOPE DIFFUSION CHAMBER

by

Keith D. Sherban

A microscope diffusion chamber has been used to determine the equilibrium and non-equilibrium osmotic response of individual cells. The chamber allowed the direct observation of a cell subjected to a change in concentration at a specified temperature. Cell systems used in this work were egg lecithin liposomes and human lymphocytes. For each cell system the normalized osmotically inactive volume, membrane hydraulic permeability (for five specific temperatures) and membrane activation energy have been determined.

The raw data generated were processed by a computer algorithm which used a parameter estimation technique to yield the best statistical estimate of the membrane permeability. The program was made "user friendly" by setting up the algorithm to run in menu format and converted to an IBM PC. In addition, the program also allowed the user to run simulations for sensitivity studies and the design of experiments.

A major finding/result is that the estimated parameters using this device and software matched previously reported results for egg lecithin liposomes and human lymphocytes. This suggests that the device and software can be applied successfully for determining water permeability of cell membranes, including temperature effects.

ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor Dr. McGrath. I am grateful for his support, guidance and friendship.

I would like to thank my wife for her support, patients and never ending encouragement.

I am deeply grateful to the members of my family who have also patiently supported me.

I would like to thank John Tu, Mark Melkerson and Mosen Shabana of the Bioengineering Transport Lab for their help and guidance.

I would like to thank the Ostrom's for their support and the use of their computer systems.

I would also like to thank God for this opportunity and His never ending patients and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

A Area

b Estimat

C Concentr

D Diffusi

Ea Activat

Hd Convect

j_i Flux of

L_{ph} Phen

n Number

Q Work Du

R Cell Me

Rest Esti

P Pressur

P Number

R Gas Co

Re Cell Re

Re Cell Re

S Entropy

S Sum of

T Tempera

t Time

V Volume

V Partial

W Source

NOMENCLATURE

A Area

b Estimated Parameter

C Concentration

D Diffusivity of Solute in Specified Medium

Ea Activation Energy

Hd Convective Mass Transfer Coefficient

J_i Flux of Species i

L_{ij} Phenomenlogical Coefficient

n Number of Data Points

Q Work Due to Heat

P Cell Membrane Water Permeability

Pest Estimated Permeability

p Pressure

p Number of Parameters

R Gas Constant

Rc Cell Radius - Predicted

Rm Cell Radius - Measured

S Entropy

S Sum of the Squares Function

T Temperature

t Time

V Volume

$\bar{v}$ Partial Molar Volume

w Solute Permeability

N Length

x Length

F Force

EI Sens

V Measure

X Length

x Length

X_i Force

Xij Sensitivity Coefficient

Y Measured Parameter

b Osmotic

c Cell

D Diffusi

i Initial

i Specie

j Specie

m Membra

p Pressu

s Solute

w Solven

1 Dialys

2 Sample

3 Inside

SUBSCRIPT NOMENCLATURE

b Osmotically Inactive

c Cell

D Diffusion

i Initial Condition

i Species i

j Species j

m Membrane

p Pressure

s Solute

w Solvent

1 Dialysis Membrane Region

2 Sample Region

3 Inside the Cell of Interest

GREEK NOMENCLATURE

β Parameter

ϵ Error

π Osmotic Pressure

τ Molar Fraction

$\in$ Volume Fraction

σ Reflection Coefficient

μ Chemical Potential

Φ Dissipation Function

θ Standard Deviation

η Predicted Parameter

ψ Covariance of Errors

1.1 Backgr

The c

organisms

units. Th

change. Is

type of s

understand

subjected

water 50

freezing

process,

or injure

occur, an

freezing

formation

stress.

intracell

warming.

cooling.

The

osmotic

that will

behavior

CHAPTER 1

Introduction

1.1 Background and Motivation for Present Work

The cell is the most basic unit of living material. All biological organisms are composed of one or more of these fundamentally similar units. Understanding the reaction of a cell, due to an environmental change, is a concern of biologists and other scientists. One particular type of scientist, namely the cryobiologist, is interested in understanding what happens to a cell and its membrane as the cell is subjected to low temperatures. Since a cell is composed primarily of water (50-90%), the temperature of $0^{o}C$ is of considerable interest: the freezing point of water (at atmospheric conditions). During the freezing process, it has been shown that the membrane of the cell becomes damaged or injured. It has been suggested that two distinct mechanisms of injury occur, and this has been formulated into the "two-factor" hypothesis of freezing injury.[1] Specifically, at low rates of cooling, all ice formation is extracellular and injury is a direct result of osmotic stress. At high rates of cooling, cells are damaged by the nucleation of intracellular ice, and by its subsequent recrystallization during warming. An optimal recovery is then observed at an intermediate rate of cooling.[2]

The important phenomenon of interest for this paper deals with the osmotic responses that occur during cooling and warming. The problem that will be addressed is the equilibrium and non-equilibrium osmotic behavior of a cell at a given temperature. To further explain, recall

from abov

extracell

experienc

quite lar

tal resp

volume.

concentra

suffers d

Typi

of cells

character

sample vo

concentra

calibrati

an indire

scatterin

determine

This tech

subjects

information

With

developed

provide q

observatio

volumes

concentra

from above that during a low rate of cooling all ice formation is extracellular. As the extracellular water freezes, the cell begins to experience an increase in extracellular solute concentration which can be quite large, depending on the environmental/experimental conditions. The cell responds by expelling water from inside thus decreasing the cell volume. In other words, the cell is trying to increase its intracellular concentration in order to reestablish an equilibrium state. The cell suffers damage due to dehydration.

Typical techniques for determining the equilibrium osmotic behavior of cells includes one or more of the following undesirable characteristics: individual cells cannot be monitored; relatively large sample volumes are required; manipulation of the extracellular solute concentration is inconvenient and/or time consuming; rather extensive calibration procedures are required because the experimental technique is an indirect one.[3] Optical techniques using light absorption and scattering have been used in stopped-flow spectrophotometers in order to determine the transient osmotic behavior of biological cells.[4,5,6] This technique is indirect, requires relatively large sample volumes, subjects cells to substantial shearing stresses, and yields no information about individual cells, only the average.[3]

With these undesirable characteristics in mind, J.J. McGrath developed the microscope diffusion chamber. The chamber attempts to provide the following ideal characteristics: (1) allows real time direct observation of individual cells in suspension, (2) requires small sample volumes (10-1000 microliters), (3) allows rapid change of extracellular concentrations (1-10 seconds), (4) allows repeated changes in

extracellular solutions, (5) allows temperature control of the medium, (6) allows the use of all standard microscope optical techniques, and (7) is simple and economical to construct and use.[3] Although other microscope diffusion chambers have been developed, none have all the above characteristics, while the chamber developed by McGrath does.

1.0 Statem

The w

microscope

at the Bio

State Univ

Shabana, C

diffusion

aid with C

liposomes

temperatu

extends th

at differe

applies th

lymphocyte

uses the s

the membr

parameters

The f

informatio

chamber.

as a cell

analysis.

of how the

To obtain

experiment

1.2 Statement of Objective

The work presented here is part of an ongoing study of the microscope diffusion chamber developed by J.J. McGrath and was conducted at the Bioengineering Transport Process Laboratory (BTPL) of Michigan State University. Previous work performed by S.P. Nowlen, S.M. Tu and M. Shabana, of the BTPL, initiated the testing and application of the diffusion chamber along with developing parameter estimation software to aid with data analysis. Their work consisted of using cell systems of liposomes and unfertilized hamster ova and was carried out at room temperature. The work in this thesis has accomplished the following: (1) extends the diffusion chamber capabilities to allow experiments to be run at different temperatures; (2) improves the software usability; (3) applies the diffusion chamber to the cell systems of liposomes and human lymphocytes; (4) uses the software to clarify previous results, and (5) uses the software to study the sensitivity of the estimated parameter (the membrane water permeability) with respect to a change in input parameters.

The first step toward these goals was to research and gather information about the work accomplished in the BTPL using the diffusion chamber. At the time, S.M. Tu was conducting experiments using liposomes as a cell system and developing a computer program to aid with data analysis. It was determined that the best way to gain an understanding of how the diffusion chamber operated was by trying to verify the results Tu obtained. This was accomplished by using liposomes under the same experimental conditions and analyzing the results using Tu's computer

program.

Once t

experimenta

different t

this consi

the inside

device use

circulatin

chamber's

control th

Figure 4.3

and a digi

distributi

at various

The c

the data a

something

during run

thrust to

explain to

this, the

user for t

so that it

with the g

PRIME 750,

Engineering

program.

Once this was accomplished, the next step was to adapt the experimental system to allow for experiments to be carried out at different temperatures. The diffusion chamber was already designed with this consideration in mind. It had a heat exchange channel running along the inside of the outer edge of the chamber body (see Figure 4.2.2). The device used to control the chamber's temperature was a refrigerated circulating bath. The bath was connected directly to the diffusion chamber's heat exchange channel. The bath's fluid was also used to control the temperature of the isotonic and hypertonic solutions (see Figure 4.3.1). Once the experimental system was set up, thermocouples and a digital display device were used to characterize the temperature distribution of the diffusion chamber and to monitor of the temperature at various points of the experimental system during an experimental run.

The computer program developed by Tu worked well, providing all of the data and the parameters were entered correctly. However, if something was not entered properly and/or the user encountered an error during run time, it was often difficult to locate the cause. The main thrust to improve the usability of the program was to have the program explain to the user what the program required and accomplished. To do this, the program was set up in a menu driven format which prompted the user for the necessary input. In addition, the program was also adapted so that it could be used with an IBM personal computer in conjunction with the graphical software PLOTIT. The original program resided on the PRIME 750, a minicomputer, available at the Case Center in the Engineering building at M.S.U.

The experimental work included conducting experiments using liposomes and lymphocytes as cell systems because results existed in literature for comparison. The types of experiments performed resulted in the data necessary to generate cell inactive volumes, membrane permeabilities and membrane activation energies for each cell system, with the exception of conducting experiments for the liposomes cell inactive volume. This work was previously performed by R. Callow of the BTPL. These results were compared with those reported by S.M. Tu and R. Callow for liposomes, and Porsche and Hempling for lymphocytes and were found to be in good agreement. Therefore this establishes validity of this approach.

Initially, the parameter estimation software was used to help clarify work previously done by M. Shabana using unfertilized hamster ova. When Shabana carried out his experiments and analyzed the data, the zero time was taken to be when the cell started to "respond", by visually detecting a fluctuation or shrinkage in the outer membrane of the cell. However, this is not the true zero time. By reexamining the recorded video tapes the delay time was approximated to be 6 seconds. Thus 6 seconds was added to each time interval for each set of data and new cell membrane permeabilities were estimated. Then these newly calculated permeabilities were compared to those calculated by Nowlen, using the same sets of data but different parameter estimation techniques, and were found to be in better agreement (see Appendix C for details).

The software was also applied to define the experimental conditions. By having an approximate value for the cell membrane permeability (from pervious work and published literature), the experimental conditions

could be e

history fo

the experi

desirable

radius shr

measuring

The m

was to ent

membrane p

of data ob

and 5.3.2.

Final

sensitivit

the result

experiment

experiment

what would

increased

'sensitive

was with r

Thus estim

could be entered and the program could calculate a simulated volume time history for the cell. Based on the outcome of the volume time history, the experimental conditions could be adjusted to give a better or more desirable volume history. The criteria used to here was to have the cell radius shrink by 15% or more because this allowed for greater ease in measuring volume history.

The most obvious use and primary reason for creating the software was to enter the actual data from experimental runs to estimate a membrane permeability for an individual cell. This was done for each set of data obtained. The results are tabulated in Section 5.3, Tables 5.3.1 and 5.3.2.

Finally, the program was used for sensitivity studies. The sensitivity studies were performed to help give a better understanding of the results generated in this thesis. The question was asked, if the experimental conditions were similar to those encountered during experiments with liposomes and lymphocytes, thus giving similar results, what would happen to the estimated permeability if an input parameter was increased or decreased? By doing such a study, one could determine how "sensitive" the estimated parameter, in this case the cell permeability, was with respect to a change in a particular input parameter of interest. Thus estimates of experimental uncertainties could be made.

2.1 Syste

A sch

Figure 2.1

chamber sa

isotonic s

continuous

boundary l

membrane.

dialysis m

practicall

well model

which has

CHAPTER 2

Analysis

2.1 System definition

A schematic representation of the diffusion chamber is given in Figure 2.1.1. Initially the cell specimen is place into the diffusion chamber sample region and the bulk flow region is filled with the isotonic solution. At zero time, the bulk flow region is flushed with a continuous flow of hypertonic solution. As a result, a concentration boundary layer develops along the bottom surface of the dialysis membrane. Solute diffuses through the boundary layer, through the dialysis membrane and into the sample region. The cell responds osmotically to the concentration increase. The analysis presented here will model the solute diffusion process and the cell specimen's response, which has been incorporated into a computer program.

Schematic Representation of the Microscope Diffusion Chamber

Schematic Representation of the Microscope Diffusion Chamber

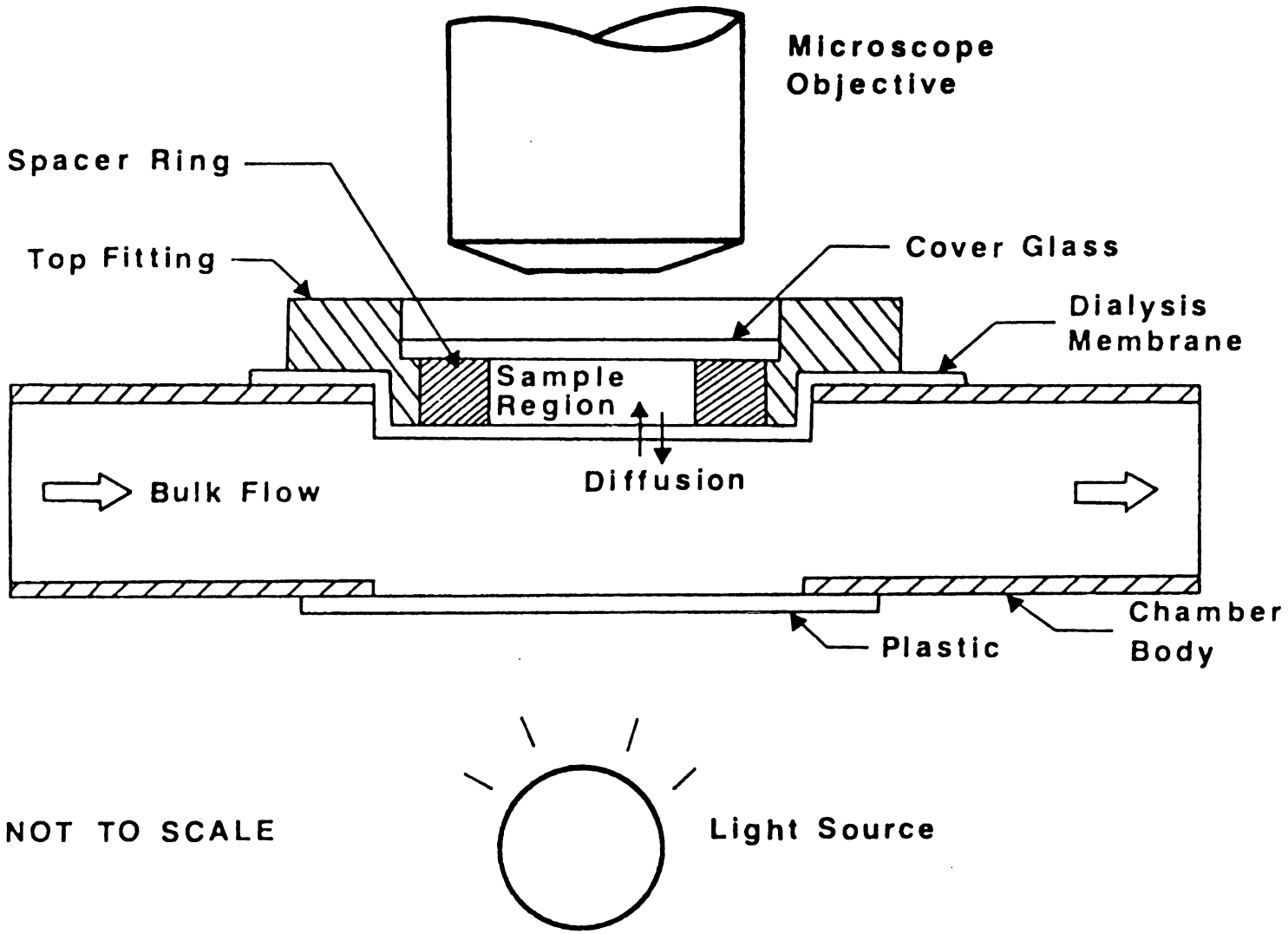


Figure 2.1.1

3.2 Model

The m

diffusion

of the cel

3.2.1 The

Since

extensive

diffusion

approach a

The a

follows:

2.2 Modeling the system

The modeling of the system can be broken into two parts: the diffusion of the solute into the sample region and the osmotic response of the cell specimen.

2.2.1 The Concentration History

Since J.J. McGrath, in conjunction with S.M. Tu, has already extensively analyzed the solute concentration history of the microscope diffusion chamber, (see reference [7] in the bibliography), their approach and results will only be summarized here.

The assumptions used to model the solute concentration are as follows:[7]

1) one dimensional transient mass transfer (developing mass transfer boundary layer is accounted for with a mean coefficient, H_d).

2) a non-selective dialysis membrane, $\sigma = 0$ [8], (i.e. solute diffuses through the membrane as it would through free solution (except the diffusivity coefficient is lower).).

3) no net volume flow in the sample region of the diffusion chamber.

4) fully developed, steady state laminar hydrodynamic conditions in the bulk flow region.

5) the diffusion chamber is isobaric and isothermal.

6) constant mass diffusivities.

7) negligible solute velocities normal to the dialysis membrane.

8) the analysis does not account for the presence of cells in the sample region.

The pr

diffusion t

impermeable

representat

mathematics

$$\frac{\delta C_{s,1}}{\delta t}$$

$$\frac{\delta C_{s,2}}{\delta t}$$

subject

H_d C_s

$C_{s,1}(z,$

$D_1 \frac{\delta C_s}{}$

$$\frac{\delta C_{s,2}(}{\delta x}$$

and th

$C_{s,1}(x,$

$C_{s,2}(x,$

$C_{s,\infty}$ =

The tec

concentratio

method. The

program SENS

1000. It st

diffusivity

surface, D_1

9) the bulk flow is assumed to step change from $C_{s,\infty}$ to C_f at the zero time.

The problem considered is therefore one-dimensional transient diffusion through two adjacent regions, one of which is bounded by an impermeable plane and the other by convective flow.[7] A schematic representation of the basic system can be seen in Figure 2.2.1. The mathematics of the problem can be described by the following equations.

$$\frac{\delta C_{s,1}(X_1,t)}{\delta t} = D_1 \frac{\delta^2 C_{s,1}}{\delta X_1^2} \quad 0 \le X_1 \le L_1 \; ; \quad t > 0 \qquad (2.2.1)$$

$$\frac{\delta C_{s,2}(X_2,t)}{\delta t} = D_2 \frac{\delta^2 C_{s,2}}{\delta X_2^2} \quad 0 \le X_2 \le L_2 \; ; \quad t > 0 \qquad (2.2.2)$$

subject to the boundary conditions:

$$H_d \left[C_{s,\infty}(t) - C_{s,1}(0,t)\right] = -D_1 \frac{\delta C_{s,1}(0,t)}{\delta X_1} \qquad (2.2.3)$$

$$C_{s,1}(L_1,t) = C_{s,2}(0,t) \qquad (2.2.4)$$

$$D_1 \frac{\delta C_{s,1}(L_1,t)}{\delta X_1} = D_2 \frac{\delta C_{s,2}(0,t)}{\delta X_2} \qquad (2.2.5)$$

$$\frac{\delta C_{s,2}(L_2,t)}{\delta X_2} = 0 \qquad (2.2.6)$$

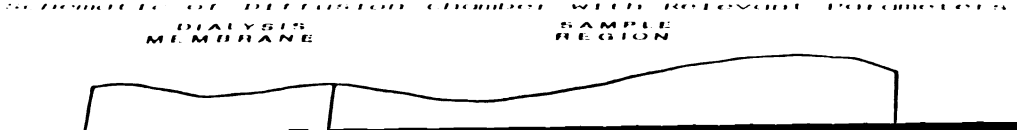
and the initial conditions:

$$C_{s,1}(X_1,0) = C_i \qquad (2.2.7)$$

$$C_{s,2}(X_2,0) = C_i \qquad (2.2.8)$$

$$C_{s,\infty} = C_i \; : \; t < 0 \qquad (2.2.9)$$

The technique used to solve these equations, and calculate the concentration history, was the backwards finite difference approximation method. These equations were formulated into a subroutine, MBCON, in the program SENS. Refer to Appendices D and E for a listing of MBCON and SENS. It should also be noted that to solve these equations the solute diffusivity in free solution, D_2, the solute diffusivity in the dialysis membrane, D_1, and the convective mass transfer coefficient, H_d, must be


DIALYSIS
MEMBRANE
SAMPLE
REGION

Schematic of Diffusion Chamber with Relevant Parameters

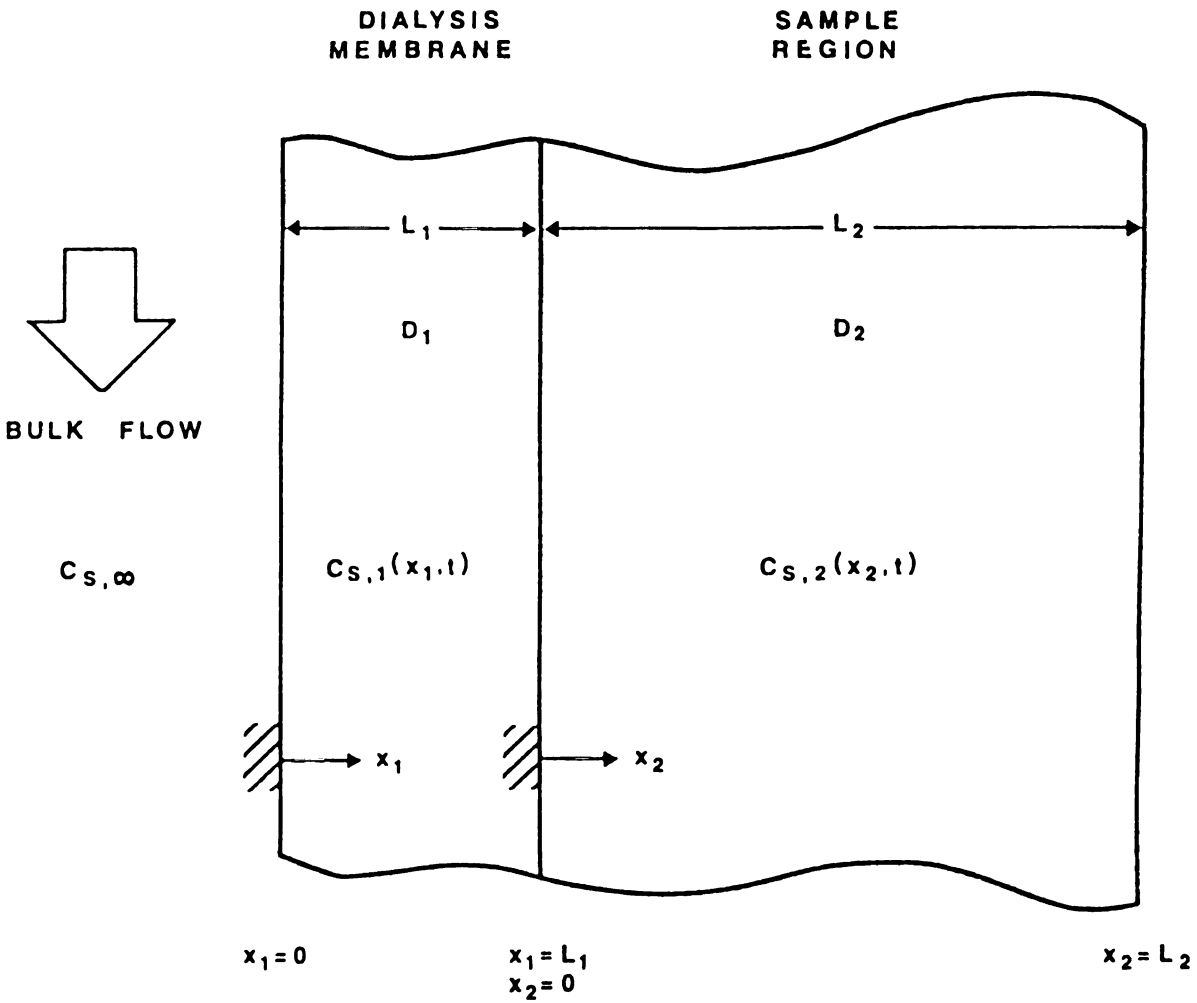


Figure 2.2.1

known. The

an appropri

The solute

manufacture

tenth of th

molecular w

dialysis me

the solute

was $D_1=D_{1,0}$

work was de

developed b

fully devel

parallel pl

approximate

which was

2.2.2 The

The ap

specimen is

Katchalsky

the classic

irreversibl

The de

flows acros

forces into

general the

known. The solute diffusivity in free solution can readily be found in an appropriate handbook (e.g. CRC Handbook of Chemistry and Physics). The solute diffusivity in the dialysis membrane has been claimed by the manufacture (ENKA AG, Product Group Membrana) to be approximately one tenth of the diffusion coefficient in free solution for compounds with a molecular weight of 300 or less.[3] (For further discussion on the dialysis membrane refer to reference [3].) Therefore, the value used for the solute diffusivity in the dialysis membrane, throughout this work, was $D_1=D_2*0.1$. The convective mass transfer coefficient used in this work was defined by J. Tu. He used an analogy from heat transfer developed by Kays and Crawford[20]. By assuming 1) hydrodynamically fully developed flow in the bulk flow region, 2) a two dimensional parallel plate system and 3) no net volume flow in the sample region, he approximated the convective mass transfer coefficient to be $H_d=D_2*10000$ (which was also used though out this work).[21]

2.2.2 The Kedem - Katchalsky Formulation

The approach used to solve for the osmotic response of a cell specimen is based on the principles developed in 1958 by Kedem and Katchalsky. Their model, the K-K Formulation, has come to be known as the classic model for membrane permeation using principles of irreversible thermodynamics[9].

The development begins by considering the forces that cause the flows across the membrane. In 1931, L. Onsager related these flows and forces into equations called the "phenomenological equations". In general these equations are written as,

$$
\begin{aligned}
J_1 &= L_{11}X_1 + L_{12}X_2 + \ldots + L_{1n}X_n \\
J_2 &= L_{21}X_1 + L_{22}X_2 + \ldots + L_{2n}X_n \\
&\vdots \qquad\qquad\qquad\qquad \vdots \\
J_n &= L_{n1}X_1 + L_{n2}X_2 + \ldots + L_{nn}X_n
\end{aligned}
\tag{2.2.10}
$$

Note that each flow, J_i (i = 1 to n), is influenced linearly by its conjugate force X_i and the nonconjugate forces X_j ($j \neq i$, j=1 to n), providing the "straight" coefficients, L_{ii}, and the "cross" or "coupling" coefficients, L_{ij} ($i \neq j$), differ form zero. Having dimensions of flow per unit force, $L_{ij} = (J_i/X_j)_{Xi}$, the coefficients have general characteristics of conductances or mobility. In addition to developing these equations Onsager also discovered the matrix of coefficients to be symmetrical; in other words $L_{ik} = L_{ki}$ for $i \neq k$. It should be noted that this linearity holds only for sufficiently slow processes occurring when the system is not too far removed from a state of equilibrium. The choice of a force conjugate to the flow J_i is restricted by the requirement that the product J_iX_i has dimensions of the rate of entropy production or decrease in free energy with time.[10] Thus this is the starting point of the thermodynamic description. The total rate of entropy change, dS/dt, is broken into the rate of entropy transfer between the system and its surroundings, d_eS/dt, and the rate of internal entropy production, d_iS/dt, which is generated by the irreversible processes occurring within the system.

$$
\frac{dS}{dt} = \frac{d_eS}{dt} + \frac{d_iS}{dt} \tag{2.2.11}
$$

If all process within a closed system occur reversibly, the rate of entropy change can be written as

$$\frac{d_e S}{dt} = \frac{1}{T} \frac{dQ}{dt} \qquad (2.2.12)$$

where dQ is the heat gained, and T is the absolute temperature. However, if the system undergoes an irreversible change the rate of entropy change is written as

$$\frac{dS}{dt} = \frac{1}{T} \frac{dQ}{dt} + \frac{d_i S}{dt} \qquad (2.2.13)$$

When dealing with irreversible processes in an isothermal system it is frequently convenient to consider the function Φ given by

$$\Phi = T \frac{d_i S}{dt} \qquad (2.2.14)$$

which Lord Rayleigh called the dissipation function. The particular processes of interest for this paper are the movements of solute and water across a cell membrane. The system which will be considered (see Figure 2.2.2) consists of 1) two compartments separated by a membrane of thickness Δx and surface area A_c and 2) two solutions of the same solvent and solute, also separated by the membrane. The outer compartment is designated by ^{2} and the inner compartment by ^{3}.

The general dissipation function for the two component system of solvent and solute, for an isothermal system, is written as

$$\Phi = J_w X_w + J_s X_s \qquad (2.2.15)$$

where w denotes the solvent and s denotes the solute. Focusing attention within the membrane of a volume element of unit area and thickness dx, the dissipation function becomes

The Two Compartment - Two Component System

The Two Compartment - Two Component System

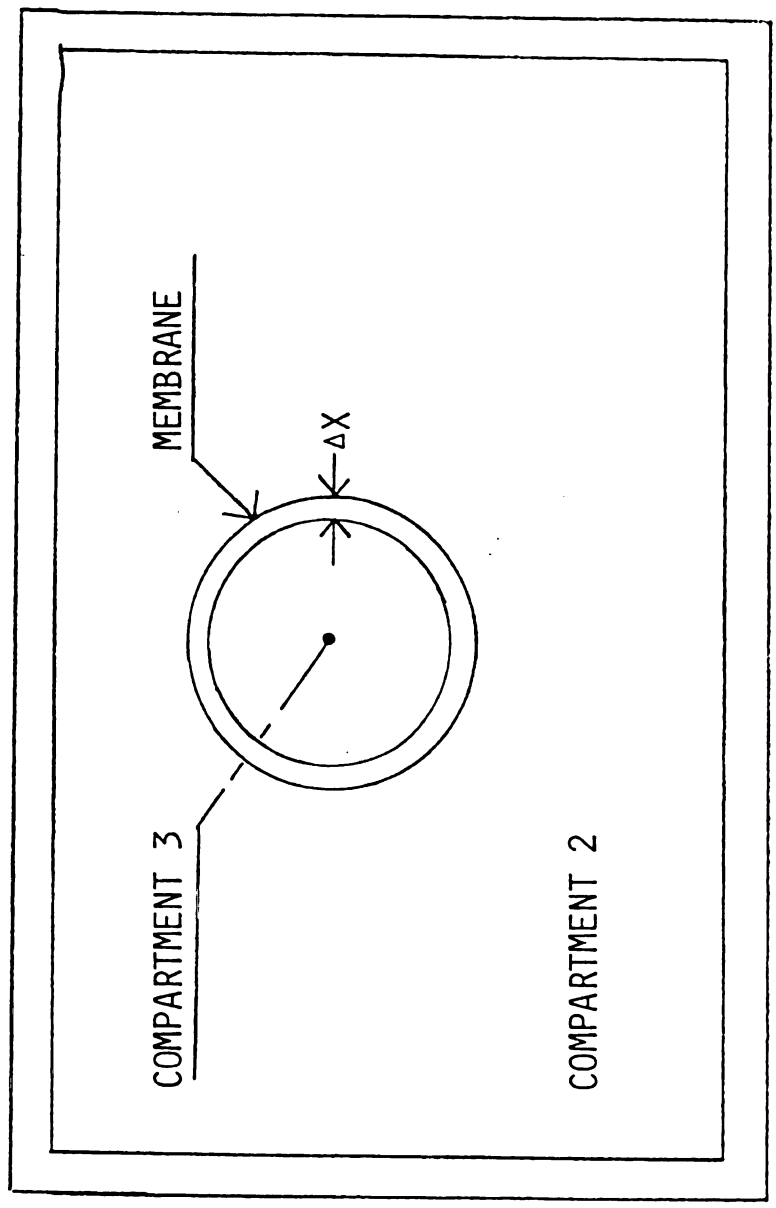


Figure 2.2.2

$$\Phi = J_w \left[-\frac{d\mu_w}{dx}\right] + J_s \left[-\frac{d\mu_s}{dx}\right] \qquad (2.2.16)$$

where μ denotes the chemical potential. Since J_w and J_s are independent of x we can integrate across the membrane from x=0 to x=Δx;

$$\Phi_m = J_w \int_0^{\Delta x} \left[-\frac{d\mu_w}{dt}\right] dx + J_s \int_0^{\Delta x} \left[-\frac{d\mu_s}{dt}\right] dx \qquad (2.2.17)$$

Making the assumption that the chemical potentials at the surfaces of the membrane are the same as those in the adjacent solutions the dissipation function becomes

$$\Phi_m = J_w\Delta\mu_w + J_s\Delta\mu_s \qquad (2.2.18)$$

where $\Delta\mu_w = \mu_{w,2} - \mu_{w,3}$ and $\Delta\mu_s = \mu_{s,2} - \mu_{s,3}$. If the solution is considered to be ideal, the chemical potential is approximated by

$$\Delta\mu_j = \bar{v}_j\Delta p + RT\Delta(\ln\gamma_j) \qquad (j= w \text{ or } s) \qquad (2.2.19)$$

where $\bar{v}$ is the partial molar volume of j, Δp in the difference in pressure between the outer and inner compartment, γ the molar fraction of constituent j. To further simplify equation (2.2.19), we also assume both solutions dilute. This implies the volume fraction of solute is small, $\epsilon = C_s\bar{v}_s \ll 1$, thus

$$\Delta\mu_s = \bar{v}_s\Delta p + RT\frac{\Delta C_s}{\bar{C}_s} \qquad (2.2.20)$$

where

$$\bar{C}_s = \frac{\bar{C}_{s,2}(x_2,t) + \bar{C}_{s,3}(t)}{2}$$

and

$$\Delta\mu_w = \bar{v}_w\Delta p + RT\frac{\Delta C_s}{\bar{C}_w} \qquad (2.2.21)$$

where

$$\bar{\bar{C}}_w = \frac{1 - \epsilon}{\bar{\bar{v}}_w} \approx \frac{1}{\bar{\bar{v}}_w}$$

Introducing (2.2.19) and (2.2.23) into equation (2.2.18), the dissipation function becomes

$$\Phi_m = J_w \left(\bar{\bar{v}}_w \Delta p - \frac{RT}{\bar{\bar{C}}_w}\Delta C_s\right) + J_s \left(\bar{\bar{v}}_w \Delta p + \frac{RT}{\bar{\bar{C}}_s}\Delta C_s\right) \quad (2.2.22)$$

Rearranging Φ_m we get

$$\Phi_m = (J_w\bar{\bar{v}}_w + J_s\bar{\bar{v}}_s)\Delta p + \left(\frac{J_s}{\bar{\bar{C}}_s} - \frac{J_w}{\bar{\bar{C}}_w}\right)RT\Delta C_s \quad (2.2.23)$$

Note in equation (2.2.20) a new set of forces and flows represent the dissipation function. The new forces are the hydrostatic pressure, $\Delta p = X_p$ and the osmotic pressure, $RT\Delta C_s = X_D$. The new conjugate forces are the total volume flow per unit area,

$$J_v = J_w\bar{\bar{v}}_w + J_s\bar{\bar{v}}_s \quad (2.2.24)$$

and the velocity of solute relative to solvent,

$$J_D = \frac{J_s}{\bar{\bar{C}}_s} - \frac{J_w}{\bar{\bar{C}}_w} \quad (2.2.25)$$

which is called the exchange flow. Writing these new flows and forces in terms of phenomenological equations we get

$$J_v = L_p\Delta p + L_{pD}\Delta\pi \quad (2.2.26)$$

$$J_D = L_{Dp}\Delta p + L_D\Delta\pi \quad (2.2.27)$$

where $\Delta\pi = RT\Delta C_s$. Making use of Onsager's reciprocal relation, $L_{pD} = L_{Dp}$, equation (2.2.26) and (2.2.27) become

$$J_v = L_p\Delta p + L_{pD}\Delta\pi \quad (2.2.28)$$

$$J_D = L_{pD}\Delta p + L_D\Delta\pi \qquad (2.2.29)$$

To further clarify the significance of these equations, the phenomenological coefficients, L_p, L_D and L_{pD}, are transformed into other coefficients that allow for more convenient comparison with experimental data, w_s, $\acute{U}_s$ and P.

Before deriving these new coefficients we must first look at the flows that are defined. Instead of studying the exchange flow, J_D, it would be more advantageous to study the solute flow J_s. By rearranging equations (2.2.24) and (2.2.25) and assuming $\bar{C}_w\bar{v}_w=1$

$$J_s = \frac{(J_s + J_D)}{(\bar{v}_s\bar{C}_s + 1)}\bar{C}_s \qquad (2.2.30)$$

Also, by assuming the solution on both sides of the membrane dilute, $\bar{v}_s\bar{C}_s \ll 1$, (2.2.30) becomes

$$J_s = (J_v + J_D)\bar{C}_s \qquad (2.2.31)$$

The first coefficient to be determined will be w_s, the solute permeability. To accomplish this experimentally, it is more convenient to have conditions of zero volume flow across the membrane, $J_v=0$. If a concentration difference exists across the membrane, a pressure difference can be applied such that $J_v=0$, according to equation (2.2.28). Rearranging (2.2.28) becomes

$$\Delta p = -\left[\frac{L_{pD}}{L_p}\right]\Delta\pi \qquad (2.2.32)$$

Substituting equation (2.2.29) and (2.2.32) into (2.2.31) yields

$$J_s = \left[\frac{L_pL_D - L_{pD}^2}{L_p}\right]\bar{C}_s\Delta\pi \qquad (2.2.33)$$

The solute permeability is defined to be:

$$w_s = \left[\frac{L_p L_p - L_{pD}^2}{L_p}\right]\bar{C}_s \qquad (2.2.34)$$

The second coefficient to be defined is the reflection coefficient, which was originally introduced by Staverman in 1951. This coefficient is defined by

$$\sigma_s = -\frac{L_{pD}}{L_p} = -\left(\frac{J_D}{J_v}\right)\Delta\pi=0 \qquad (2.2.35)$$

Imposing similar conditions as described above, where J_v=0, (2.2.32) becomes

$$\Delta p = \sigma_s RT\Delta C_s$$

and rearranging

$$\sigma_s = \frac{\Delta p}{RT\Delta C_s} \qquad (2.2.36)$$

The value of σ_s ranges between 0 and 1 where for an ideal semipermeable membrane σ_s=1. In other words, all of the solute is reflected. Thus, for σ_s<1 implies only some of the solute is reflected.

Finally the last coefficient to be described is P, the membrane hydraulic permeability. This coefficient is defined letting

$$P = \frac{L_p RT}{\bar{v}_w} \qquad (2.2.37)$$

where $\bar{v}_w$ is the molar volume of the solvent.

By using these definition for w_s, σ_s and P, J_v and J_s can be written in a more useful form. Recall form (2.2.28)

$$J_v = L_p\Delta p + L_{pD}\Delta\pi$$

Substituting in (2.2.35) and (2.2.37), the volume flux becomes

$$J_v = \bar{v}_w P \left[\frac{\Delta p}{RT} - \sigma_s \Delta C_s\right] \qquad (2.2.38)$$

Also, recall the solute flux, J_s, from (2.2.31)

$$J_s = (J_v + J_D)\bar{C}_s$$

By substituting (2.2.28), (2.2.29), (2.2.34) and (2.2.35) into (2.2.31) we get

$$J_s = \bar{C}_s(1 - \sigma_s)J_v + w_s\Delta\pi \qquad (2.2.39)$$

Thus equations (2.2.38) and (2.2.39) are the equations commonly used to determine membrane characteristics and are known as the K-K formulation.

Summarizing the conditions for which these equations are valid are as follows:

1) The system is two compartment - two component

2) The system is in thermal equilibrium

3) The membrane is permeable to the solvent and may or may not be permeable to the solute.

4) The solutions are assumed to be ideal and dilute

5) The driving forces are considered to be sufficiently small such that a linear relation exists between the driving forces and the resulting flows.

2 - 2 - 3 The Volume Flow of a Cell

Recall from Figure 2.1.1, the cell specimen resides in the sample region of the diffusion chamber. As hypertonic solution is flushed into the bulk flow region the solute diffuses into the sample region and the cell responds osmotically. The equation that is most useful for describing the response of the cell is the equation of volume flow (2 - 2 . 38)

$$J_v = \bar{v}_w P \left[\frac{\Delta p}{RT} - \sigma_s \Delta C_s\right]$$

To further simplify this equation we must take a closer look at the experimental conditions. As mentioned previously equation (2.2.38) implies that the volume flow across the cell membrane can be caused by a hydrostatic pressure difference, Δp, and/or a concentration difference ΔC_s. However with the experimental set up used in this work it is not likely for a hydrostatic pressure difference to exist across the membrane. Therefore Δp is assumed to be zero. Also the cell's membrane is assumed to be an ideal semipermeable membrane to the solute that were used which implies σ_s=1. Taking into account these assumptions, equation (2-2-38) can be rewritten as

$$J_v = -\bar{v}_w P \Delta C_s \qquad (2.2.40)$$

This equation can be further simplified into a more useful form. First recognize that

$$J_v = \frac{1}{A_c(t)} \frac{dV_c(t)}{dt}$$

where $A_c(t)=4\pi R_c(t)^2$ for spherical systems (which was assumed), the surface area of the cell and $V_c(t)=(4/3)\pi R_c(t)^3$, the volume of the cell. Therefore (2.2.40) can be rearranged into

$$\frac{dR_c(t)}{dt} = -\bar{v}_w P \left[C_{s,2}(X_2,t) - C_{s,3}(t)\right] \qquad (2.2.41)$$

With the equation in this form the only unknowns are P and $C_{s,3}(t)$. $R_c(t)$, the radius of the cell, can be measured, $\bar{v}_w$, the partial molar volume of the solvent is a constant which can be found in the appropriate tables and $C_{s,2}(X_2,t)$ the concentration of solute outside the cell, is

obtained for from the equations described in section 2.2.1. However, the concentration inside the cell $C_{s,3}(t)$ can be written in terms of the cell volume (which in related to the cell radius). This is accomplished by using the definition of concentration (written here in terms of molality):

$$C_s = \frac{\text{moles of solute}}{\text{volume of solvent}}$$

Letting $N_{s,3}$ represent the moles of solute inside the cell and $V_w(t)$ represent the volume of solvent inside the cell, the concentration is now written as

$$C_{s,3}(t) = \frac{N_{s,3}}{V_w(t)}$$

Note, $V_w(t)$ can be written as

$$V_w = V_c(t) - V_b$$

where $V_c(t)$ is the total volume of the cell and V_b is the "inactive volume". The inactive volume of the cell is that which is not "free" solvent of the cell, i.e. the solute, bound solvent and any cell organelle material. This parameter is a constant and can be determined experimentally (see Appendix A). Therefore, the concentration within the cell is

$$C_{s,3}(t) = \frac{N_{s,3}}{V_c(t) - V_b} \qquad (2.2.42)$$

The initial concentration (t=0) within the cell is

$$C_i = \frac{N_{s,3}}{V_c(0) - V_b} \qquad (2.2.43)$$

Solving for $N_{s,3}$ and substituting into (2.2.42), the instantaneous concentration becomes

$$C_{s,3}(t) = C_i \left[\frac{V_c(0) - V_b}{V_c(t) - V_b}\right] \qquad (2.2.44)$$

by substituting (2.2.44) into (2.2.41) we get

$$\frac{dR_c(t)}{dt} = -P\bar{v}_w\left[C_{s,2}(X_2,t) - C_i\left[\frac{V_c(0) - V_b}{V_c(t) - V_b}\right]\right] \qquad (2.2.45)$$

Thus, the volume flow, J_v, has been written in a more convenient form which now describes the rate of change of the cell radius. This equation is also used in the program SENS. The method used to solve this equation was the numerical method of Runge-Kutta (4^{th} order). Since the concentration history within the sample region is known as a function of position and time, $\bar{v}_w$ is a constant (the partial molar volume of water), and V_b can be determined experimentally, the only unknown is P, the cell membrane hydraulic permeability, in equation (2.2.45).

2 - 3 The Estimation of Parameter P

As mentioned above in section 2.2.3 the membrane hydraulic permeability, P, is unknown and must be solved for. The method used to solve for the parameter P is a technique known as parameter estimation. Parameter estimation is a discipline that provides tools for the efficient use of data in the estimation of constants appearing in mathematical models and for aiding in modeling phenomena.[11] Typically the solution to an equation is thought of as solving for the state of a system given the initial conditions, the boundary conditions and the constants, or parameters, which can be found in appropriate handbooks.

However in many circumstances the parameter(s), in this case P, is (are) unknown. This is the problem that can be addressed using methods of parameter estimation.

It should be noted that there is more than one parameter estimation method available. This work involves using the ordinary least squares (OLS) method. This method, like the other estimation methods, attempts to minimize the error between the functional values generated by the mathematical model and the experimentally measured functional values, with respect to the parameter being estimated. The function used in the OLS method is

$$S = \Sigma_{i=1}^{n} (Y_i - \eta_i(\bar{\beta}))^2 \qquad (2.3.1)$$

where n is the number of data points. S is referred to as the sum of the squares function. This equation states that the differences between the measured data values, Y_i, and the corresponding predicted values from the model, η_i, are squared and summed. The goal is to minimize S with respect to the vector of parameters, $\bar{\beta}$. This is accomplished by taking the derivative of S with respect to the parameters and setting it equal to zero.

$$\frac{\delta S}{\delta \bar{\beta}} = \bar{0} \qquad (2.3.2)$$

When the values of $\bar{\beta}$ are found that satisfy equation (2.3.2) the sum of squares function has been minimized. In some cases it is possible to have more than one set of parameters that satisfy (2.3.2) due to the presence of local minimums. The ultimate desire would be to find the

global minimum. In general this is not a problem because most models that are well posed only have one minimum.

Using equation (2.3.1), the sum of squares function for this work is

$$S = \Sigma_{i=1}^{n} [Rm_i(t) - Rc_i(t)]^2 \qquad (2.3.3)$$

where $Rm_i(t)$ is the experimentally measured radius and $Rc_i(t)$ is the predicted radius, using the model equation (2.2.45), at time t. Recall the procedure normally used to minimize (2.3.3) would be to set the derivative of S with respect to P equal zero and solve for P. However, the method used to minimize S, in the program SENS, was not as direct due to the difficulty of solving for Rc(t) explicitly. Given a set of experimental data, (i.e. (t_i, Rm_i) for i=1 to n), S can be minimized by having a computer program, SENS, generate theoretical sets of data, as mentioned in section 2.2, for a likely range of P's supplied by the user, which can be found in the published literature. Each set of data can then be plugged into (2.3.3) to calculate a S. The S with the smallest value, S_{min}, corresponds to the set of data with the best curve fit of R v - s - t and the best estimate of the cell permeability, P_{est}.

Now that a method exists for estimating P, it would be beneficial to know how good of an estimate P_{est} is. This is accomplished by calculating the standard deviation of P_{est}. However, this is not an easy task because of the nonlinearity of S with respect to P. (S is said to be nonlinear in P if the sensitivity coefficient,

$$\frac{\delta S}{\delta P}$$

is a function of the parameter, P.[11]) To get around this, J. Beck formulated an approximate standard deviation by examining the covariance matrix of the parameters for models that are linear with respect to the parameters. To calculate the standard deviation of P we must start with the general sum of squares function for a linear model

$$S = (Y - X\beta)^T(Y - X\beta) \qquad (2.3.4)$$

where $\eta = X\beta$ and

$$X = X_{jk}(i) = \frac{\delta\eta_j(i)}{\delta\beta_k} \; ; \; i=1,\ldots,n; \; j=1,\ldots,n; \; k=1,\ldots,p$$

Taking the derivative of S with respect to β, setting the matrix of derivatives equal to zero and solving for β=b we get

$$b = (X^TX)^{-1}XY \qquad (2.3.5)$$

By making the following assumptions

1) $\hat{Y}_i = \eta_i + \epsilon_i$; the error, ϵ_i, is in the measuring of Y_i and is additive,
2) $E(\epsilon_i) = 0$; the expected mean value of the error is zero, (the function E(x) is the statistical function of the expected value of the variable x.)
3) Errorless independent variables,
4) Nonrandom parameters and no prior information regarding the parameters,

the covariance of (2.3.5) is

$$cov(b) = (X^TX)^{-1}X\psi X(X^TX)^{-1} \qquad (2.3.6)$$

where $\psi = E(\epsilon\epsilon^T)$. Equation (2.3.6) can now be used to approximate the covariance matrix of the parameters for nonlinear models;

$$cov(b) \approx (X^TX)^{-1}X^T\psi X(X^TX)^{-1} \qquad (2.3.7)$$

With the additional assumptions of

1) constant variance errors,
2) uncorrelated errors,
3) covariance matrix of errors is known to within a multiplicative constant,

the estimate of (2.3.7) becomes

$$\text{cov}(b) \approx (X^T X)^{-1} s^2 \; ; \quad s^2 \approx \frac{(Y - \hat{Y})^T (Y - \hat{Y})}{(n - p)} \qquad (2.3.8)$$

where n is the number of measurements, or data points, recorded, Y is the experimental measured value, $\hat{Y}$ is the predicted value and p is the number of parameters. Recall, in this work, there is only one parameter (P). Rewriting (2.3.8) for one parameter the covariance matrix simplifies to

$$\text{cov}(b) = V(b) \approx (X^T X)^{-1} s^2 \; ; \quad s^2 \approx \frac{(Y - \hat{Y})^T (Y - \hat{Y})}{(n - 1)} \qquad (2.3.9)$$

where V(b) is the statistical function variance, which is related to the standard deviation by $\theta^2(P_{est}) = V(b)$, (where θ in the standard deviation). Therefore, applying (2.3.9) the square of the standard deviation becomes

$$\theta^2(P_{est}) \approx \frac{\Sigma_{i=1}^{n} [Rm_i(t) - Rc_i(t)]^2}{\Sigma_{i=1}^{n} \left(\frac{\delta Rc_i}{\delta P_{est}}\right)^2 (n-1)} \qquad (2.3.10)$$

where $\delta Rc_i / \delta P_{est}$ is the so called sensitivity coefficient.

2.4 The Effect of Temperature on P, (The Activation Energy)

Several workers have studied the temperature dependence of the movement of water across cell membranes. The relationship used to describe this dependence obeys the classical Arrhenius type equation

$$P = k \exp[-\Delta E_a/RT] \qquad (2.4.1)$$

where k is a constant (frequency factor) and E_a is the activation energy. This equation can be rewritten as

$$\ln(P) = \ln(k) - \frac{\Delta E_a}{RT} \qquad (2.4.2)$$

By plotting ln(P) v.s. 1/T, the activation energy can determined from the slope.

CHAPTER 3

The Parameter Estimation/Simulation Program, SENS

3.1 Introduction and Background (of J. Tu's Program)

As mentioned in Section 1.2, J. Tu developed a FORTRAN computer algorithm, SENS, to be used in conjunction with the microscope diffusion chamber system to estimate membrane water permeabilities. Specifically, the program was designed to estimate the permeability of a cell membrane or run a simulation (pseudo) experiment to see what would happen during an experiment under specified conditions. Prior to running the program the (actual or simulated) experimental data and parameters were entered into an input file. The input file was read by SENS and processed. The output consisted of four optional graphs (sent to the screen and a graphics file) and an output file containing tables. The first graph plotted the normalized concentration change, in the dialysis membrane and sample region, as a function of the normalized time. The second graph plotted the sensitivity coefficient, $\delta Rc/\delta P$, as a function of time. The third plot was the normalized cell volume as a function of time. The last graph plotted the sum of the squares function versus the permeability. The first table in the output file was the input data, the second a table containing the concentration distribution, in the dialysis membrane and the sample region, as a function of time, the third a table of the experimentally measured radius as function of time and finally the best statistical estimate of the membrane hydraulic permeability, the

standard deviation of the best estimate of the permeability and the corresponding minimum sum.

Tu's program worked quite well, providing all of the parameters and data were entered into the input file correctly. Therefore, the basic structure of his program was left intact i.e. the processing of the parameters and data. The major modifications made were at the input and output stages. The input stage was revised to allow the user an option of entering the necessary input by one of two ways: 1) a (modified) input file or 2) by answering the prompted questions. The output was arranged in a more logical manner and labelled more clearly as to what was being (graphically) plotted and what tables were being generated. Also, the best estimate of the permeability, the standard deviation and the minimum sum were sent to the screen and to two output files.

3.2 The Modified SENS

At first, the concept of making SENS user friendly seemed a relatively simple task. But during the modification process it was discovered that this would not be the case. The program grew 716 (FORTRAN) programming lines (which is about 24K) to 2002 programming lines (which is about 68K).

The most efficient way to describe how SENS works would be to look at the flow chart of SENS in Figure 3.2.1. The actual program can be found in Appendix D. In addition, sample output, of the four graphs describe in Section 3.1 and of one output file, can be seen in Figures 3.2.2 - 3.2.6. Note: this sample output is from actual experimental data for an experiment using a lymphocyte at 25^{o}C.

Once the modifications were made, the next step was to convert SENS to an IBM Personal Computer.

Figure 3.2.1 - Flow Chart of SENS - PRIME Version

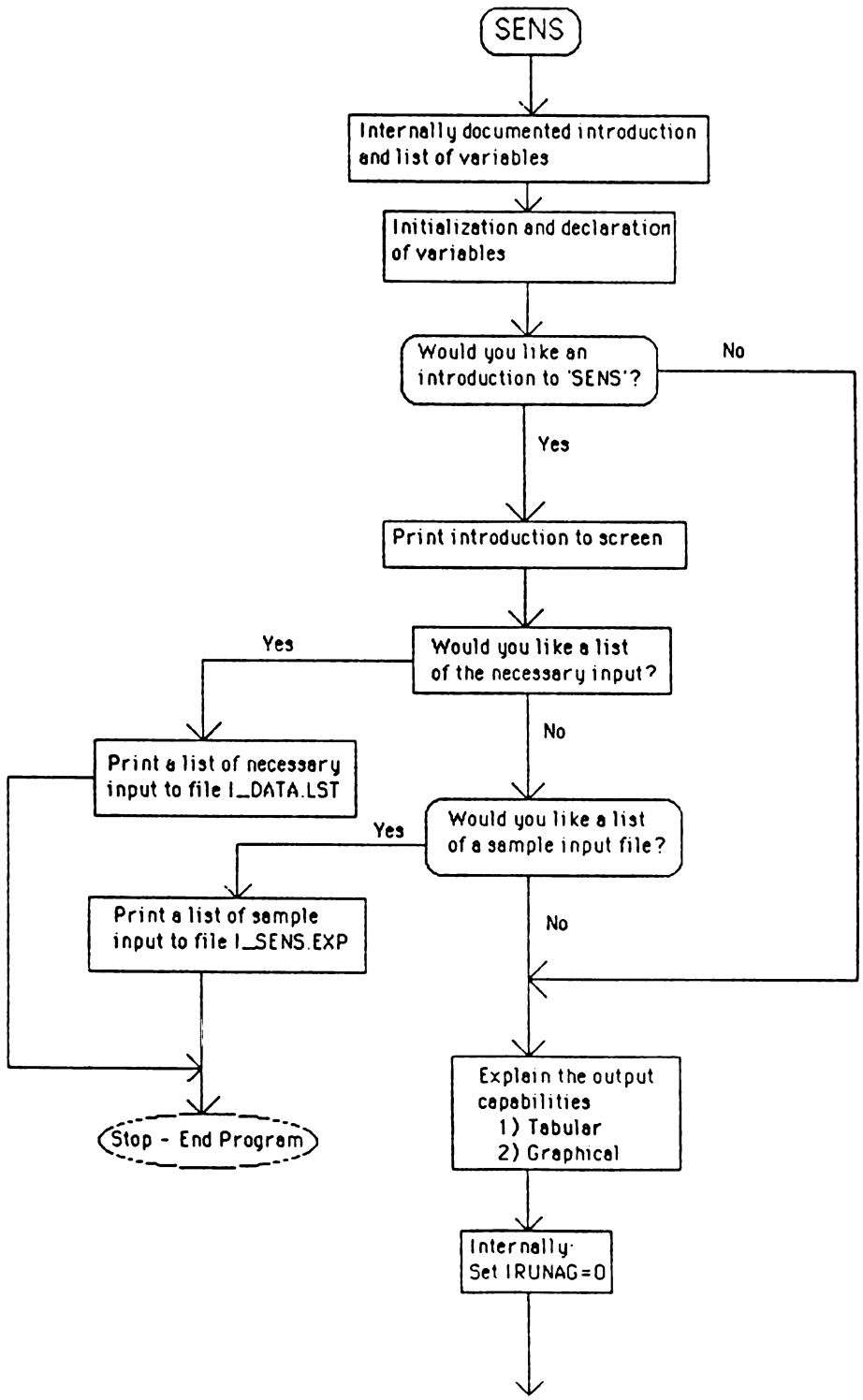


Figure 3.2.1 (cont'd.)

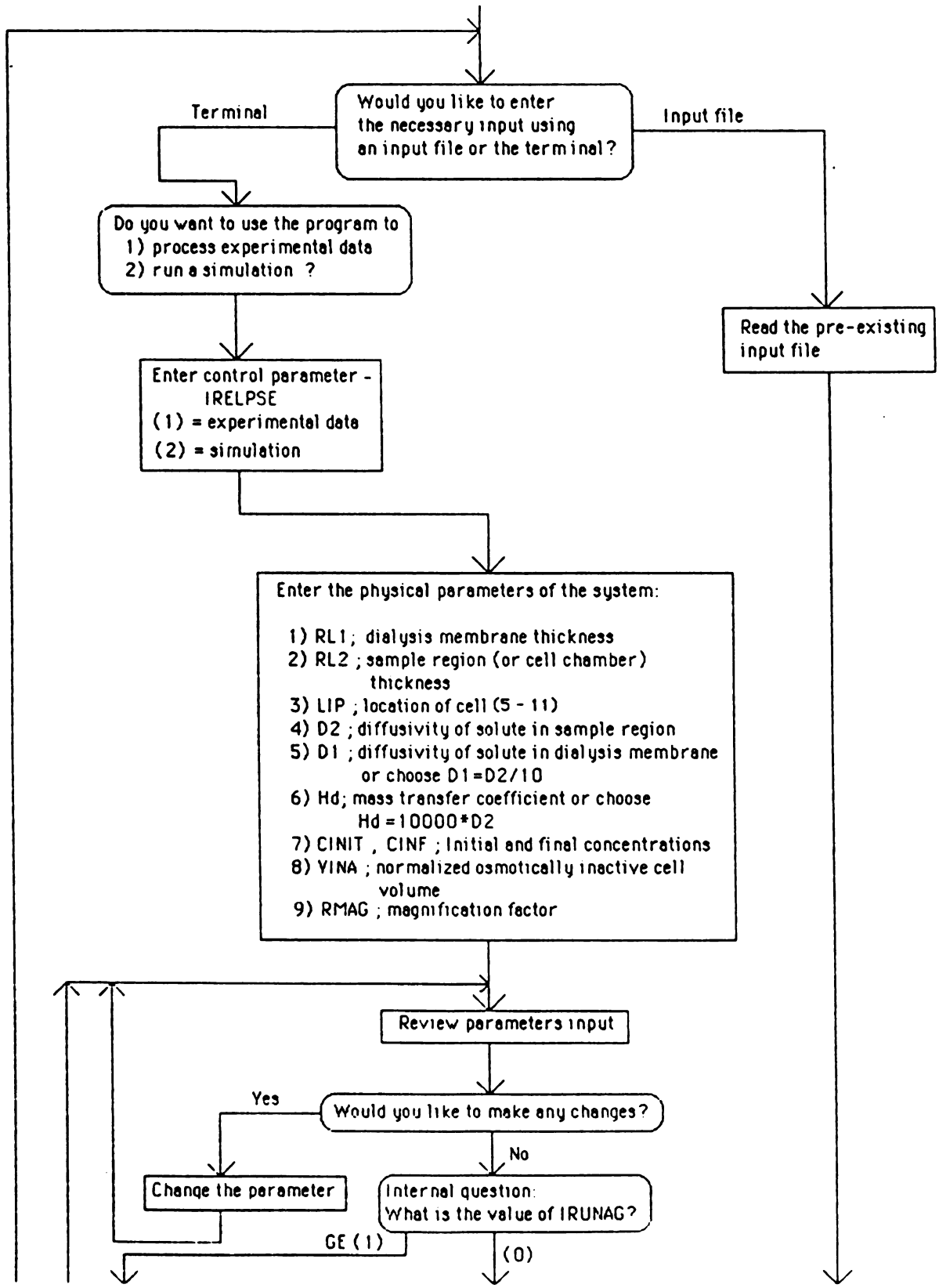


Figure 3.2.1 (cont'd)

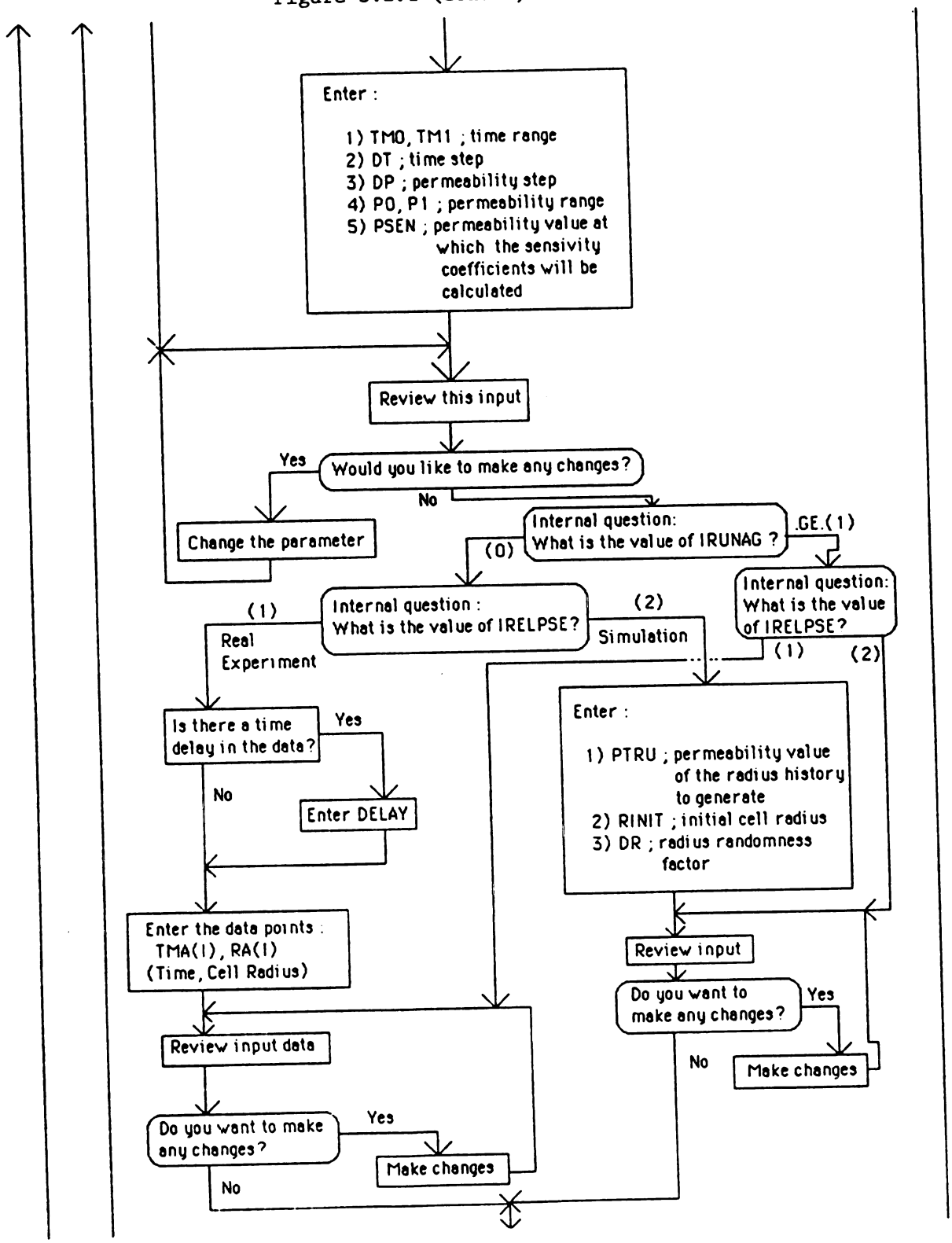


Figure 3.2.1 (cont'd.)

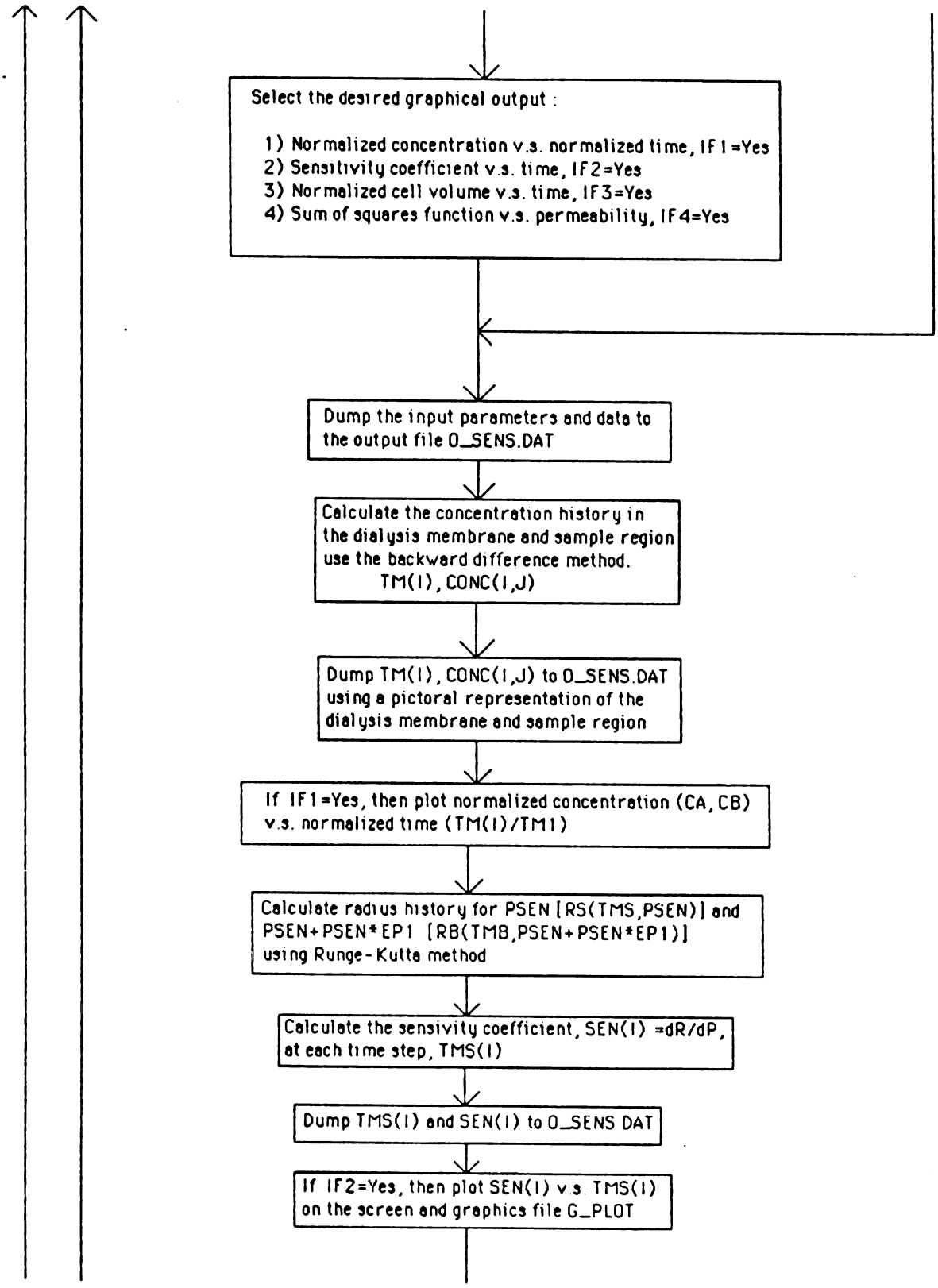


Figure 3.2.1 (cont'd)

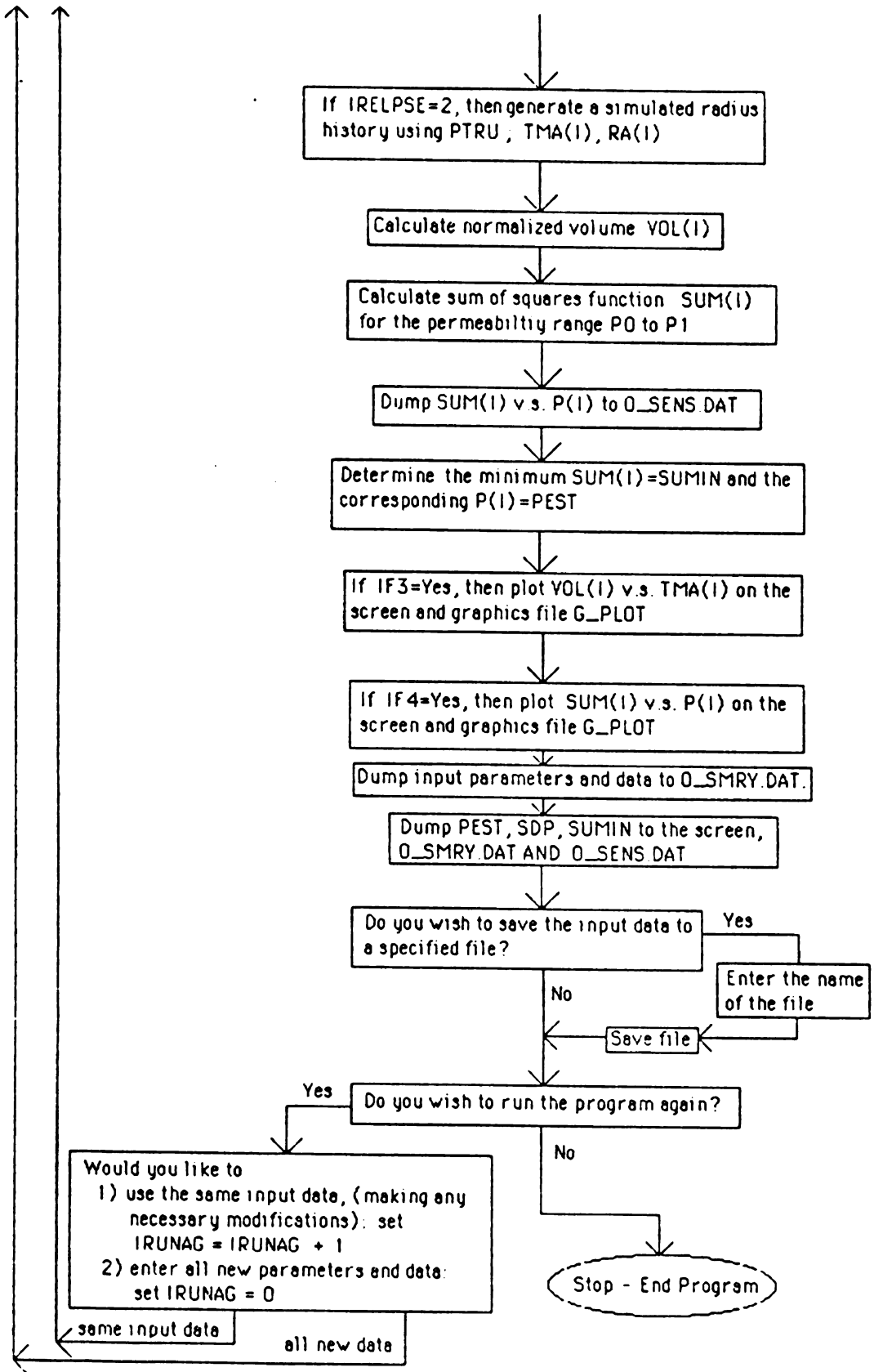


FIGURE 3.2.2 - Sample Output File of SENS - O_SMRY.DAT

```
********************  THIS IS FILE O_SMRY.DAT  ************************

 THE INPUT PARAMETERS AND DATA WERE:

  THE VALUES ENTERED SO FAR ARE:
  1) DIALYSIS MEMBRANE THICKNESS -     .160E+02 MICRONS
  2) CELL CHAMBER THICKNESS -     .915E+02 MICRONS
  3) LIPOSOME POSITION (5-11) -  5
  4) DIFFUSIVITY IN CELL CHAMBER -     .148E-08 M*M/SEC.
  5) DIFFUSIVITY IN DIALYSIS MEMBRANE -     .148E-09 M*M/SEC.
  6) MASS TRANSFER COEFFICIENT -     .148E-04
  7) INITIAL CONCENTRATION -     .291 OSMOLALITY
  8) FINAL CONCENTRATION -     .725 OSMOLALITY
  9) INACTIVE VOLUME % - 34.70
  10) MAGNIFICATION FACTOR - 11290.0

  THE VALUES ENTERED ARE:
  1) TIME STEP -     1.00 SEC.
  2) TIME RANGE -        .0 SEC. TO     60.0 SEC.
  3) PERMEABILITY STEP -      .10 MICRONS/SEC.
  4) PERMEABILITY RANGE -      .00 MICRONS/SEC. TO
                            15.00 MICRONS/SEC.
  5) INVESTIGATING PERMEABILITY -    9.30 MICRONS/SEC.

  THE DATA POINTS ENTERED WERE:
  (INCLUDING THE TIME DELAY OF     .0000 SEC.)

    J        TIME(J)       RADIUS(J)
             (SEC.)        (CENTIMETERS)
    1          .20           4.69
    2         1.40           4.69
    3         3.00           4.65
    4         4.40           4.65
    5         7.60           4.61
    6        11.80           4.52
    7        13.90           4.43
    8        15.30           4.38
    9        18.40           4.34
   10        22.00           4.25
   11        26.60           4.16
   12        31.40           4.12
   13        37.60           4.07
   14        51.80           4.03
   15        54.30           3.99
   16        58.90           3.99
   17          .00            .00
```

FIGURE 3.2.2 (cont'd.)

********** THE RESULTING PERMEABILITY **********

THE LOCAL MINIMUM OCCURS AT P = 8.600 MICRONS/SEC.

THE STANDARD DEVIATION OF ESTIMATED P IS .267E+00 MICRONS/SEC.

THE MINIMUM VALUE OF SUM IS .004

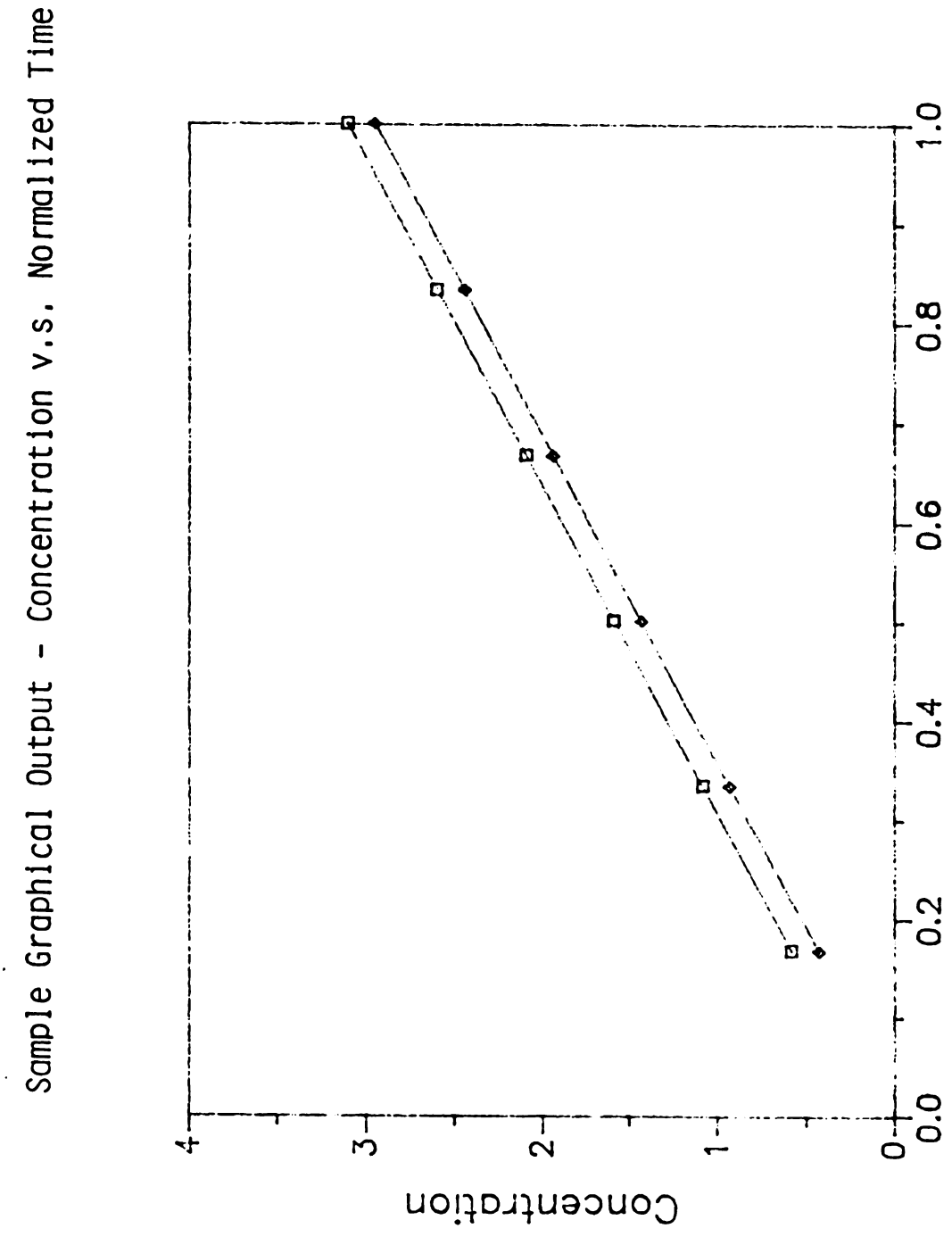

Sample Graphical Output - Concentration v.s. Normalized Time
Concentration
0
1
2
3
4
0.0
0.2
0.4
0.6
0.8
1.0
Normalized Time

Figure 3.2.3

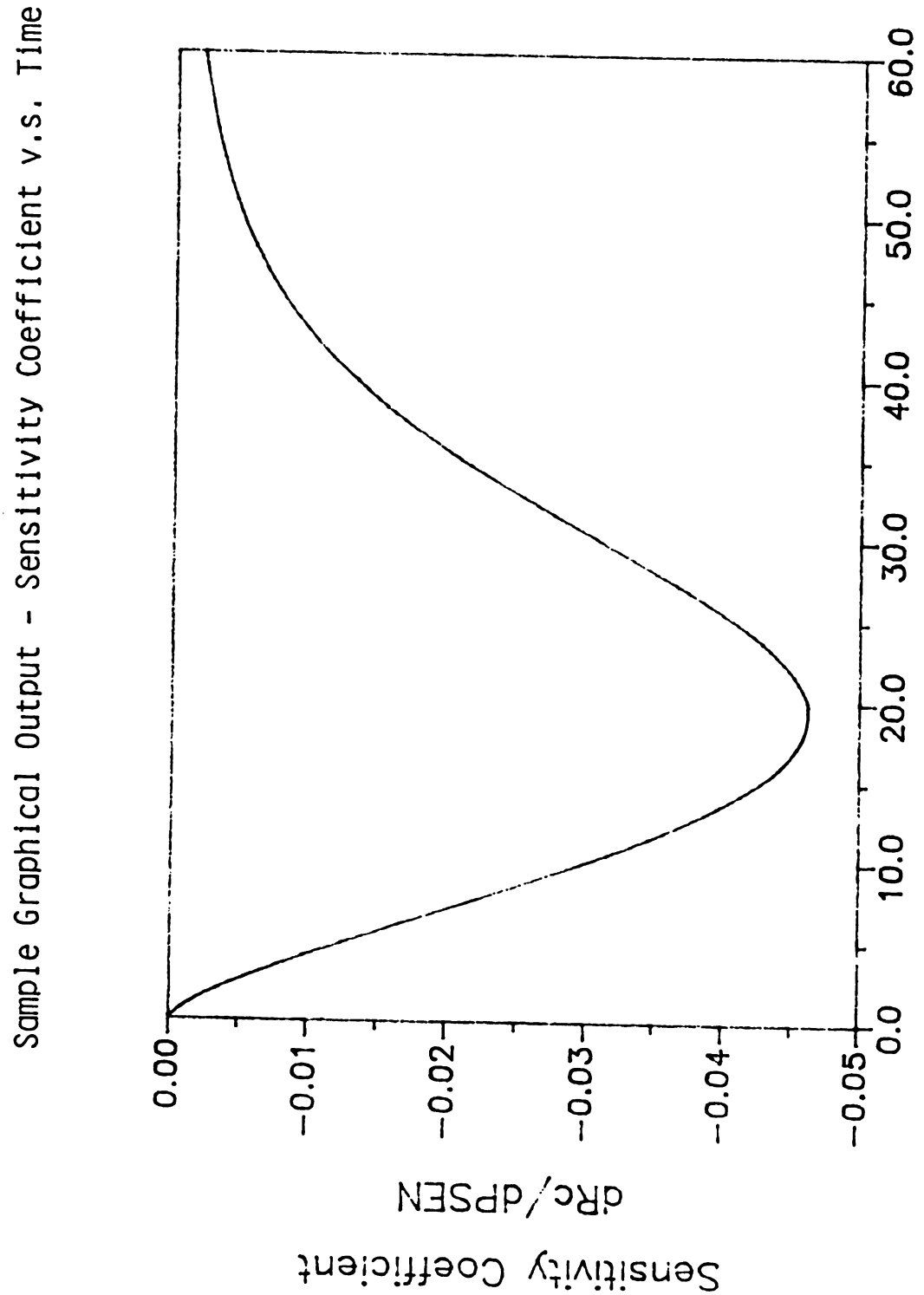

Sample Graphical Output - Sensitivity Coefficient v.s. Time
Sensitivity Coefficient
dRc/dPSEN
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
0.0
10.0
20.0
30.0
40.0
50.0
60.0
Time (sec.)

Figure 3.2.4

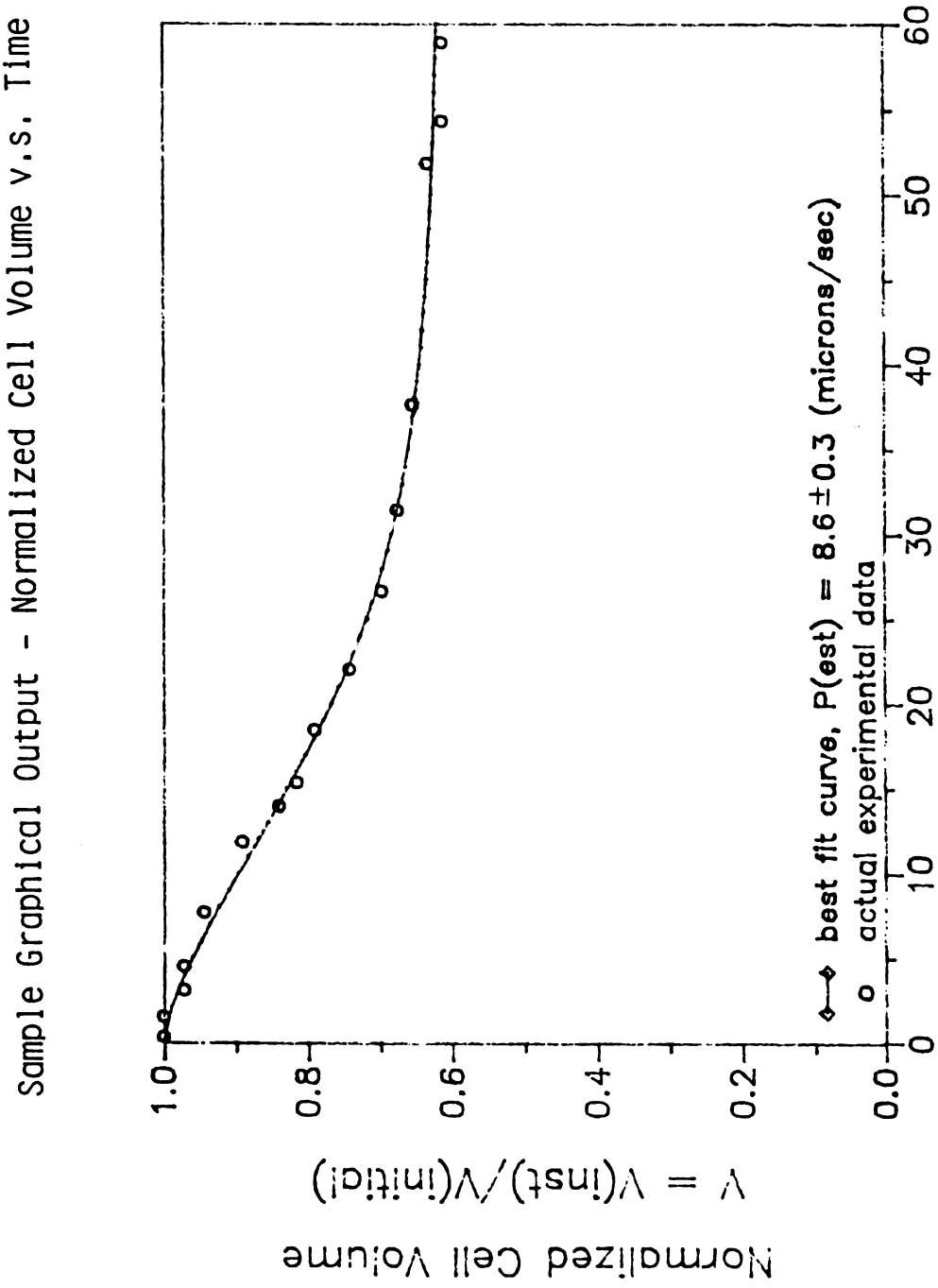

Sample Graphical Output - Normalized Cell Volume v.s. Time
Normalized Cell Volume
V = V(inst)/V(initial)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Time (sec.)
best fit curve, P(est) = 8.6±0.3 (microns/sec)
actual experimental data

Figure 3.2.5

Sample Graphical Output - Sum of the Squares v.s. Permeability

Sample Graphical Output - Sum of the Squares v.s. Permeability

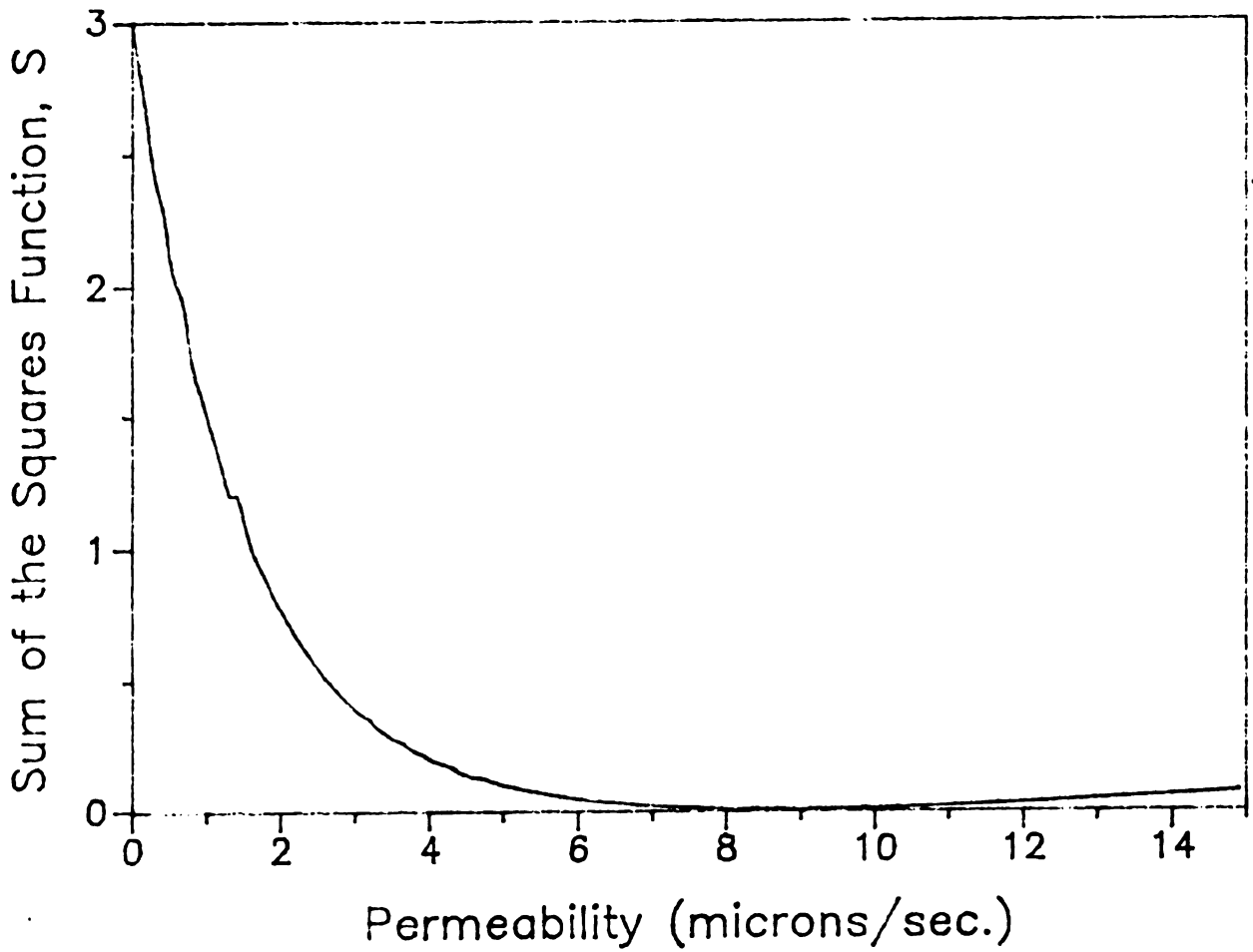


Figure 3.2.6

3.3 Converting SENS to the IBM PC

Before SENS was adapted to the IBM PC, the primary concern that needed to be addressed was which graphical software package should be used. Originally SENS resided on the PRIME, which allowed the use of the interactive graphical software, PRINTEX. The graphical software package that was chosen to be used with the converted SENS version was PLOTIT. PLOTIT was chosen because of its ease of use, the quality of graphs produced (using a Hewlett Packard Plotter) and its availability at the Engineering Computer Facility PC room.

Converting SENS from the PRIME to the IBM PC involved a two step process. First SENS was adapted to function as though it were on an IBM PC but was actually still on the PRIME. In other words, the interactive graphical programming code was stripped from the original version of SENS and replaced with code to generate four (optional) separate output files which could be used with PLOTIT (on the PRIME). Then once all the "bugs" were worked out SENS was converted to the IBM PC.

Another important consideration addressed was which FORTRAN compiler should be used. The compiler that was used was MICROSOFT FORTRAN. This compiler was chosen because the author was familiar with its operation and it seemed to have a good error detecting mechanism which was very helpful when SENS was converted.

Once the conversion process was completed, a sample input file was used as a test to make sure both versions yielded the same results. It should also be noted that after the conversion some minor programming changes were made to both versions resulting in the programs recorded in

Appendices D and E.

CHAPTER 4

Experimental Equipment and Procedures

4.1 Preparing Cells for Experimental Runs

The methods used to prepare the cells used in this work will now be described.

4.1.1 Preparing Egg Lecithin Liposomes

Before experiments using egg-lecithin liposomes were conducted, the liposomes, the isotonic sucrose solution and the hypertonic sucrose solution had to be prepared. The solutions were made by adding the proper amount of sucrose to a known amount of distilled water. The desired concentrations were 0.02 osmolality for the isotonic solution and 0.04 osmolality for the hypertonic solution. The concentrations were measured using an osmometer.

Once these solutions were made the liposome cells were prepared. 0.07 grams of the egg lecithin lipid was measured into a 25 ml erlenmeyer flask. Next, the lipid was dissolved using 25 ml of a 2:1 chloroform:methanol solution and a vortex mixer. Then 0.5 ml of this solution was pipetted into a 50 ml round bottom flask which was fastened to a rotavac and a vacuum was applied for approximately ½ hour. The chloroform:methanol solution evaporated leaving a thin film of lipid dispersed on the bottom of the flask. The flask was detached and 10 ml of isotonic solution was gently added, being careful not to disturb the lipid film, and stoppered. The circulating bath, which had been

preheated to 60°C, was turned off, the flask was placed inside and steadied, and the bath was closed. This solution was left in the bath overnight and by morning a small cloud of lipid had formed on the bottom near the middle of the flask.

After a few tries, it was discovered that the best results for obtaining what was believed to be "unilamillar" vesicles was to use a pipetman, which was preadjusted to 18 μl samples, and extract a sample from "near" the edge of the cloud.

4.1.2 Preparing Human Lymphocytes

Prior to conducting experiments using lymphocytes, whole blood was obtained and separated, and isotonic and hypertonic salt (sodium chloride) solutions were prepared. Again, these solutions were prepared in a similar fashion as those that were used in the liposome experiments. The desired concentrations for the lymphocyte experiments was 0.291 osmolality for the isotonic solution and 0.725 osmolality for the hypertonic solution. 0.291 osmolality was chosen for the isotonic solution because this is the approximate osmolality of human blood. 0.725 was chosen because by increasing the concentration 2.5 times allowed the final cell size to decrease about 40%, with respect to the initial cell volume, which allowed for greater ease in recording the change in the cell radius. Also, this was the approximate range used by Hempling and Porsche, which allowed for comparisons to be made. In addition to measuring the concentrations of the solution, the pH was also measured. The pH of the solutions ranged form 7.0 to 7.3. The pH measurements were made using an Orion pH electrode probe and meter.

The blood used was either obtained from the Red Cross in Lansing, Michigan or was drawn from the author by a medical technologist on campus. The blood was collected in vacuum tubes containing EDTA. Therefore the age of the drawn blood ranged between 1 and 24 hours old by the time the blood had undergone separation. Initially 3 ml of Histopaque-1077 (SIGMA DIAGNOSTICS) medium was placed in 15 ml test tube. Next 6 ml of blood was carefully layered on top of the medium. The tube was then placed into a swing-bucket centrifuge and set at 387 G's for 30 minutes. This resulted in four distinct layers. The top layer contained

primarily plasma and platelets, the next layer contained the desired lymphocyte cells, while the last two layers contained the medium and other blood cells (including red blood cells), respectively. The top layer was suctioned off to approximately 5 mm above the lymphocyte layer. Next the lymphocyte layer was pipetted off and placed into another 15 ml test tube. These cells were then washed with 5 ml of Bacto Hemagglutination buffer solution [(0512-33-2) DIFCO LABORATORIES] (PH 7.3±0.1) and centrifuged at 387 G's for 10 minutes. The lymphocytes remained at the bottom of the tube while platelets were suspended in the buffer solution. The top layer of platelets was then suctioned off. This washing procedure was repeated two more times. Note, clumping sometimes occurred at any step of the washing. Clumping occurred approximately one out of every five separation attempts. Sometimes the clumps could be shook loose and sometimes the procedure was started over with another sample of blood because the clumps would not readily break apart. Finally, 0.8 ml of isotonic solution was added to the lymphocyte/platelet medium and stored at $4^{o}C$ for an average of one hour.

4.2 Description of the Microscope Diffusion Chamber

List of parts:

1) Chamber body, with heat exchange ducts
2) Clear plastic bottom cover slip
3) Dialysis membrane, (Cuprophan M80, ENKA AG, Product Group Membrana)
4) Rubber membrane retaining ring
5) Top fitting
6) Top cover glass
7) Plastic membrane retainer

Schematic representations of the diffusion chamber are shown in Figure 4.2.1 and 4.2.2. The chamber body was made of copper to allow for effective heat transfer. There were two separate flow channels built into the chamber body. The inner bulk flow channel ran down into the entrance, near the middle area of the chamber body, across the bottom clear plastic cover slip and up and out the exit. This channel provided the introduction of the hypertonic solution during an experimental run. The outer flow channel ran along the outer edge of the chamber, in a square pattern, surrounding the inner channel region. This was the channel used to control the temperature of the diffusion chamber. The dialysis membrane separated the bulk flow region and the sample region. The membrane has a dual purpose: 1) absorbing the shear of the bulk flow region, thus keeping the cell specimen relatively stationary while 2) providing a mechanism for solute transport (i.e. diffusion). The rubber retaining ring held the dialysis membrane firmly to the top fitting, made of brass, and also provided a seal between the top fitting and the chamber body. The top cover glass was glued to the top fitting providing a solid stationary boundary. The area between the dialysis membrane and

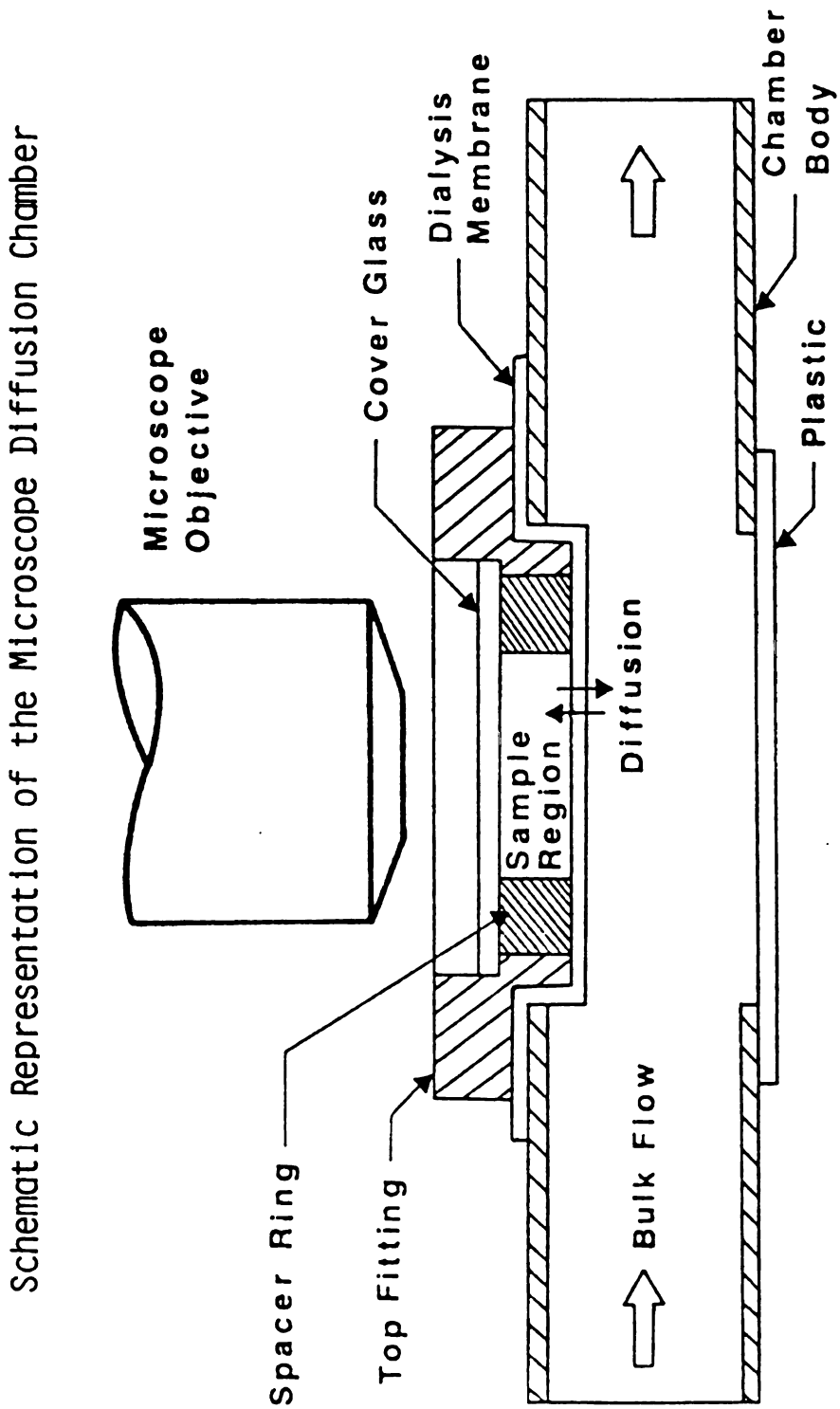
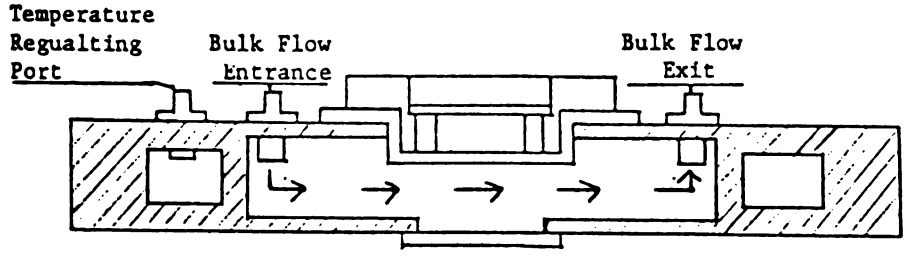
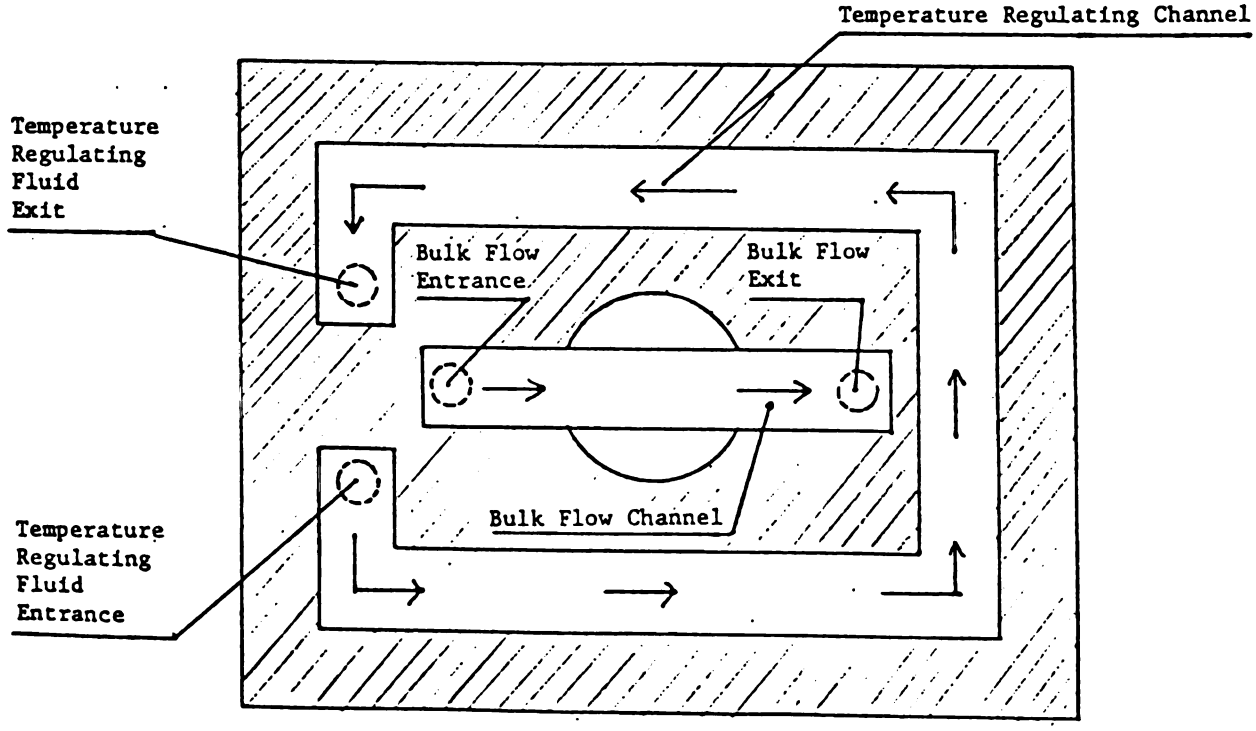

Schematic Representation of the Microscope Diffusion Chamber
Microscope Objective
Cover Glass
Dialysis Membrane
Chamber Body
Plastic
Spacer Ring
Top Fitting
Sample Region
Diffusion
Bulk Flow
Light Source
NOT TO SCALE

Figure 4.2.1

Figure 4.2.2 - Schematic Cross Sectional View of Microscope Diffusion Chamber



SIDE CROSS SECTIONAL VIEW



TOP CROSS SECTIONAL VIEW

the top cover glass was called the sample region. This is where the cell specimen resided.

4.3 Description of the Overall Experimental System

List of Equipment Used During Experimental Runs

1) Microscope diffusion chamber
2) Microscope, (Ziess Universal Research D-7082)
3) Pumping system - isotonic and hypertonic solutions
4) Discharge beaker (1000 ml)
5) Pressurized air supply (Engineering Building)
6) Vibration damping table
7) Digital temperature display device, (OMEGA Digicator C)
8) Copper-Constantan thermocouples, (OMEGA Engineering, Inc., Model No. TT-T-24)
9) Endocal refrigerated circulating bath, (RTE-8DD, NESLAB)
10) Insulated Tub
11) Video monitor, (19" RCA Color Television)
12) Video camera, (Color JVC or Black and White)
13) Video cassette recorder (Sony-βI or Sony U-matic,VO-5600)
14) Video tape (Beta or 3/4")
15) Timer (Midwest Telecommunication)
16) Vacuum grease

After having isolated and prepared the cells of interest in the isotonic solution, the experimental system was set up. A schematic representation of the experimental system is shown in Figure 4.3.1. The system consisted primarily of three units: 1) the microscope diffusion chamber and pumping system, 2) the temperature control system and 3) the data recording equipment.

The microscope was set up on a vibration damping table. This table was used due to the focusing problems that occurred when the microscope was on a bench or counter top. Vibrations from other engineering labs were conducted throughout the building which caused a blurring effect when attempting to focus the microscope on a cell specimen.

First the microscope diffusion chamber body was placed on the specimen stage of the microscope and fastened. Next the electronic solenoid valves of the pumping system were connected to the specimen stage. Then

Schematic of the Overall System

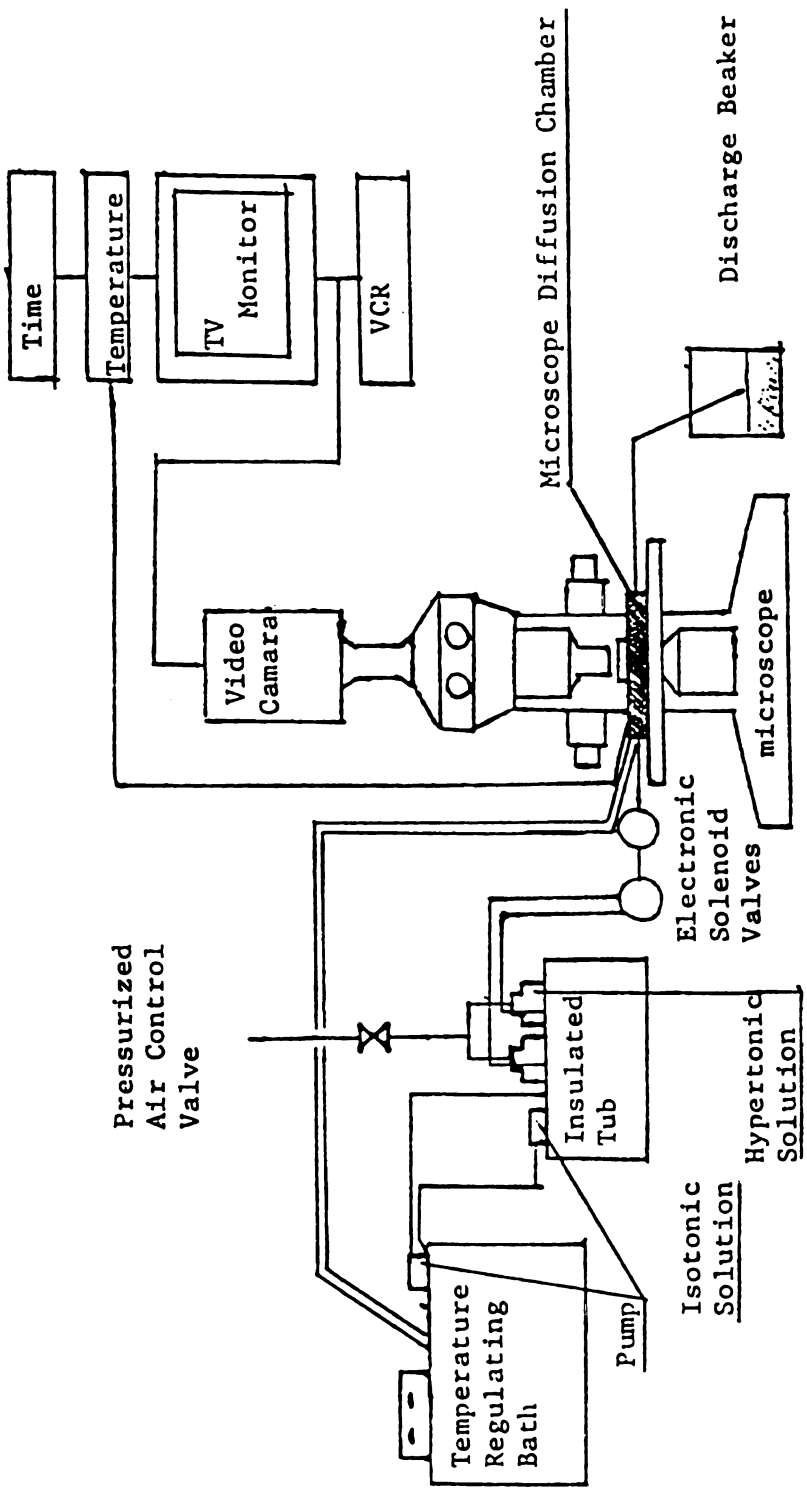


Figure 4.3.1

the pumping system was connected to the entrance for the inner bulk flow channel, a discharge hose was connected from the exit bulk flow port to a discharge beaker and the air supply was connected to the pumping system. Subsequently the temperature control equipment was set up. The circulating bath was placed on a table next to the vibration damping table. The bath provided the fluid medium necessary to control the temperature of the diffusion chamber and the isotonic and hypertonic solution bottles. An internal circulating pump dispensed fluid to and from the diffusion chamber while two external pumps provided and removed the fluid necessary to heat/cool the solution bottles in the insulated tub. The hoses connected to the external entrance and exit ports of the diffusion chamber were insulated to minimized the heat transfer to/from the surrounding lab environment. As mentioned above, the solution bottles were placed in an insulated tub. The reason the solution bottles were placed in a separate tank, and not directly into the circulating bath, was to avoid vibration transfer from the compressor in the circulating bath to the solution bottles and ultimately to the sample region of the diffusion chamber. In addition, a series of thermocouples were used to monitor the temperature at various points of the experimental system. More specifically, the temperatures of the top fitting of the diffusion chamber, the fluid in the insulated tank, the fluid in the circulating bath, the fluid at the entrance of the inner bulk flow channel and the fluid in each solution bottle were monitored. Each of these thermocouples were connected to a digital temperature display device.

Finally the data recording equipment was set up. First the video camera was attached to the vertical tube on top of the microscope. The video cable from the camera was connected to timer. The timer projected a digital stop watch on the upper left corner of the video monitor. The timer cable was then connected to the video cassette recorder, which was connected to the video monitor.

4.4 Description of an Experimental Run

After the system was set up one or more experimental runs could be conducted. (It should be noted here that before any experiments were performed some preliminary tests were conducted, see Appendix B.) Initially the temperature controlling bath was started and allowed to equilibrate to a desired temperature. This included having the diffusion chamber, solution bottles and circulating fluid at approximately the same temperature. Typically these three temperatures were equal to within 0.5°C. During this temperature transient, the dialysis membrane was prepared. The membrane was shipped from the manufacture (ENKA) in sheets measuring 8½ x 11 inches, which were cut into 1½ x 2 inch sections and presoaked in isotonic solution for 30 minutes. After these preliminary steps were taken, hoses leading from the solution bottles were preflushed, using manual release valves, being careful to remove all air bubbles from the lines. Next, the top fitting was removed and the chamber body was flushed with isotonic solution. The top fitting was then inverted, cover glass facing up, and an 18 μl sample, containing the cell specimen, was pipetted on to the center of the glass. Then a section of presoaked dialysis membrane was placed across the membrane retainer. Extra care was taken in handling the dialysis membrane being careful not to rip it. The membrane was then carefully lowered on to the top fitting using the plastic membrane retainer. Holding the membrane retainer in place, the rubber O-ring was applied to the fitted groove in the top fitting. This O-ring held the membrane firmly in place. Next, a small amount of vacuum grease was applied to the O-ring and then the top fitting was placed into the chamber body. Note, at this point it was

also very important to make sure no air bubbles were present in the bulk flow region. Air bubbles in the bulk flow region would cause a pulsing motion during an experimental run. The microscope was next focused on the sample region. Then the pumping system was turned on flushing the isotonic solution through the bulk flow region. This allowed for the operator to check for air bubbles and any leaks before the hypertonic solution was introduced thus "preserving" the cell specimen. The isotonic solution was shut off and a search for a desirable specimen was conducted. Once a cell was located the sample region thickness (RL2) and cell position (LIP) were determined. The sample chamber thickness was calculated using the grid marks on the fine adjustment focusing knob. Each grid mark was calculated to be 1.5 μm deep. (This measurement was performed by J. Tu). The number of grid marks counted between the top cover glass and the dialysis membrane gave an approximate sample region thickness. Note in the analysis it was assumed that the sample region thickness remained constant, however in practice the sample region thickness sometimes increased, on the average, 5 - 7%. Therefore an average thickness was used based on the initial and final sample region thicknesses. Also, the cell position was noted by counting the number of grid marks from the dialysis membrane or the top cover glass. Again, in the analysis the cell position was assumed constant; however in practice the cell sometimes moved, on average 5%. Therefore an average cell position was used based on the initial and final cell position. After these calculation were made the isotonic solution was started again, final focusing adjustment were made and the video recorder was started. At the desired time (t=0), the timer was started and the switch for the

hypertonic solution was engaged, which also cut the supply of isotonic solution. Note, the reason hypertonic solution was started while the isotonic solution was flowing, and not from a dead start, was because the shock from a dead start sometimes caused the cell to move out of the plane of focus. The transition from flowing isotonic to hypertonic was less abrupt. The cell response was recorded for a length of time based upon calculations made using the simulation mode of the data reduction program using reasonable estimates of anticipated permeability. At the end of the time duration, the pumping system, timer and video recorder were stopped.

At this point the experimental system could be set up for another run or solution bottles could be exchanged and the system started up again, further reducing the cell size.

4.5 Description of Data Measurements

After each run or after a set of runs, the data measurements were made. It should be noted that all experimental runs had a time delay. This was due to the small piece of tubing which connected the flow and the diffusion chamber bulk flow entrance port. In the description above, it was mentioned that a timer was started at the time the hypertonic solution was switched on. At that moment, the solution in the connecting tube still had isotonic solution in it. Based upon the length of the tube, the tube diameter and the flow rate of the hypertonic solution, a "time delay" was calculated (at the beginning of each experimental day). The time delay did not need to be subtracted here because the program SENS allowed the user to have a time delay (DELAY) subtracted from the data if necessary. The typical time delay was between 2.2 seconds and 3.1 seconds with an average of 2.7 seconds.

The recorded video tape was played back and using the pause function the time of the timer and cell radius was recorded. The number of data points collected was about 20 to 30 for each cell. It should also be noted here that during the play back of the tape, measuring the cell radius was sometimes a difficult task for three reasons: 1) the cell sometimes fluctuated such that the outer membrane of the cell was no longer spherical in shape, particularly when experiments using liposomes were conducted, (note: this fluctuation tended to diminish as the temperature at which the experiment was conducted was decreased), 2) the cell outer membrane was not clearly defined on the TV monitor, and 3) the pause function for the VCR, particularly the SONY Beta machine, caused the projected image on the TV monitor to vibrate slightly. These effects

will be studied in Sections 6.2.7 and 6.3.7. It should also be noted that when experiments using lymphocytes were conducted no distinction was made between T-cells and B-cells (which could obviously lead to a variability in the results presented in this paper).

The temperature was also recorded for each experimental run by using a copper-constantan thermocouple which was placed on the top fitting. During an experimental run the temperature sometimes changed slightly, particularly if the temperature of the experiment was removed from room temperature. However, this temperature change, on average, was no more than 1.5^{o}C (which occurred when the temperature of the experimental run was either at 10^{o}C or 37^{o}C).

4.6 Types of Experiments Performed

As described previously, the experimental system was set up either to do multiple experiments with different cells, at the same experimental conditions, or to use the same cell and subject it to different experimental conditions, e.g. continue to increase or decrease the extracellular concentration or temperature. Using these two basic configurations the normalized osmotically inactive volume of the cell, the cell membrane water permeability and the cell membrane water permeability activation energy could be determined.

To obtain the inactive volume of a cell, the cell was initially subjected to the isotonic solution and the radius of the cell was recorded. Then the cell was subjected to an increase in concentration and allowed to come to an equilibrium cell volume. The cell radius was again recorded for this new specified concentration. Next the original isotonic solution was replaced by another solution with an even higher concentration with which the cell had equilibrated. This procedure was repeated until the cell had undergone five increases in concentration. The inactive volume of the cell was obtained by developing a Boyle-Van't Hoff plot (see Appendix A).

To obtain the permeability of a cell type at a specified temperature, the cell was initially subjected to the isotonic solution at this specified temperature. At desired time a hypertonic solution was introduced and the radius history of the cell was recorded as mentioned in Section 4.5. The parameter characterizing the system and the measured data were entered into SENS and an estimated permeability was formulated.

To obtain the activation energy of a cell type, experiments were performed at five different temperatures. At any given temperature, five individual permeabilities were recorded and averaged to generate a mean permeability. Recall by plotting the natural logarithm of the mean permeability as a function of the inverse absolute temperature the activation energy was obtained.

CHAPTER 5

The Experimental Results and Discussion

5.1 Introduction

As mentioned previously, the objective of these experiments was to apply the microscope diffusion chamber to cell systems that had previously been examined by other scientists. The results obtained from the diffusion chamber would then be compared with the results of these other scientists. The specific parameters that were compared were the normalized osmotically inactive cell volume, cell membrane water permeability and cell membrane activation energy. The cells systems tested were egg-lecithin liposomes and human lymphocytes.

5.2 The Normalized Osmotically Inactive Cell Volume

The normalized osmotically inactive cell volume of egg-lecithin liposomes was not determined as a part of this work. Experiments using the diffusion chamber were performed by R. Callow [18] of the BTPL, using similar experimental conditions. The normalized osmotically inactive volume reported by Callow was 6.0%. Therefore, this was the values used to determine the liposome cell membrane permeability and activation energy, (see Figure 5.2.1 for a schematic of Callow's results).

The normalized osmotically inactive volume for human lymphocytes was determined to be $\hat{V}_b = 34.7\%$. See Figure 5.2.2 for the graphs of $\hat{V}_{cell}$ v.s. $1/C_s$. This result is in good agreement with the inactive volumes published by Hempling [14] (32.0%) and Porsche [15] (36.9%) (see Table 5.2.1). Therefore this inactive volume (34.7%) was used to formulate the membrane permeability and activation energy for lymphocytes.

Normalized Cell Volume v.s. Normalized Inverse Concentration - Liposome

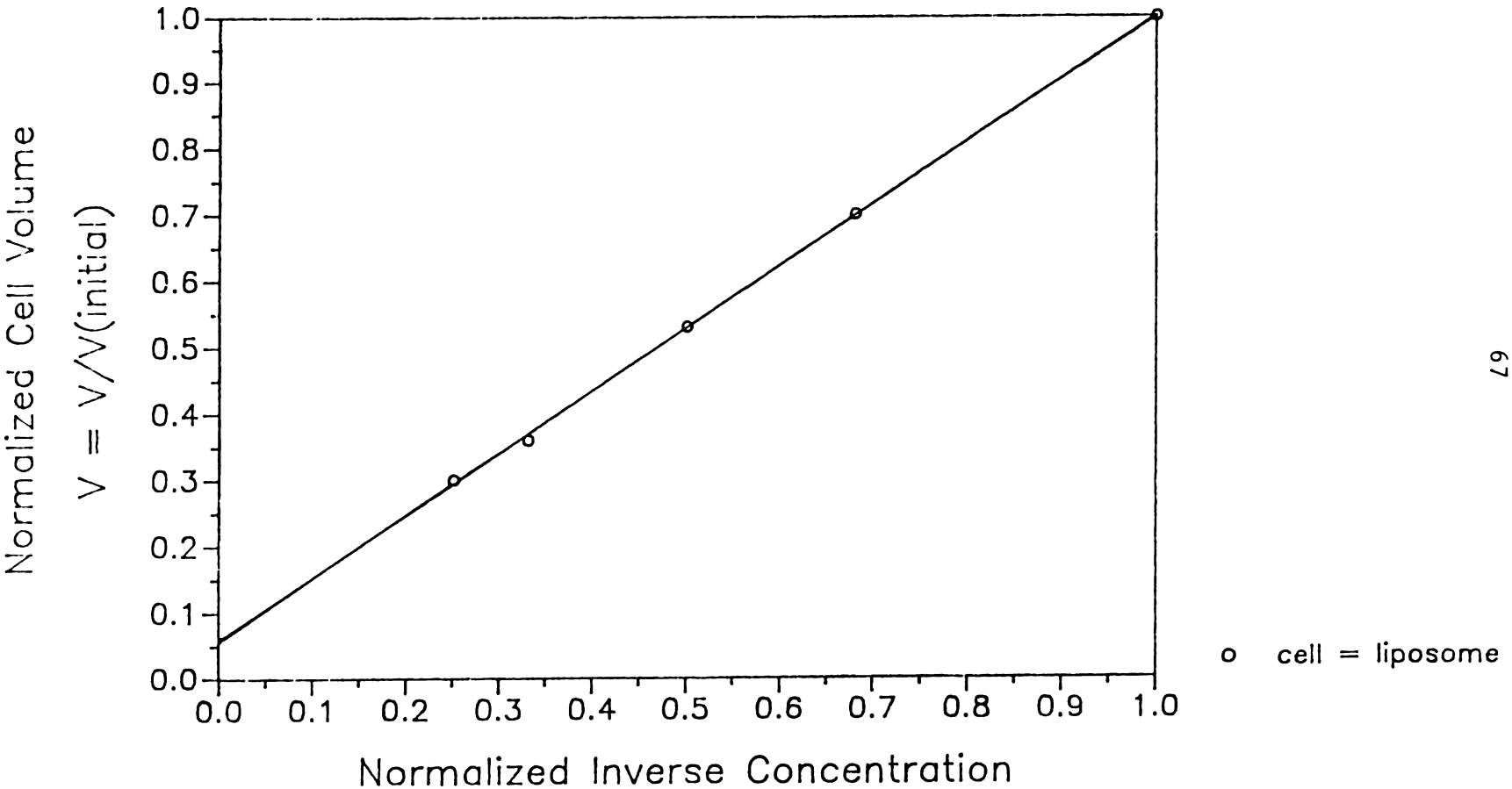


Figure 5.2.1

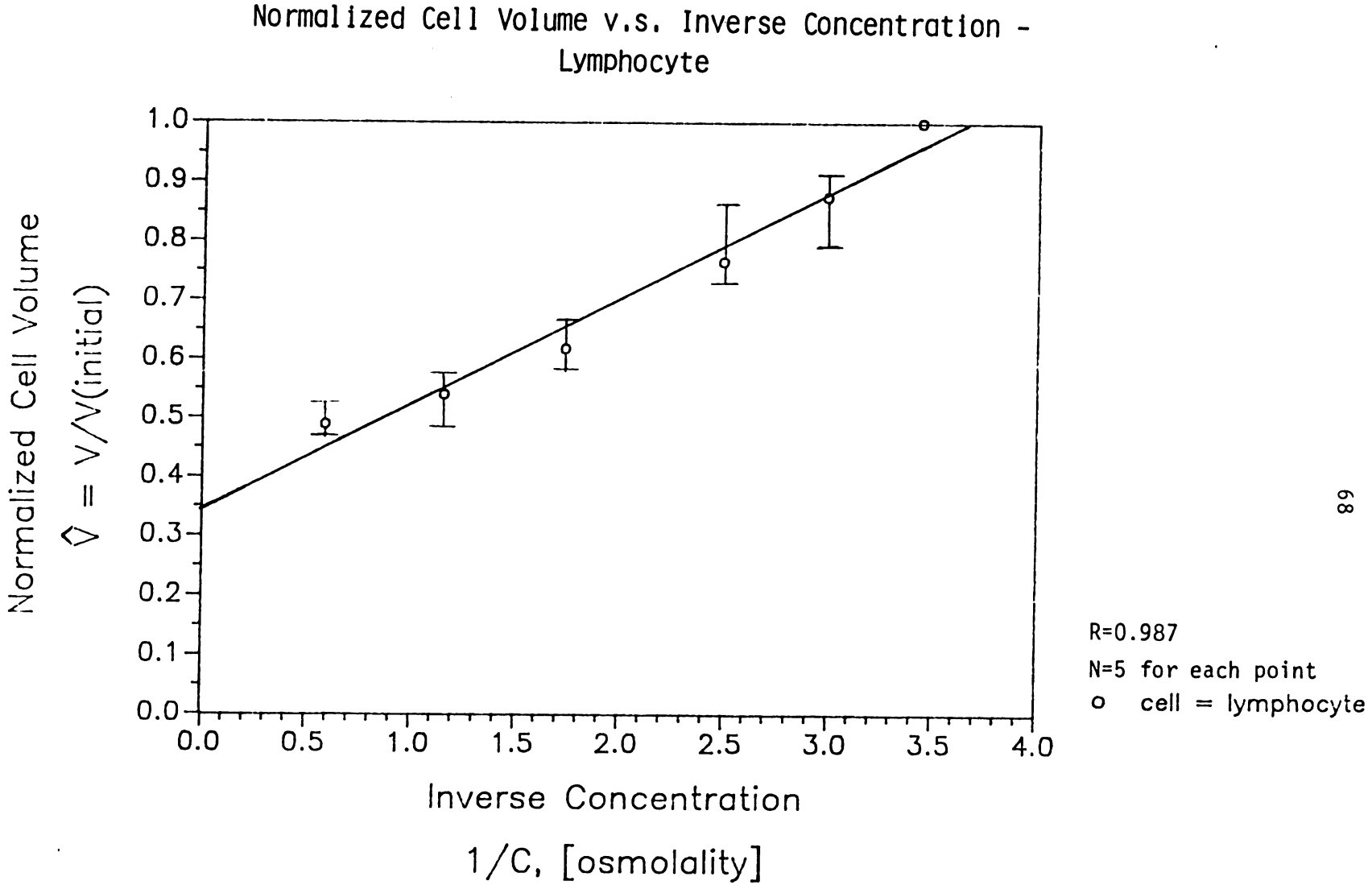

Normalized Cell Volume v.s. Inverse Concentration - Lymphocyte
Normalized Cell Volume
◇ = V/V(initial)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Inverse Concentration
1/C, [osmolality]
R=0.987
N=5 for each point
o cell = lymphocyte

Figure 5.2.2

TABLE 5.2.1 - Normalized Osmotically Inactive Cell Volume

cell type: human lymphocyte

Investigator	Normalized Volume (%)
Hempling	32.0
Porshce	36.9
Sherban (*)	34.7

5.3 The Cell Membrane Water Permeability

The membrane water permeability was calculated for 25 individual liposome cells and 25 individual lymphocyte cells. The temperatures at which the permeability was generated were 10°C, 16°C, 25°C, 30°C and 37°C for liposomes and 10°C, 16°C, 25°C, 30°C and 35°C for lymphocytes, five at each temperature. These results are summarized in Tables 5.3.1 and 5.3.2. The mean permeability at each temperature is also recorded.

A comparison can be made between the permeability calculated at 25°C from this work and the work published by other scientists, for both liposomes and lymphocytes (see Table 5.3.3). For example, Boroske [12] reported the mean permeability (P_m) for egg-lecithin liposomes to be 41±4.9 μm/sec, Callow [13] reported P_m = 40.5±8.4 μm/sec, Melkerson [13] reported P_m=41.0±3.1 μm/sec and Tu [7] reported P_m = 39.0±3.3 μm/sec. The mean permeability generated for this work was 40.2±6.9 μm/sec. This is in excellent agreement with the mean permeability reported by the above investigators.

For the case of the lymphocyte, a similar comparison can be made. Hempling [14] reported the mean permeability at 25°C to be 10.4±0.45 μm/sec, while Porshce [15] reported P_m = 4.2±0.42 μm/sec at 25°C. The mean permeability calculated for this work was 9.3±1.9 μm/sec at 25°C. This is in good agreement with the permeability reported by Hempling. However, there seems to be a discrepancy with the resulting mean permeability reported by Porshce. By reviewing the paper published by Porshce it was discovered that she devised a method to directly measure the concentration change within the sample region of a diffusion chamber similar to the one developed by McGrath. The solute she used was sodium

chloride and the dialysis membrane had a wetted thickness of 20 μm. Her system was also similar in that there was a time delay of approximately 1 second due to the hypertonic solution front passing from the (switched) valve to the chamber. Based on the information she reported in reference [15] it was concluded that she treated the sample region (cell chamber) as a "lumped" system. The thickness the sample region during these concentration measurements was never specified. When the concentration measurements were made she discovered that the concentration-time history could be approximated by an exponential function. She calculated the time constants with and with out a dialysis membrane to be τ=2.08 sec and τ=1.48 sec, respectively. Therefore, she claimed the dialysis membrane only played a secondary role as it did not cause much addition to the delay and that the deviation from a step-like behavior seemed to be caused mostly by a disturbance of the concentration profile due to turbulence on its way toward the cell chamber. These results also indicated that 95% of the final concentration was reached after about 5.6 seconds, when the cell has not yet started to shrink, and 99% was reached after 8.6 seconds. In addition, she also stated that the zero time was defined when shrinkage of more that 2% was detected. However, she did realize that an under estimate of the delay time would result in an underestimate of the membrane permeability. Based on the above argument she concluded that the approximation of the measured concentration-history by a step function impose at the corrected zero time seemed therefore justified. These same experimental conditions that Porsche used were entered into SENS, (i.e. wetted dialysis membrane thickness = 20 μm, sample region thickness = 100 μm, the sodium chloride diffusivity

in free solution, D_2, = 1.49E-9 m^2/sec (at 25°C), the sodium chloride diffusivity in the dialysis membrane, D_1=0.1*D_2, the convective mass transfer coefficient, Hd=10000.*D_2, the initial concentration = .310 osmol and the final concentration = 0.478 osmol), to see what the 95% and 99% concentration-time history results would be. Note, two assumptions were made in this analysis since these parameters were not specified; specifically, the sample region thickness was estimated at 100 μm and the concentration readings were taken at the surface of the dialysis membrane in the sample region, LIP-5. This analysis showed the 95% concentration reading did not occur until approximately 45 seconds, while the 99% concentration reading did not occur until approximately 85 seconds. Consequently Porsche's analysis would lead to an underestimated membrane permeability. Therefore, this is probably the cause for the discrepancy.

Note in Tables 5.3.1 and 5.3.2 two types standard deviations are recorded: 1) the individual standard deviation for a single cell at a specific temperature and 2) the standard deviation for the "population" of cells at a specified temperature. The results show that the standard deviation was less for an individual cell than for the population of cells, at a specified temperature. Therefore it can be concluded that there is a variation among the population.

Another way to look at it would be that for these cell types (liposomes and lymphocytes) a given cell will have a specific cell membrane permeability and that this membrane permeability will vary from cell to cell (at a specified temperature). This result was not anticipated for the liposome study but was not surprising for the lymphocyte study because no distinction was made between B and T cells.

Another interesting observation was the ratio of the standard deviation, both individual and population, to the resulting mean permeability. These results show this ratio for the liposomes ranged from 0.005 to 0.11 and on the average was 0.04 (for individual cells), while this ratio for the lymphocytes ranged from 0.03 to 0.10 and on the average was 0.05 (for individual cells). This ratio was higher for the population because of the variance in population (0.14 for liposomes, on the average and 0.17 for lymphocytes, on the average). There did not appear to be any apparent patterns or trends.

TABLE 5.3.1 - Permeability Results for Liposomes

solute: sucrose
concentration: 0.02 - 0.04 osmolality

Temperature (C^o)	Permeability ($\mu m/sec$)	Standard Deviation (S.D.) (for an individual cell) ($\mu m/sec$)	S.D./Pm
10	25.4	2.6	0.11
10	26.5	1.6	0.07
10	22.0	0.6	0.03
10	24.2	0.5	0.02
10	21.9	0.5	0.02
16	27.7	1.7	0.06
16	32.0	0.7	0.02
16	33.2	1.0	0.03
16	30.4	1.6	0.05
16	32.2	1.6	0.05
25	39.0	0.7	0.02
25	37.2	2.4	0.06
25	38.0	1.4	0.03
25	45.9	0.2	0.005
25	41.0	1.0	0.02
30	65.6	3.7	0.06
30	62.0	4.1	0.06
30	66.0	3.4	0.05
30	64.8	2.8	0.04
30	70.3	3.0	0.05
37	92.5	2.1	0.02
37	104.2	1.9	0.02
37	95.8	8.5	0.09
37	95.8	3.9	0.04
37	94.1	3.5	0.04

TABLE 5.3.1 (cont'd.)

Average Permeability For Each Specified Temperature

Temperature (C^o)	Permeability, Pm (μm/sec)	Standard Deviation, $S.D._p$ (for population) (μm/sec)	$S.D._p/Pm$
10	24.0	4.1	0.17
16	30.7	4.8	0.16
25	40.2	6.9	0.17
30	65.7	6.0	0.09
37	96.5	9.1	0.09

TABLE 5.3.2 - Permeability Results for Lymphocytes

solute: sodium chloride
concentration: 0.291 - 0.725 osmolality

Temperature (C^o)	Permeability ($\mu m/sec$)	Standard Deviation (S.D.) ($\mu m/sec$)	S.D./Pm
10	2.7	0.1	0.04
10	3.0	0.1	0.04
10	2.8	0.1	0.04
10	2.7	0.1	0.04
10	2.7	0.1	0.04
16	5.1	0.1	0.02
16	4.0	0.1	0.02
16	4.3	0.3	0.07
16	4.4	0.2	0.04
16	5.2	0.3	0.07
25	8.6	0.3	0.03
25	9.2	0.5	0.05
25	8.2	0.3	0.03
25	10.2	0.5	0.05
25	10.3	0.3	0.03
30	17.5	1.4	0.09
30	16.4	1.1	0.07
30	15.4	1.0	0.06
30	17.9	1.4	0.09
30	14.5	0.9	0.06
35	23.9	1.3	0.05
35	24.5	2.5	0.10
35	25.8	1.9	0.08
35	22.7	1.2	0.05
35	26.9	2.0	0.08

TABLE 5.3.2 (cont'd.)

Average Permeability For Each Specified Temperature

Temperature (C^o)	Permeability, Pm (μm/sec)	Standard Deviation, $S.D._p$ (for population) (μm/sec)	$S.D._P/Pm$
10	2.8	0.3	0.11
16	4.6	1.1	0.24
25	9.3	1.9	0.20
30	16.3	2.8	0.17
35	24.8	3.3	0.13

TABLE 5.3.3 - Comparison of Permeability Results

cell type: egg lecithin liposomes
temperature: 25°C
solute: sucrose

Investigator	Concentration (osmolality)	Mean Permeability (μm/sec)	N
Boroske	0.0 - 0.04	41.1 ± 4.9	13
Callow	0.0 - 0.02 0.0 - 0.04	40.5 ± 8.4	23
Melkerson	0.0 - 0.04	41.0 ± 3.1	6
Tu	0.02 - 0.04	39.0 ± 3.3	9
Sherban (*)	0.02 - 0.04	40.2 ± 6.9	5

cell type: human lymphocytes
temperature: 25°C
solute: sodium chloride

Investigator	Concentration (osmolality)	Mean Permeability (μm/sec)	N
Hempling	0.315 - 0.600	10.4 ± 0.45	?
Porshce	0.310 - 0.478	4.2 ± 0.42	?
Sherban (*)	0.291 - 0.725	9.3 ± 1.9	5

Note: N is the number of cells used to determine the mean permeability.

5.4 The Cell Membrane Water Permeability Activation Energy

From the permeability data accumulated, the cell membrane activation energy was formulated for both egg-lecithin liposomes and human lymphocytes. Each activation energy, ΔE_a, was obtained from the slope of the lines in Figures 5.4.1 and 5.4.2. The activation energy resulting from this work, ΔEa=8.9 Kcal/mole, for egg-lecithin liposomes compared well with those published by other scientists. Specifically, Blok [16] reported 9.5 Kcal/mole, while Reeves [17] cited 8.25 Kcal/mole. In addition, the activation energy calculated from this work for human lymphocytes was 15.1 Kcal/mole. This result was in good agreement with the activation energy reported by Hempling, ΔEa=14.1 Kcal/mole. However, the activation energy reported by Porsche, ΔEa=3.4 Kcal/mole, did not compare well. She reported that her result was in good agreement with the results reported by Hempling in reference [23], i.e. the activation energy for lymphoid cells was 4.4 Kcal/mole, while the activation energy for tumor cells was 5.7 Kcal/mole. Yet, the activation energies reported by Hempling in reference [23] ranged between 13 and 18 Kcal/mole, rather than 4.4 and 5.7 Kcal/mole. Therefore it is difficult to make a comparison. These results are summarized in Table 5.4.1.

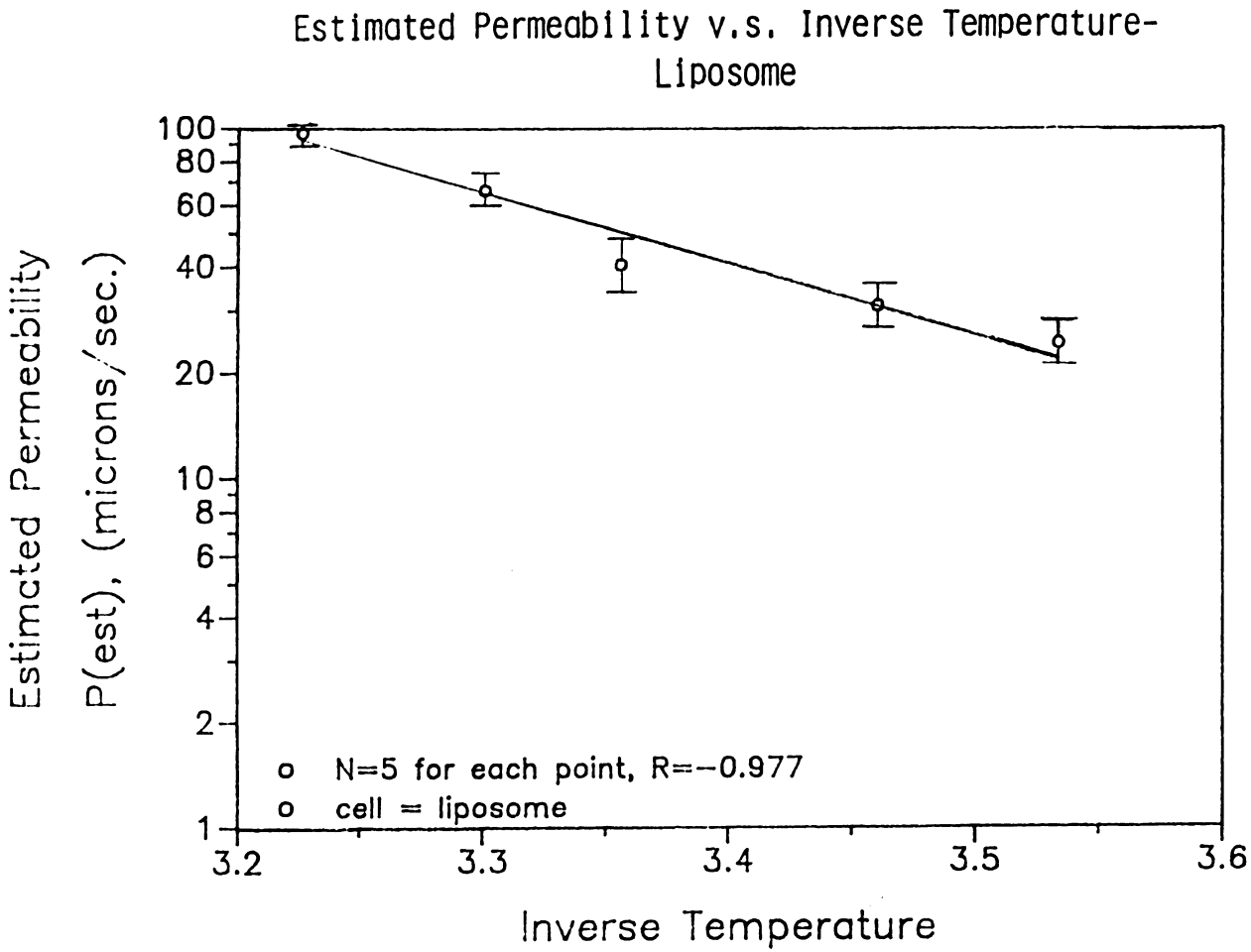

Estimated Permeability v.s. Inverse Temperature-
Liposome
Estimated Permeability
P(est), (microns/sec.)
100
80
60
40
20
10
8
6
4
2
1
o N=5 for each point, R=-0.977
o cell = liposome
3.2
3.3
3.4
3.5
3.6
Inverse Temperature
1/T E3, [degrees kelvin]

Figure 5.4.1

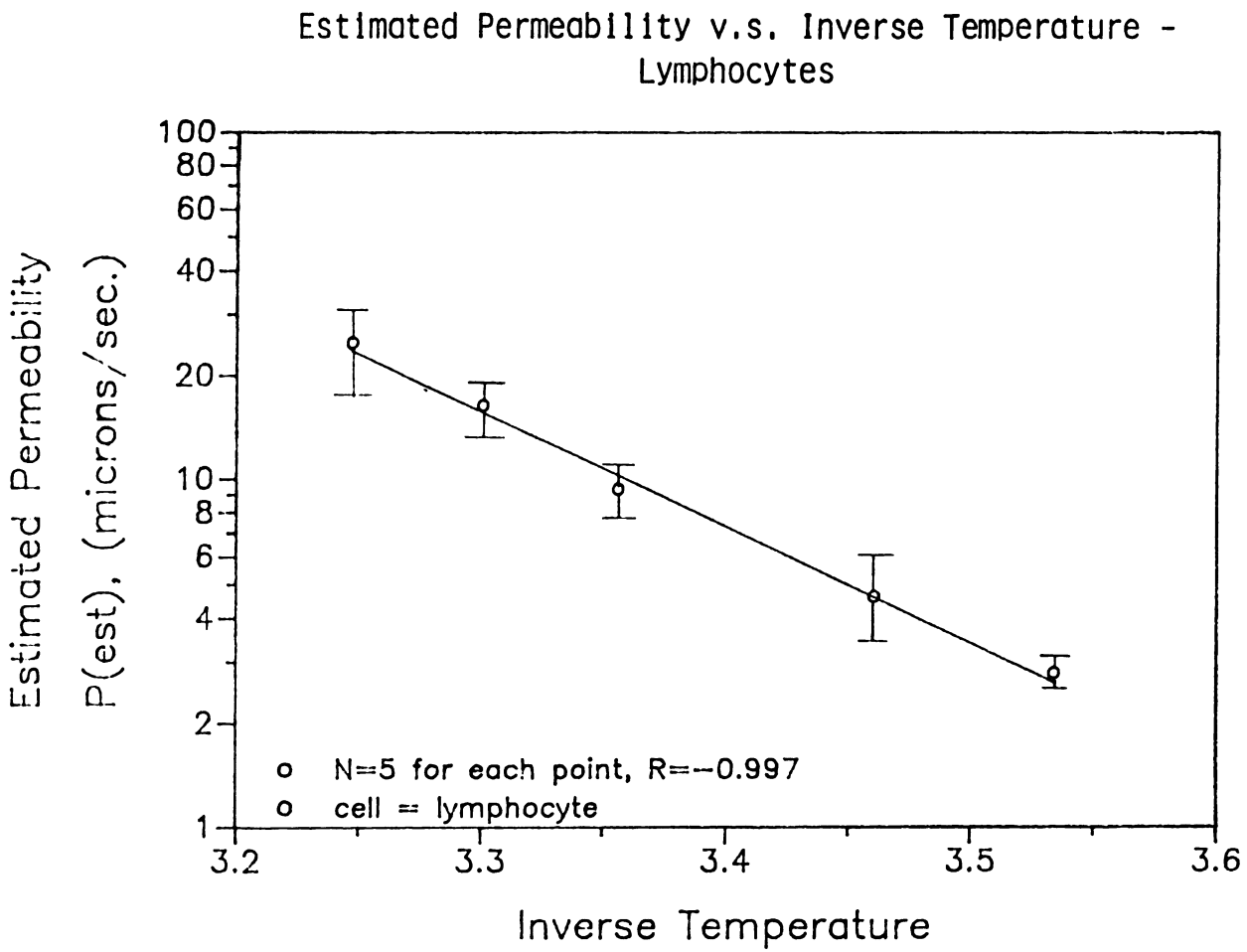

Estimated Permeability v.s. Inverse Temperature -
Lymphocytes
Estimated Permeability
P(est), (microns/sec.)
100
80
60
40
20
10
8
6
4
2
1
o N=5 for each point, R=-0.997
o cell = lymphocyte
3.2
3.3
3.4
3.5
3.6
Inverse Temperature
1/T E3, [degrees kelvin]

Figure 5.4.2

TABLE 5.4.1 - Activation Energy Results

cell type: egg lecithin liposomes

Investigator	Activation Energy (kcal/mole)
Blok	9.5
Reeves	8.25
Sherban (*)	8.9

cell type: human lymphocytes

Investigator	Activation Energy (kcal/mole)
Hempling	14.1
Porsche	3.4
Sherban (*)	15.1

(*) = Results from this thesis

CHAPTER 6

Sensitivity Studies

6.1 Introduction

As mention in the objective statement of this thesis (Section 1.2), the program SENS was used to study the sensitivity of the estimated parameter with respect to a change in input parameters. This can more clearly be stated by asking a key question. Specifically, what effect would an under or over specified input parameter, which describes some aspect of the experimental conditions, have on the resulting estimated permeability, standard deviation and minimum sum produced by SENS? The approach used to answer this question will be termed "sensitivity studies". Since there are an infinite number of possible experimental cases which could be studied, this discussion will be limited to two "base" cases, which are relevant to the work presented in this paper. From the study of these base cases some basic trends can be seen and some generalized statements can be made. The two cases presented here involve one for a liposome and one for a lymphocyte, both at room temperature. Also, only those input parameters that could possibly have a value different from the base case were studied. In other words, a value that was specified for an input parameter for which there was confidence (for that being the actual value) was not studied. More specifically, the wetted dialysis membrane thickness (RL1), the diffusivity of the solute in free solution (D2), the magnification factor (RMAG) and the initial and final concentrations (CINIT and CINF) were not investigated.

6.2 The Liposome Base Case

The experimental conditions which were used to describe the base case for the liposome were conditions commonly encountered in the lab when experiments were performed at room temperature, i.e. 25°C. Initially SENS was used, in the simulation mode, to generate a radius history of a liposome which had an estimated permeability of 39.9 μm/sec. The experimental conditions specified were as follows, (unless otherwise specified):

TABLE 6.2.1 - The Liposome Base Case

cell type: liposome
solute: sucrose
temperature: 25°C

Variable	Description of Variable	Specified Value
RL1	Wetted dialysis membrane thickness	16 μm
RL2	Sample region thickness	100 μm
LIP	Cell position in sample region	5
D2	Diffusivity of solute in free solution	$0.521*10^{-9}$ m^2/sec
D1	Diffusivity of solute in dialysis membrane	D2*0.1
Hd	Mass Transfer coefficient	D2*10000
VINA	Normalized osmotically inactive volume	6.0%
RINIT	Initial cell radius	10 μm
DR	Imposed randomness factor	0.0 μm
RMAG	Magnification factor	1
CINIT	Initial (isotonic) concentration	0.02 osmol
CINF	Final (hypertonic) concentration	0.04 osmol
DP	Permeability step	0.1 μm/sec
DT	Time step	2.5 sec
TM0	Starting time	0.0 sec
TM1	End time	500 sec
DELAY	Time delay	0.0 sec

The resulting estimated permeability generated was 39.9 ± 0.000 μm/sec (with a minimum sum of 0.000). This generated data was then reentered, using the (real) experimental parameter estimation mode, to yield a permeability of 39.9 ± 0.037 μm/sec (with a minimum sum of 0.000). Note, when comparing the standard deviations there appears to be a discrepancy. However, only the first three significant figures of a calculated radius, $Rc_i(t)$, were stored and the rest were truncated. Therefore, when this radius history generated, in the simulation mode, reentered into SENS, in the (real) experimental parameter estimation mode, the absolute value of the difference between the simulated (pseudo) radius and the predicted radius was greater than zero.

In each of the following sections (6.2.1 through 6.2.8) an input parameter was varied (by increasing and then decreasing the value of the parameter) to investigate the effect that this variation would have on the resulting estimated permeability and standard deviation. Only one input parameter was varied at a time and no other changes were made. The resulting estimated permeability, standard deviation and minimum sum have been tabulated for each varied input parameter. In addition, the varied parameter has been plotted versus the resulting estimated permeability (for most cases) or the standard deviation.

In the last section (6.2.9) the question was asked, what would be the effect on the membrane activation energy of the liposome if the activation energy of the dialysis membrane was changed? The results are tabulated in Table 6.2.10. This is important because if the activation energy of the dialysis membrane reported by the manufacture is not

correct, it could have a dramatic effect on the results presented in this thesis. The magnitude of this difference would determine the severity.

6.2.1 The Effect of Varying D1

TABLE 6.2.2 - The Effect of Varying D1

	D1 E10 (m^2/sec)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	10.0	31.3 ± 0.400	0.077
	3.0	32.2 ± 0.348	0.054
	2.0	32.9 ± 0.311	0.040
	1.5	33.6 ± 0.274	0.029
	1.0	35.1 ± 0.201	0.014
*	0.521	39.9 ± 0.037	0.000
	0.3	49.5 ± 0.225	0.006
	0.25	55.2 ± 0.286	0.006
	0.16	81.6 ± 0.832	0.013

* = original base case

The results show (Table 6.2.2 and Figure 6.2.1) that the estimated permeability was inversely related to D_1 in a non-linear way. For example, by doubling D_1 the estimated permeability decreased approximately 10%, while increasing D_1 by a factor of 20 only decreased P(est) another 10%, (i.e a total of 20% more than the base case). However, decreasing D_1 by a factor of 2 increases P(est) by almost 40% and decreasing D_1 by a factor of 4 increase D_1 by over 200%. Therefore the value of the sucrose diffusivity in the dialysis membrane was on the border line of being critically important and not so important. That is to say that if D_1 was under estimated the resulting estimated permeability, P(est), would increase dramatically, while if D_2 was over estimated P(est) would not have a major effect. Thus it would be desirable to increase D_1 by some means. The manufacture (ENKA) has claimed that the solute diffusivity in the dialysis membrane is equivalent to the permeability of the membrane, P_{mem} times the wetted

membrane thickness, Δx, (i.e. $D_1 = P_{mem} * \Delta x$). Therefore if the permeability of the membrane was increased, (possibly by making the dialysis membrane out of a more permeable substance), the effect D_1 would have on P(est) would be diminished. Note, the maximum value D_1 could every be, which would not be very likely, would be the same value of the diffusivity of sucrose in free solution (in this case water) 5.21E-10 m^2/sec (at 25°C).

P(est) v.s. D_1 - Liposome

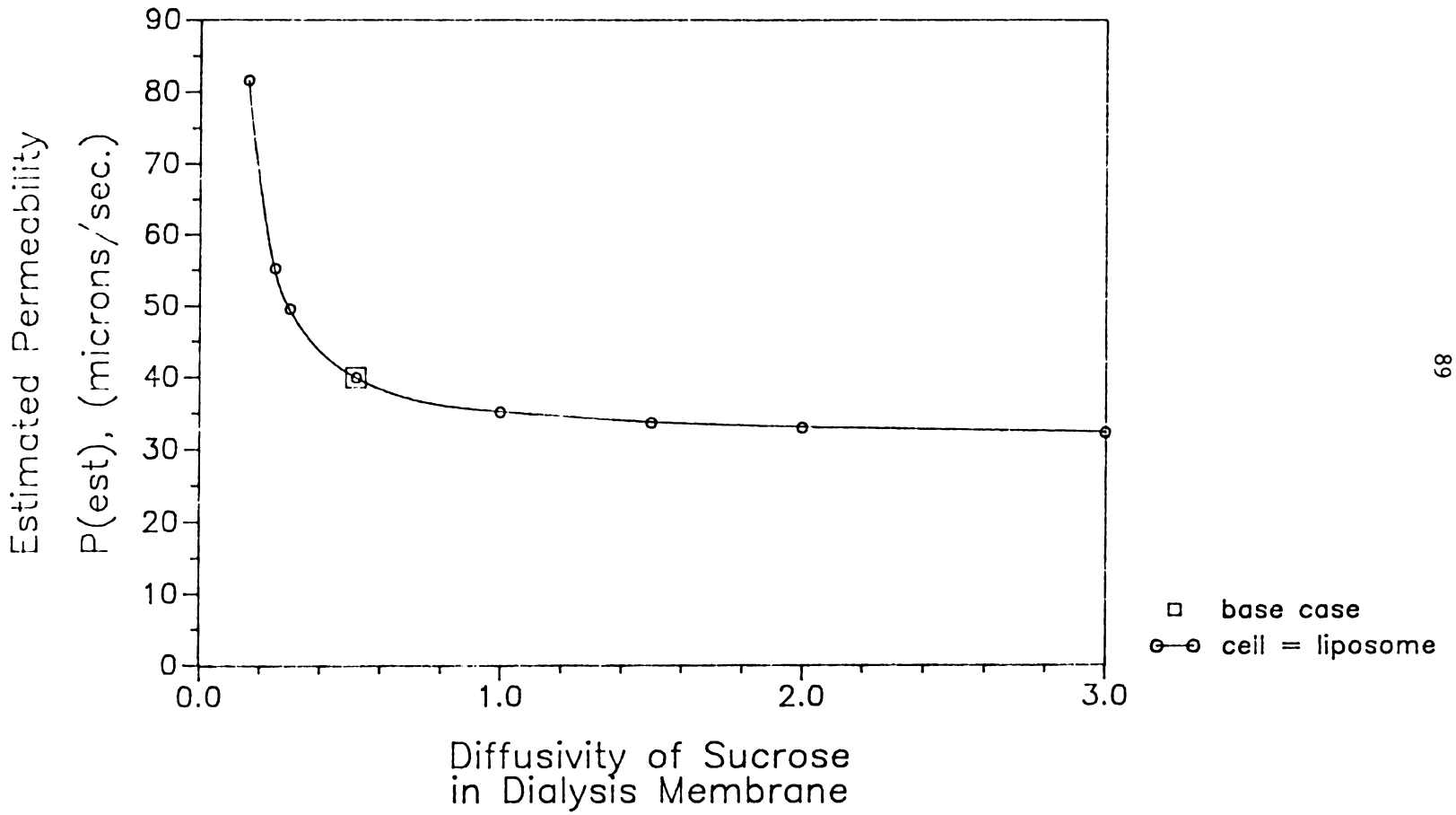


Figure 6.2.1

6.2.2 The Effect of Varying Hd

TABLE 6.2.3 - The Effect of Varying Hd

Hd E5 (m/sec)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
15.0	33.6 ± 0.272	0.029
5.21	34.0 ± 0.254	0.024
1.0	36.6 ± 0.138	0.006
* 0.521	39.9 ± 0.037	0.000
0.25	49.3 ± 0.214	0.005
0.11	88.5 ± 1.372	0.027

* - original base case

These results (Table 6.2.3 and Figure 6.2.2) show that the convective mass transfer coefficient was also inversely related to P(est) in a non-linear way. Similarly, when Hd was doubled, P(est) decreased approximately 8%, while when Hd was decreased by a factor of 2, P(est) increased by approximately 25%. Physically what this meant was that when Hd was decreased, the concentration boundary layer developing on the surface of the dialysis membrane, in the bulk flow region, was becoming large and visa versa, (i.e. when Hd was increased, the concentration boundary layer was becoming small). Obviously it would be more desirable to decrease the concentration boundary layer, thus minimizing the importance of correctly estimating Hd, with respect to estimating the membrane permeability. Experimental Hd can be increased by increasing the flow rate in the bulk flow region.

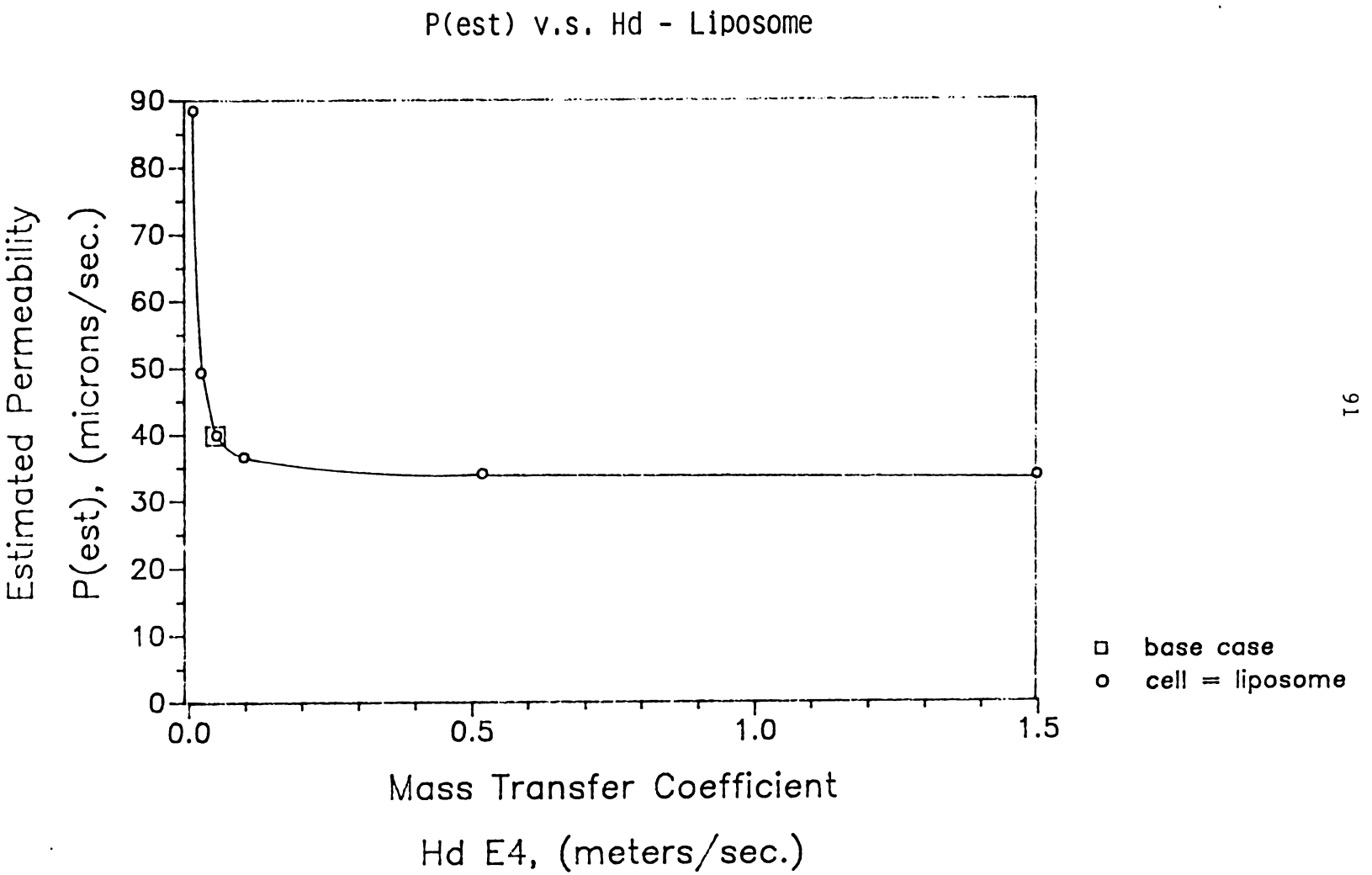

P(est) v.s. Hd - Liposome
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
Mass Transfer Coefficient
Hd E4, (meters/sec.)
base case
cell = liposome

Figure 6.2.2

6.2.3 The Effect of Varying RL2

TABLE 6.2.4 - The Effect of Varying RL2

	RL2 (μm)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	10	28.4 ± 0.562	0.206
	50	32.7 ± 0.283	0.034
	75	36.1 ± 0.129	0.005
*	100	39.9 ± 0.037	0.000
	150	49.0 ± 0.090	0.001
	200	59.2 ± 0.699	0.028
	250	68.8 ± 2.092	0.145
	300	76.7 ± 4.088	0.372

* = original base case

For the above case, the relationship that exists between the sample region thickness and P(est) appears to be slightly non-linear (see Figure 6.2.3) and was directly proportional. At first glance one might expect that there should not be much of an effect here because the cell was at the surface of the dialysis membrane; therefore the cell should be experiencing the same increase in extracellular concentration no matter what the sample region thickness. However, the developing concentration boundary layer, at the surface of the dialysis membrane, and dialysis membrane must also be taken into account. For example, let's say the sample region thickness was small and at the beginning of an experiment the bulk flow region was flushed with a hypertonic solution, thus creating a step change in concentration in the bulk flow region. The developing concentration gradient inside the sample region would be short lived and the sample region can essentially be treated as a lumped

system. On the other hand if the sample region thickness was infinitely large, a concentration gradient would always exist; therefore the cell in the sample region, even though it's at the surface of the dialysis membrane, would never experience the final hypertonic solution concentration. Looking at Figure 6.2.3 the curve appears to almost have an S shape where the "ends" of the S will eventually approach asymptotes. These two asymptotes are the two limiting cases discussed above. In most of the experiments performed in this thesis the value of RL2 was usually between 50 and 150 microns, which, for this case, could decrease P(est) by 20% and increase P(est) by 20%, respectively. Ideally RL2 should be made as small as possible (with respect to the cell diameter) because this would decrease the concentration gradient across the sample region thus decreasing the likelihood of incorrectly estimating RL2. However, this was not an easy task to accomplish because the presoaked dialysis membrane always has some unknown amount of isotonic solution on its surface, which adds to the volume of the sample region. Note liposome position was 5 (at the surface of the dialysis membrane) for all of the above data sets.

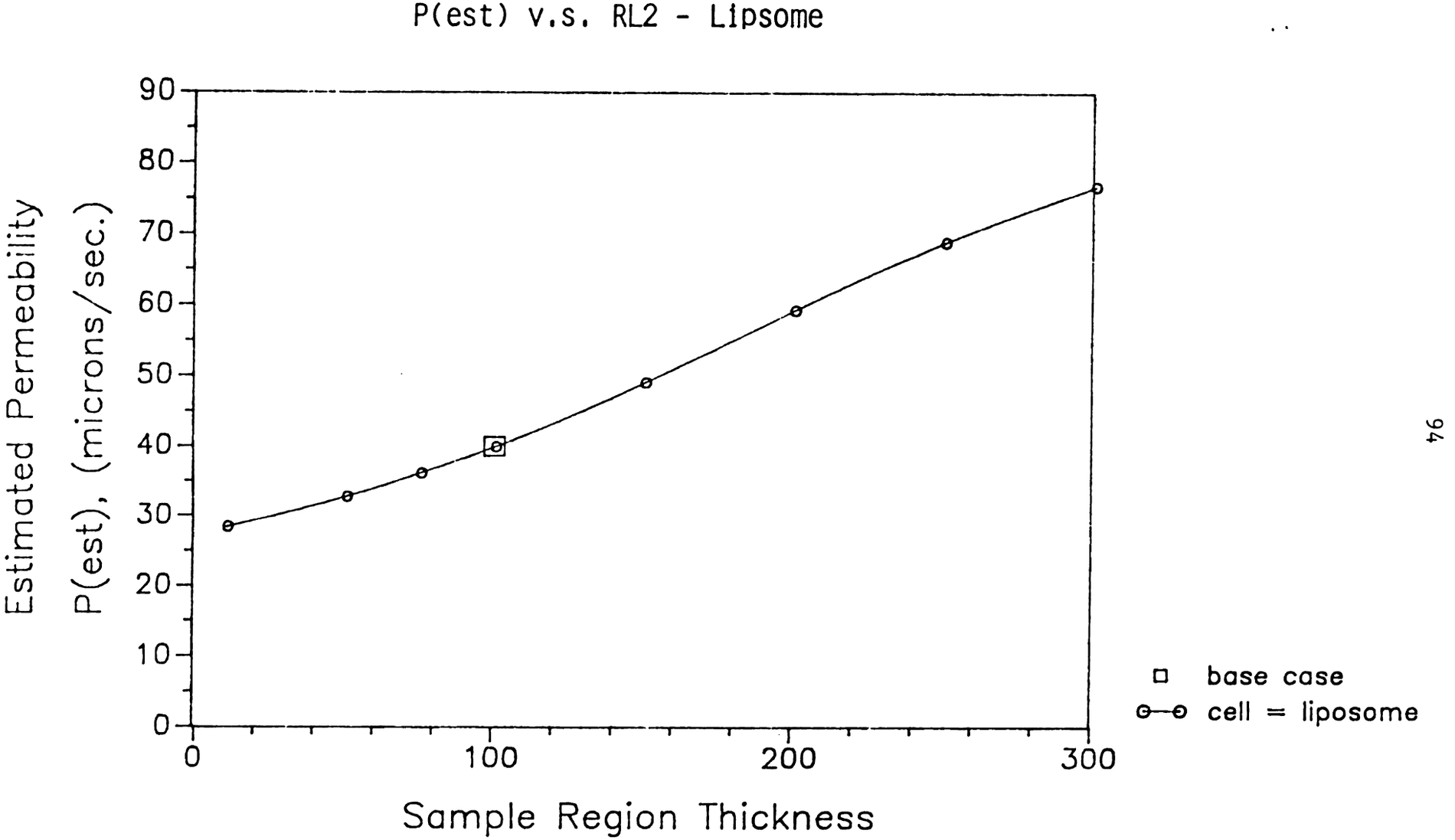

P(est) v.s. RL2 - Lipsome
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0
100
200
300
Sample Region Thickness
RL2, (microns)
base case
cell = liposome

Figure 6.2.3

6.2.4 The Effect of Varying LIP

TABLE 6.2.5 - The Effect of Varying LIP

	LIP	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	5	37.7 ± 0.198	0.010
	6	38.6 ± 0.122	0.061
	7	39.4 ± 0.061	0.001
**	8	40.1 ± 0.045	0.000
	9	40.5 ± 0.072	0.001
	10	40.8 ± 0.096	0.002
	11	40.9 ± 0.105	0.002

** : The base case was modified here in order to have the cell position be in the middle, i.e. LIP=8, instead of 5.

The results in Table 6.2.5 show that the liposome position was directly proportional to P(est) via an approximate linear relationship. Clearly from this investigation of the effect of the cell position in the sample region the estimated permeability did not change significantly (see Figure 6.2.4) and would not be considered an important effect (for this case). By mistakenly perceiving the cell to be at the middle position (LIP=8) when the cell really was at the surface of the dialysis membrane or at the surface of the top cover glass only decreases P(est) by 6% or increases P(est) by 2%, respectively. Note, this trend, of RL2 not greatly effecting P(est), would continue if RL2 were decreased. However, if RL2 were increased LIP would play a more significant role because of the developing concentration gradient in the sample region. Therefore, this would be another good reason to keep RL2 as small as possible.

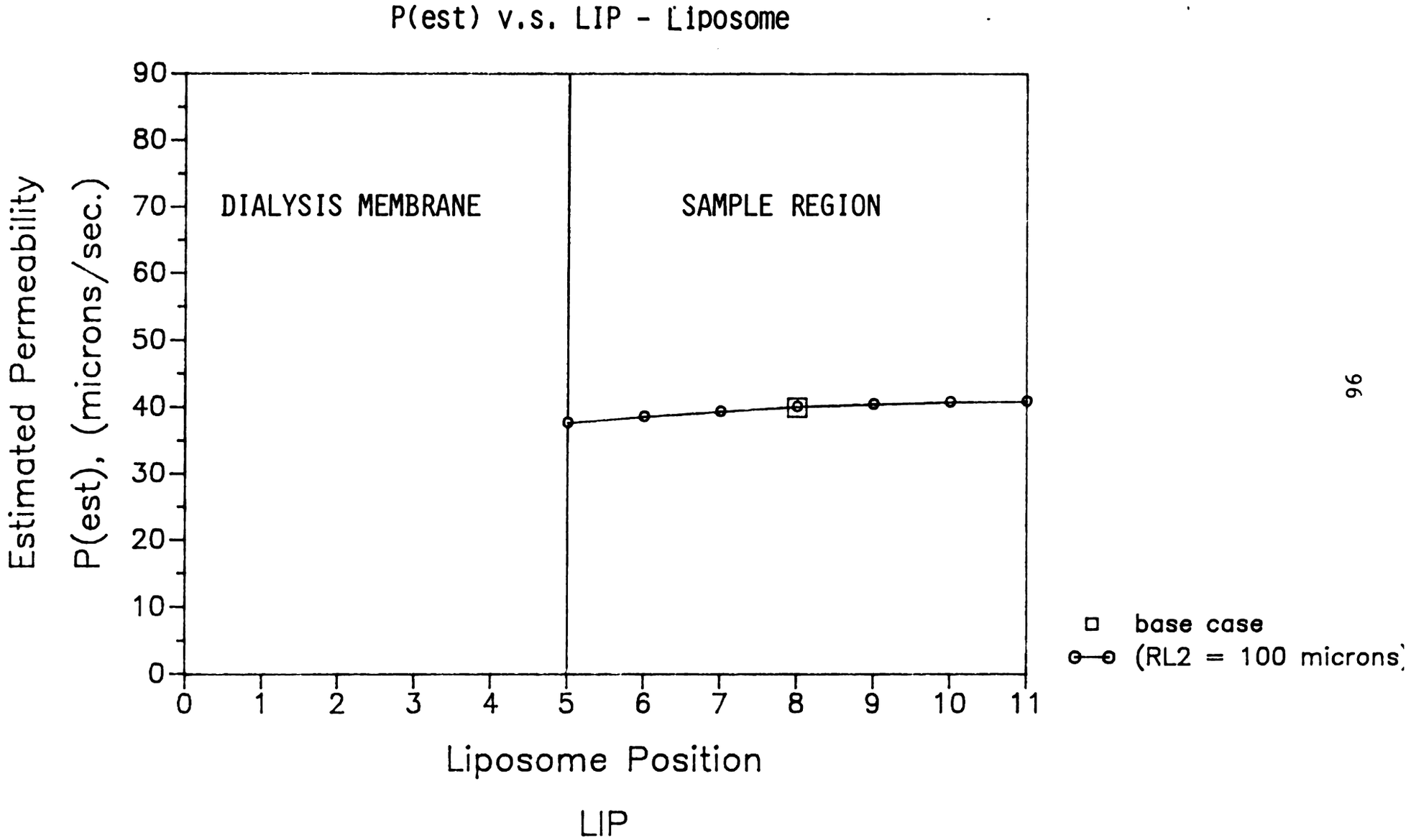

P(est) v.s. LIP - Liposome
Estimated Permeability
P(est), (microns/sec.)
DIALYSIS MEMBRANE
SAMPLE REGION
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
6
7
8
9
10
11
Liposome Position
LIP
base case
(RL2 = 100 microns)

Figure 6.2.4

6.2.5 The Effect of Varying VINA

TABLE 6.2.6 - The Effect of Varying VINA

	VINA (%)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	0.0	35.7 ± 0.632	0.158
	3.0	37.7 ± 0.381	0.044
*	6.0	39.9 ± 0.037	0.000
	10.0	42.9 ± 0.811	0.100
	15.0	46.5 ± 2.420	0.588
	20.0	50.0 ± 4.930	1.472
	30.0	56.5 ± 13.90	4.863

* = original base case

The results from this study show that the normalized osmotically inactive volume of a cell was directly proportional to P(est) in a linear fashion (see Figure 6.2.5). Decreasing VINA by a factor of 2 decreases the estimated permeability by about 5% and increasing VINA by a factor of 2 increases P(est) by about 13%. Therefore an inaccurate VINA only has a small to moderate effect on P(est).

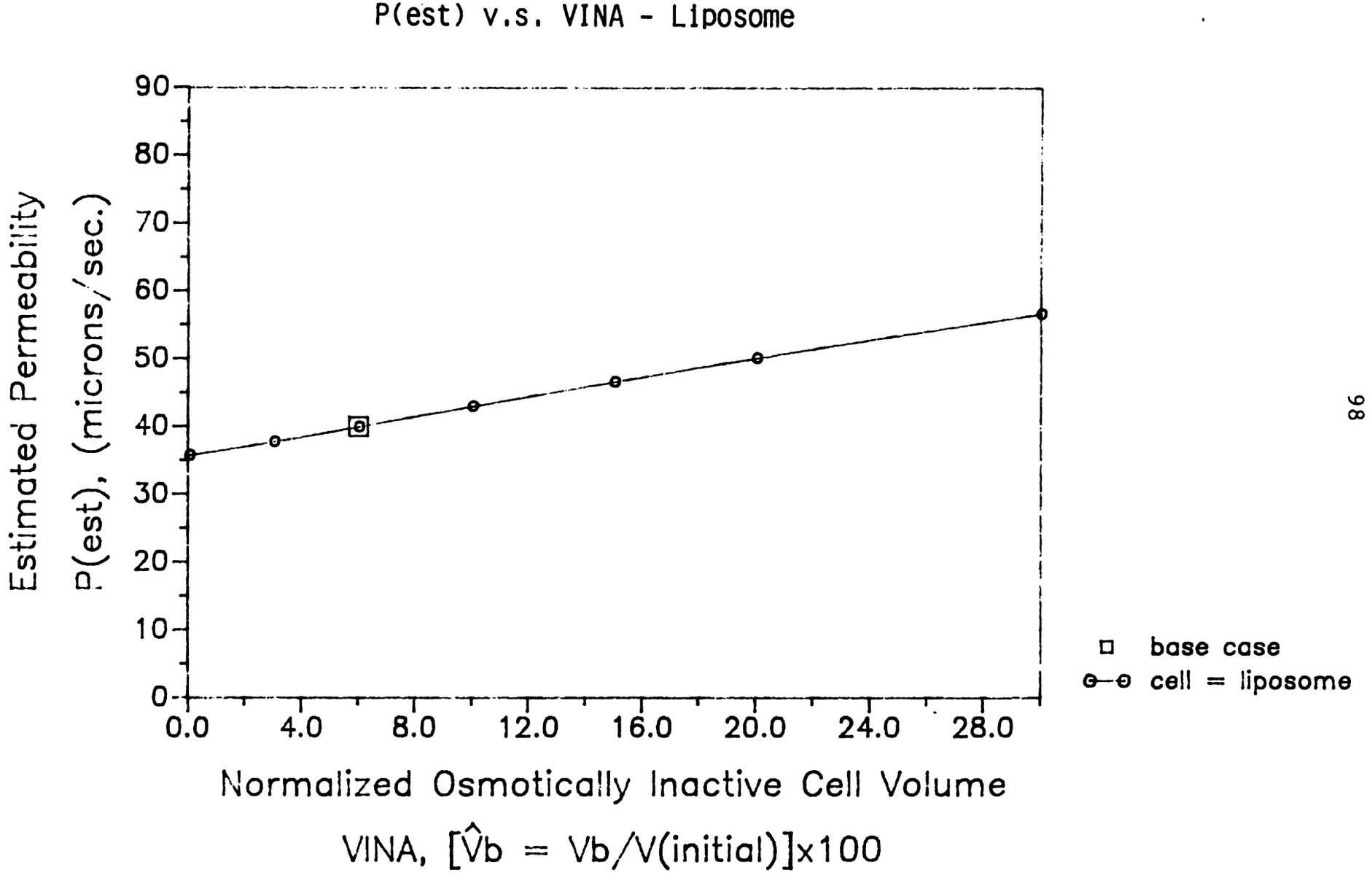

P(est) v.s. VINA - Liposome
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0.0
4.0
8.0
12.0
16.0
20.0
24.0
28.0
Normalized Osmotically Inactive Cell Volume
VINA, [V̂b = Vb/V(initial)]x100
base case
cell = liposome

Figure 6.2.5

6.2.6 The Effect of Varying DELAY

TABLE 6.2.7 - The Effect of Varying DELAY

	DELAY (sec)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	0.0	38.9 ± 0.110	0.003
	1.0	39.2 ± 0.080	0.002
	2.0	39.6 ± 0.054	0.001
**	3.0	39.9 ± 0.037	0.000

** : The base case was modified here because during an actual experimental run there was a time delay (DELAY - defined in Section 4.5) when the hypertonic solution was started, (due to the time it took for the hypertonic solution to travel from the electronic solenoid switch valve to the bulk flow region under the dialysis membrane). Based on the volumetric flow rate of the hypertonic solution, an average time delay was calculated to be about 2.7 sec. A time delay of 3.0 sec. was chosen for the new base case to allow for a little extract delay in case the flow rate decreased.

The results from this study show the DELAY was directly and nearly linearly related to P(est). By not accounting for a time delay when the data was recorded P(est) would only ben under estimated approximately 3%. Therefore, relationship that exists between the time delay and P(est) does appear to be critically important (for this case) because the slope of the line in Figure 6.2.6 is very small.

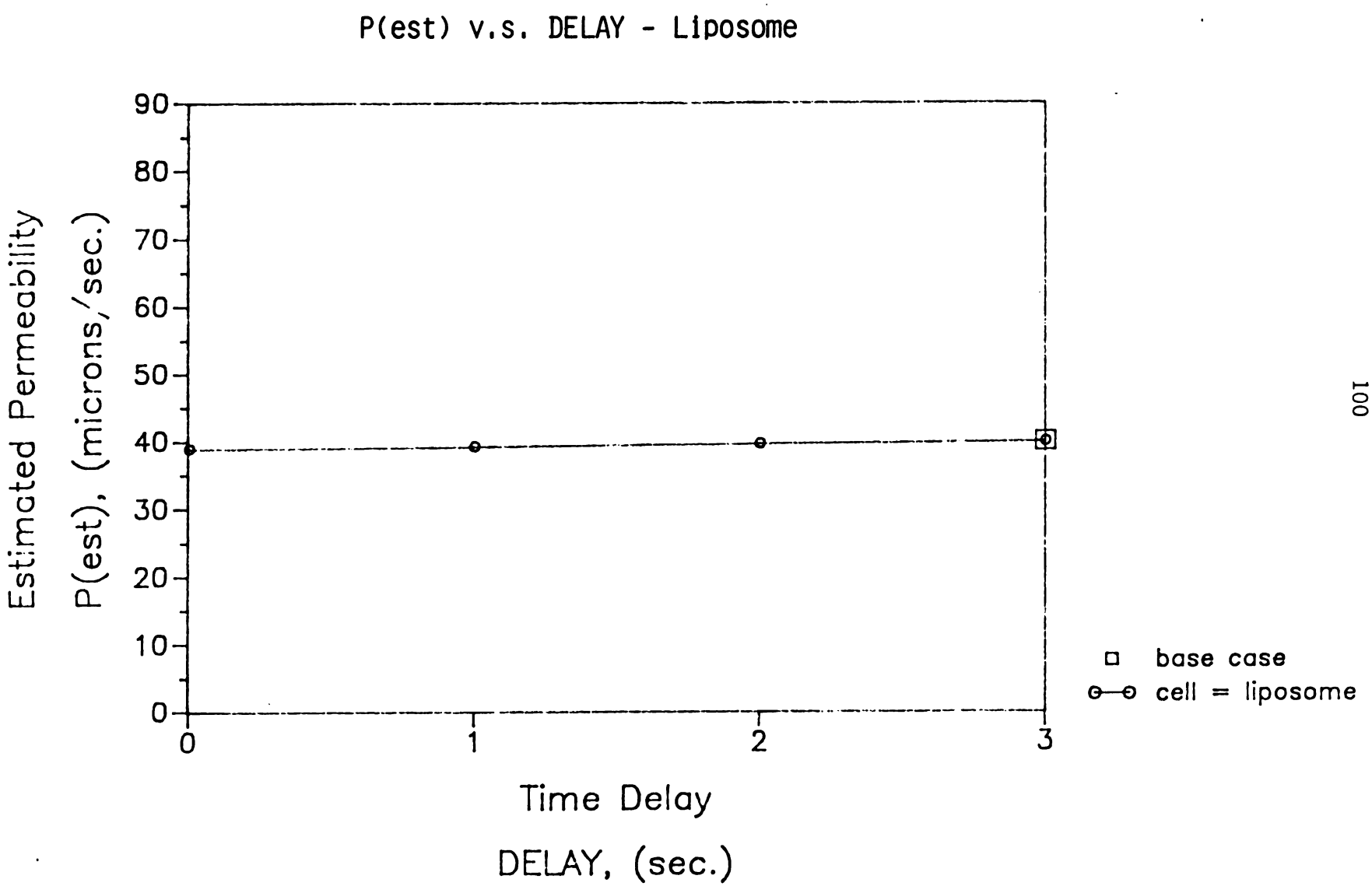

P(est) v.s. DELAY - Liposome
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0
1
2
3
Time Delay
DELAY, (sec.)
base case
cell = liposome

Figure 6.2.6

6.2.7 The Effect of Varying DR

TABLE 6.2.8 - The Effect of Varying DR

	DR (μm)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
*	0.000	39.9 ± 0.037	0.000
	0.001	40.0 ± 0.040	0.000
	0.005	40.0 ± 0.083	0.002
	0.01	39.8 ± 0.152	0.005
	0.05	39.8 ± 0.768	0.134
	0.1	39.6 ± 1.513	0.524
	0.2	39.2 ± 2.983	2.105
	0.5	38.0 ± 7.144	13.260
	1.00	36.5 ± 13.43	52.895

* = original base case

The results from this study show that P(est) was not greatly effected by DR, while the standard deviation increased linearly as DR increased (see Figure 6.2.7). Recall from Figure 3.2.1, p.31 that DR was defined to be the radius randomness factor. DR was implemented into SENS to impose a (pseudo) randomness factor on the generated radius history of a cell. This randomness factor was meant to incorporate the uncertainties mentioned in Section 4.5, (i.e. the fluctuations of the outer membrane of the cell and/or measurement errors that may have occurred. An attempt was made to estimate the maximum magnitude of this inaccuracy. Based on the TV monitor screen size, the video camera used and the lens in the microscope the cell was magnified 5080 times. The uncertainty in measuring the cell radius was estimate by approximating the range the cell radius could be. For example, a cell with a 20 μm diameter would measure 10.16 cm in the TV monitor. Based upon the

apparent thickness of the membrane projected on the screen and the "steadiness" of the cell on the screen, which vibrated slightly on the screen when the pause function was used, the uncertainty was approximated to be ±0.1 cm which corresponds to 0.1 μm uncertainty in the cell radius.

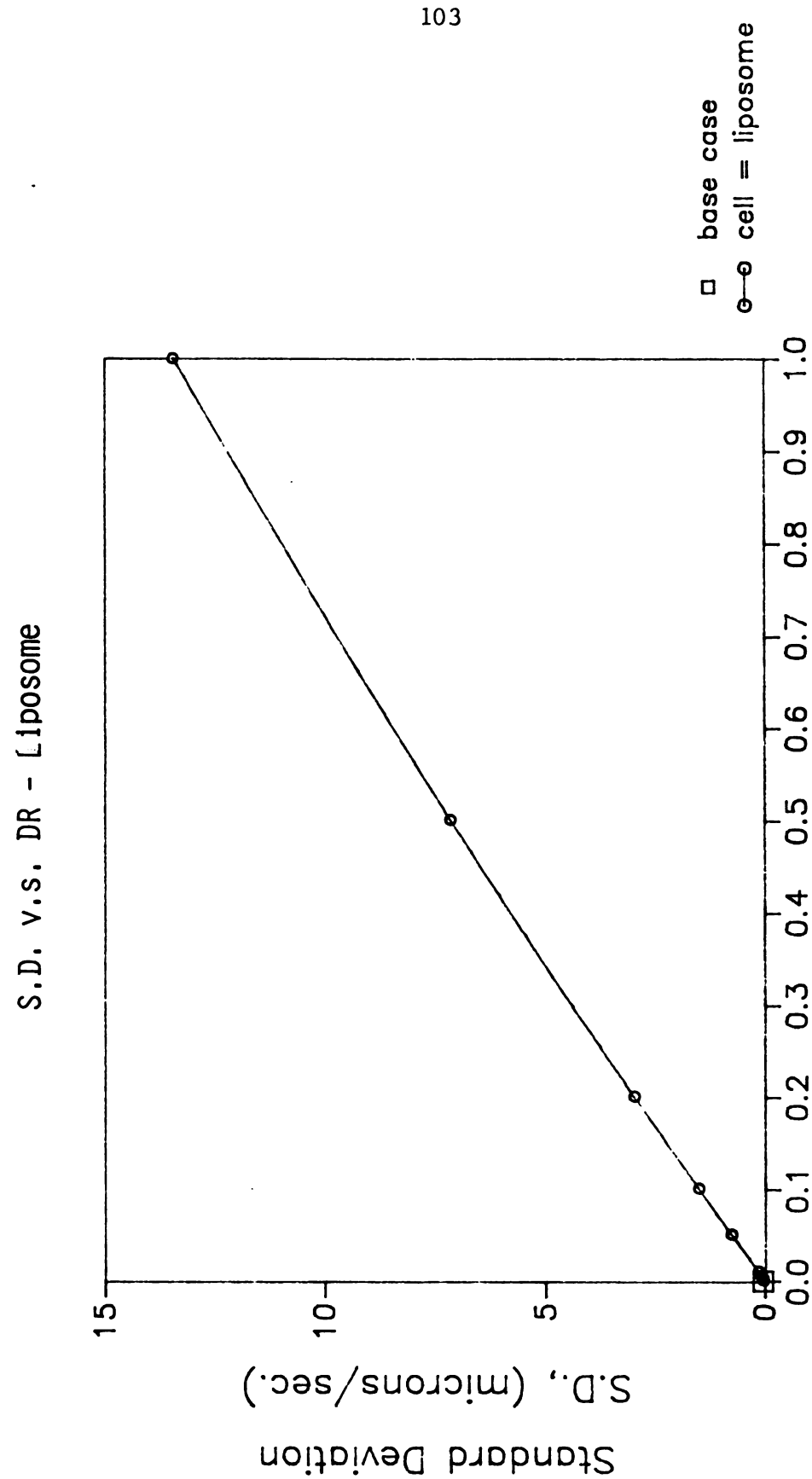

S.D. v.s. DR - Liposome
Standard Deviation
S.D., (microns/sec.)
15
10
5
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Imposed Randomness
DR, (microns)
base case
cell = liposome

Figure 6.2.7

6.2.8 The Effect of the Number of Data Points

TABLE 6.2.9 - The Effect of the Number of Data Points

# of points	Permeability and Standard Deviation (μm/sec)	Minimum Sum
4	51.4 ± 2.85	0.006
6	49.4 ± 5.63	0.064
10	46.6 ± 3.74	0.088
15	42.4 ± 3.25	0.242
20	40.0 ± 2.48	0.305
25	39.3 ± 2.06	0.350
30	39.2 ± 1.77	0.375
35	39.3 ± 1.65	0.422
40	39.4 ± 1.57	0.527
45	39.4 ± 1.45	0.570
50	39.5 ± 1.38	0.644
100	39.6 ± 0.93	1.151

Note: All of the input parameters used for this case were the same as described for the base case except DR was set at 0.1 μm. The rational for using 0.1 μm for DR was described in Section 6.2.7, (i.e. this was the best estimate for the uncertainty when the cell radius measured). If DR was kept at 0.0 μm the result would always be P(est) = 39.9 μm/sec, S.D. = 0.0 μm/sec and the SUMIN = 0.000. It should also be note that the data points used for each of the above cases were equally space within the 0 - 500 sec time interval.

The above results appear to show that the number of data points, used to generate a P(est), was related to the estimated permeability in a non-linear way. The estimated permeability started at 51.4 μm/sec and decreased until the number of data points was approximately 30, where P(est) appears to level off around 39.5 μm/sec (see Figure 6.2.8). Intuitively one would think that P(est) would oscillate about 39.9 μm/sec when only a few data points were used and then level off, at approximately 39.9 μm/sec, as the number of points was increased. It

should also be noted that it did not take very many points to get a reasonably good estimate for the membrane permeability - approximately 15 points or so. Also, recall from Section 4.5 that the number of data points recorded for each experiment conducted was about 20 - 30.

The above results also show that the standard deviation was inversely related to the number of points, with the exception of the case for the number of points equal to 4. When the standard deviation was plotted as a function of the inverse square root of the number of points the relationship was approximately linear, (if the case for the number of points equal to 4 was ignored), (see Figure 6.2.9). This was expected based on the relationship between the number of data points and the standard deviation given by equation (2.3.10).

Finally the number of data points was found to be related to the minimum sum in an increasingly linear manner (see Figure 6.2.10). This was also anticipated based on the relationship described in equation (2.3.3).

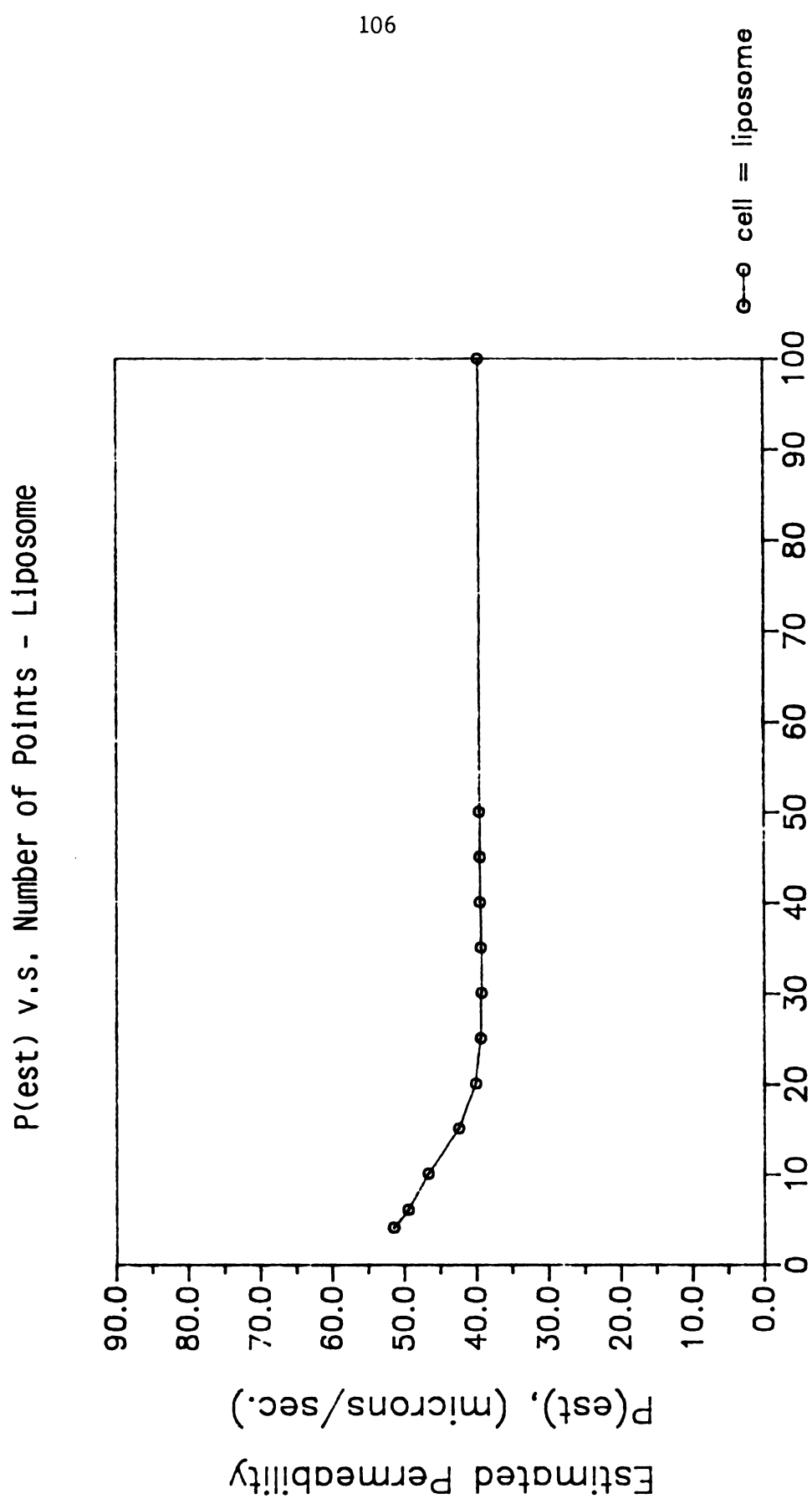

P(est) v.s. Number of Points - Liposome
Estimated Permeability
P(est), (microns/sec.)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
10
20
30
40
50
60
70
80
90
100
Number of points
cell = liposome

Figure 6.2.8

S.D. v.s. 1/(Number of Points)$^{\frac{1}{2}}$

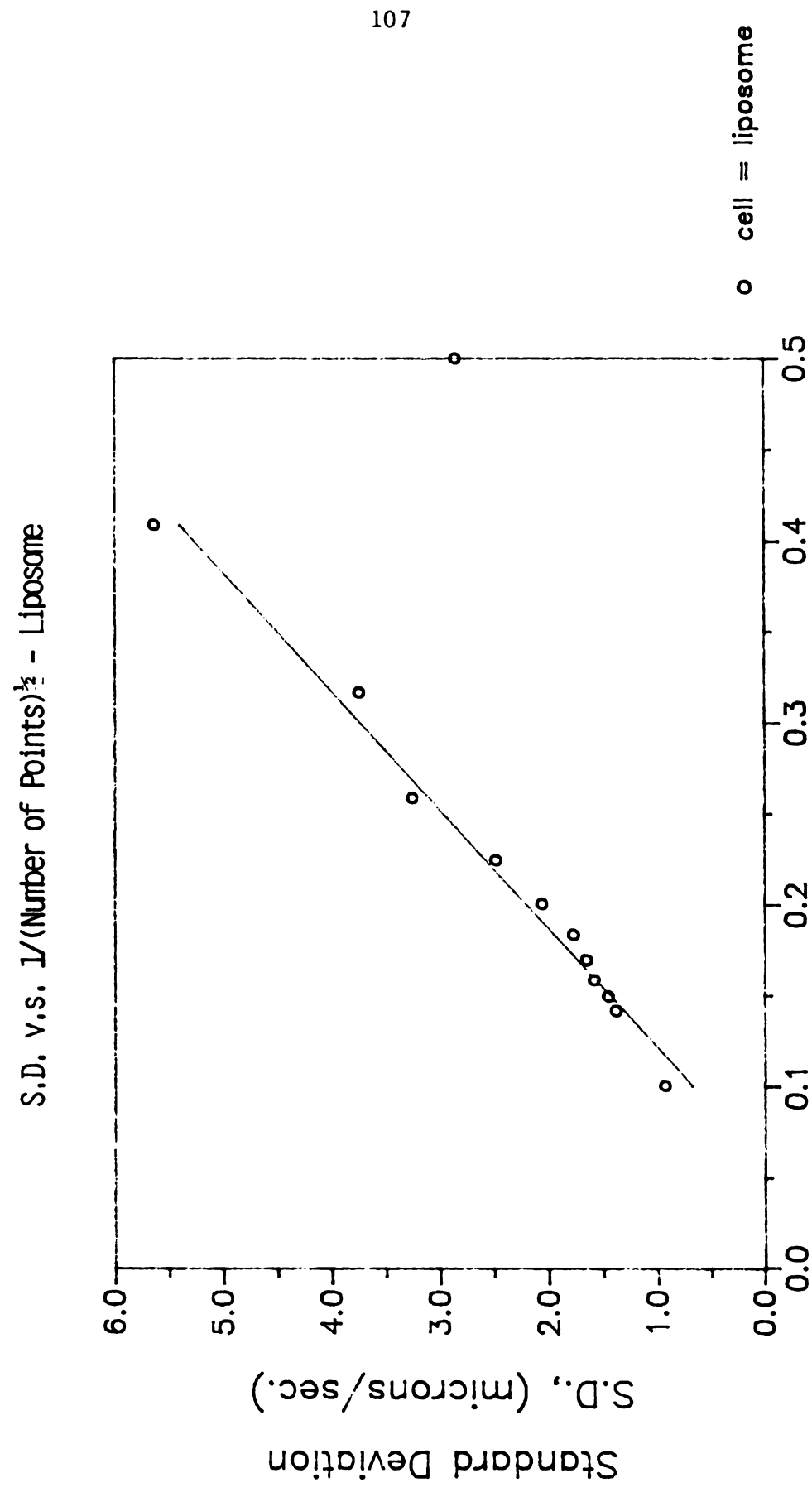

S.D. v.s. 1/(Number of Points)½ - Liposome
o cell = liposome
Standard Deviation
S.D., (microns/sec.)
1/[Number of points]½
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0.1
0.2
0.3
0.4
0.5

Figure 6.2.9

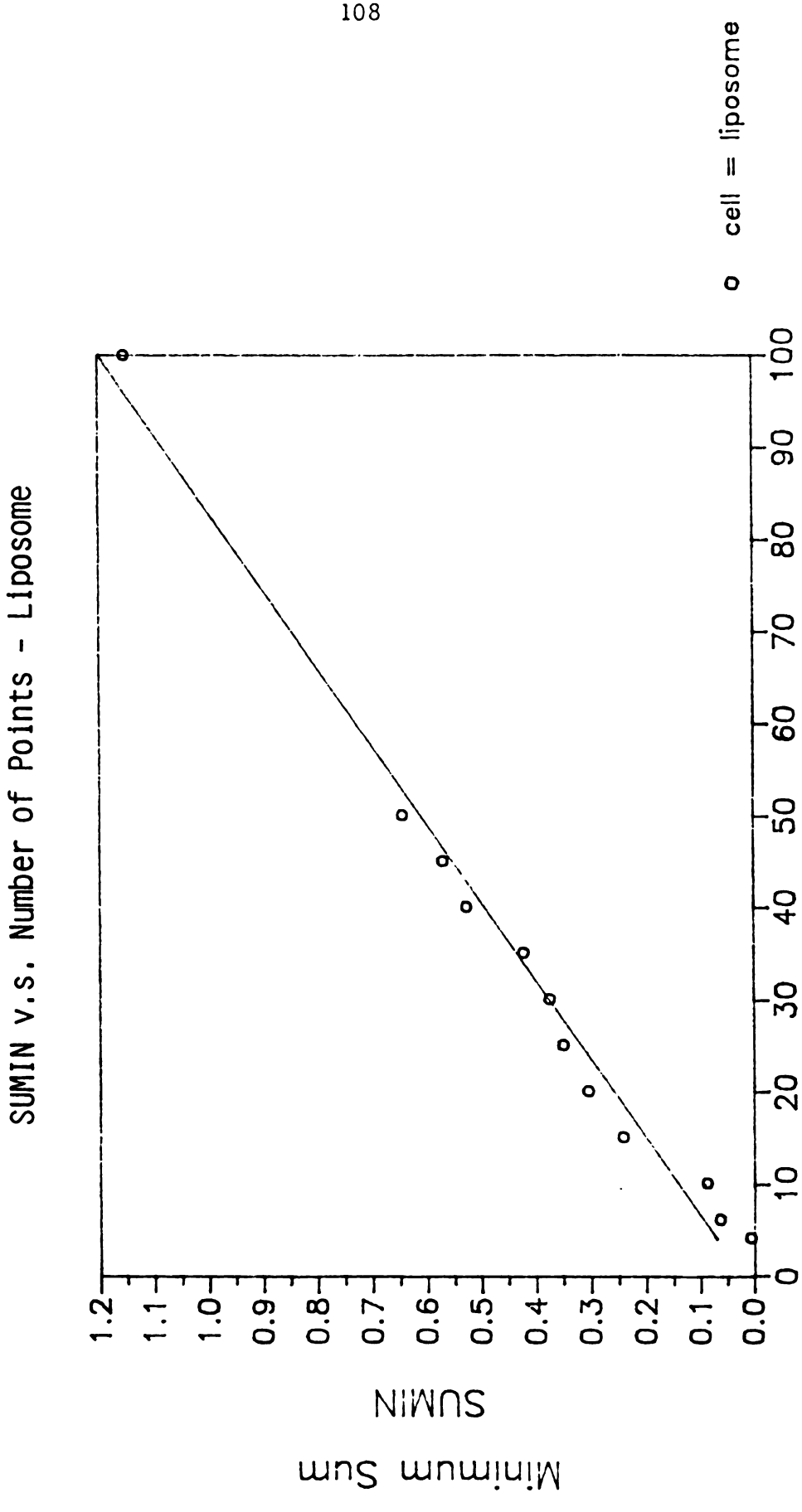

SUMIN v.s. Number of Points - Liposome
o cell = liposome
Minimum Sum
SUMIN
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
100
Number of points

Figure 6.2.10

6.2.

* -

mem

pro

the

act

act

ree

ori

was

[3]

6.2.9 The Effect of Varying Ea of the Dialysis Membrane on the Ea of the Cell

TABLE 6.2.10 - The Effect of Varying Ea of the Dialysis Membrane on the Ea of the Cell

	Ea, Dialysis Membrane (Kcal/mole)	Ea, Liposome Membrane (Kcal/mole)
	0.5	12.0
	1.0	11.7
	2.0	11.2
	4.0	10.2
*	6.53	8.9
	8.0	8.1
	10.0	7.0
	12.5	5.5
	15.0	3.7

* = original base case

This investigation shows that the relationship between the two membrane activation energies was slightly non-linear and inversely proportional (see Figure 6.2.11). What this study suggested was that if the manufacturer incorrectly stated the temperature effects, i.e. the activation energy, with respect to the dialysis membrane, then the activation energy reported in this work for the liposome would have to be reevaluated. However, this is not likely but it is possible. The original base case dialysis membrane E_a was 6.53 Kcal/mole. This data was obtained from the manufacture (ENKA) and was published in reference [3].

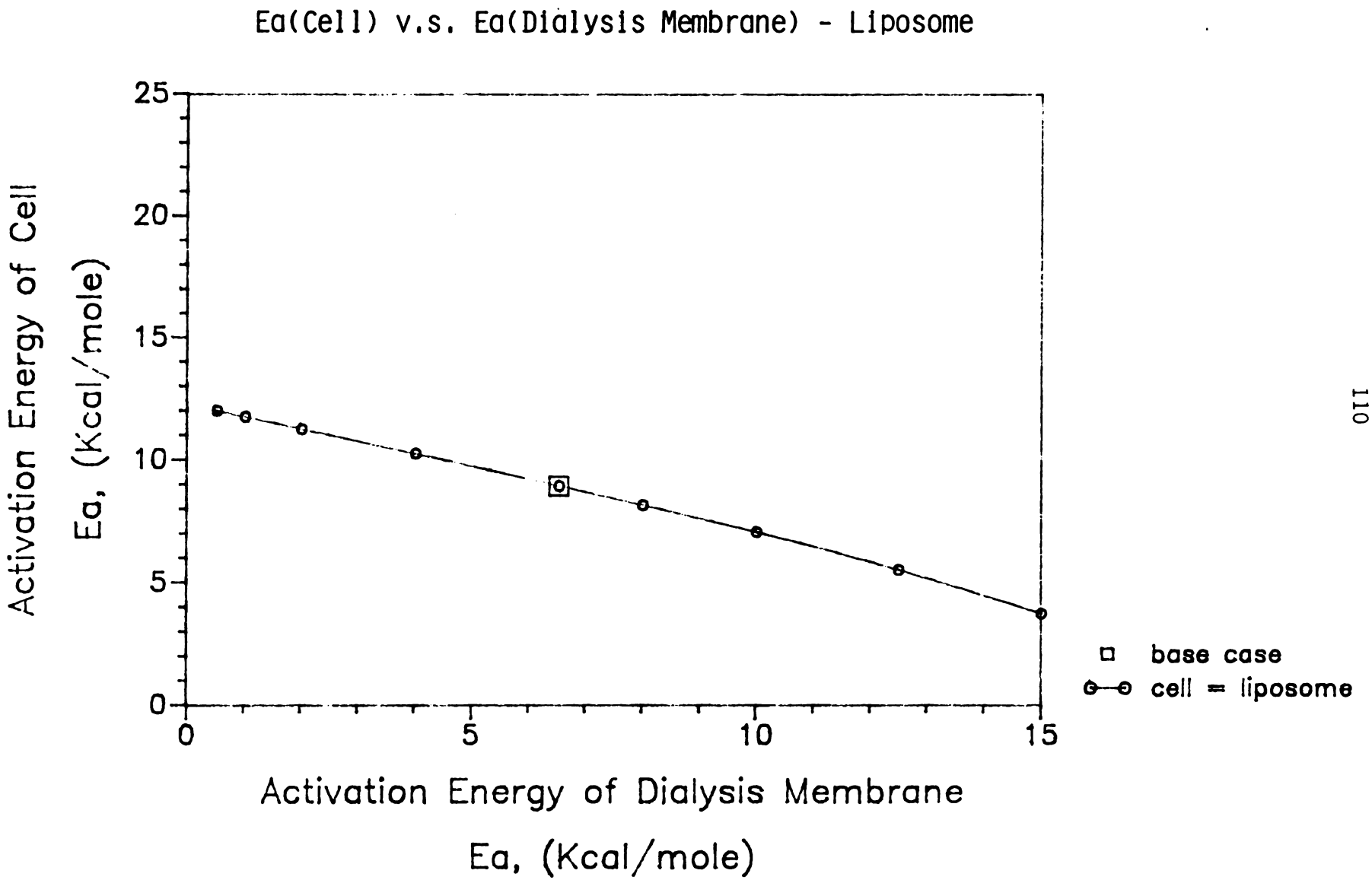

Ea(Cell) v.s. Ea(Dialysis Membrane) - Liposome
Activation Energy of Cell
Ea, (Kcal/mole)
25
20
15
10
5
0
0
5
10
15
base case
cell = liposome
Activation Energy of Dialysis Membrane
Ea, (Kcal/mole)

Figure 6.2.11

6.3

show

enco

at 2

radiu

Var

H

V

R

D

R

C

C

D

D

T

T

D

6.3 The Lymphocyte Base Case

The experimental conditions used for the lymphocyte base case are shown below in Table 6.3.1. The conditions specified were also commonly encountered conditions when experiments were performed using lymphocytes at 25°C. Again, SENS was used in the simulation mode to generate a radius history for a cell with an estimated permeability of 9.3 μm/sec.

TABLE 6.3.1 - The Lymphocyte Base Case

cell type: lymphocyte
solute: sodium chloride
temperature: 25°C

Variable	Description of Variable	Specified Value
RL1	Wetted dialysis membrane thickness	16 μm
RL2	Sample region thickness	100 μm
LIP	Cell position in sample region	5
D2	Diffusivity of solute in free solution	$1.483*10^{-9}$ m^2/sec
D1	Diffusivity of solute in dialysis membrane	D2*0.1
Hd	Mass Transfer coefficient	D2*10000
VINA	Normalized osmotically inactive volume	34.7%
RINIT	Initial cell radius	5.5 μm
DR	Imposed randomness factor	0.0 μm
RMAG	Magnification factor	1
CINIT	Initial (isotonic) concentration	0.291 osmol
CINF	Final (hypertonic) concentration	0.725 osmol
DP	Permeability step	0.1 μm/sec
DT	Time step	1.0 sec
TM0	Starting time	0.0 sec
TM1	End time	100 sec
DELAY	Time delay	0.0 sec

Whe

into SENS

permeabil

Th

the same

through 6

deviation

tabulated

When these parameters and generated radius history data were reentered into SENS, in the (real) experimental parameter estimation mode, the resulting permeability was 9.3 ± 0.042 μm/sec (with a minimum sum of 0.000).

The approach used to investigate the input parameter of interest was the same approach as described for the liposome base case in Sections 6.2.1 through 6.2.9, (i.e. only the input parameter of interest was varied and any deviation from this approach was specifically stated). The results were also tabulated and graphs were produced where appropriate.

6.3.1

*

* = or

T

D_1 in

decrea

P(est)

case t

simila

value

the go

thus d

would

(at 2s

6.3.1 The Effect of Varying D1

TABLE 6.3.1 - The Effect of Varying D1

	D1 E9 (m^2/sec)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	1.5	5.2 ± 0.055	0.002
	1.0	5.4 ± 0.060	0.002
	0.5	5.9 ± 0.070	0.002
	0.2	7.8 ± 0.068	0.001
*	0.148	9.3 ± 0.042	0.000
	0.1	12.7 ± 0.348	0.004
	0.05	35.8 ± 11.38	0.106
	0.04	73.5 ± 63.06	0.211

* = original base case

The results show that the estimated permeability was inversely related to D_1 in a non-linear way (see Figure 6.3.1). When D_1 was doubled P(est) decreased approximately 30%, while when D_1 was increased by a factor of 10 P(est) only decreased another 14%. When the value of D_1 was half the base case the estimated permeability increased approximately 250%. Therefore, a similar conclusion that was made in 6.2.1 can be made here also, i.e. the value used for D_1 was on the border line of being critically important. Also the goal here would be to either increase the dialysis membrane permeability, thus diminishing the effect D_1 has on P(est). The maximum value D_1 could have would be the same for the sodium chloride diffusivity in water, 1.48E-9 m^2/sec (at 25°C), however this would not be very realistic.

P(est) v.s. D_1 - Lymphocyte

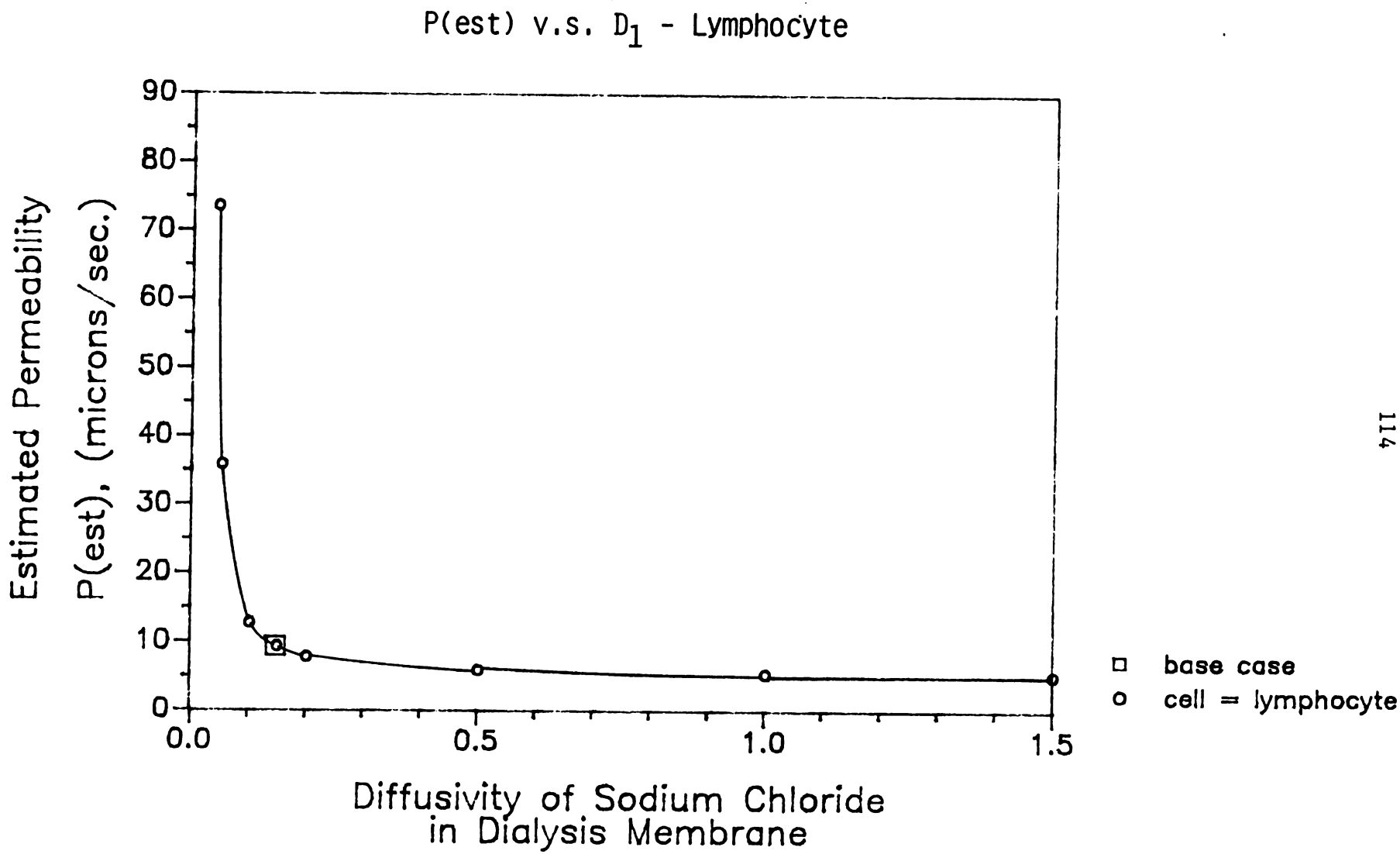

P(est) v.s. D_1 - Lymphocyte
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
Diffusivity of Sodium Chloride
in Dialysis Membrane
D1 E9, (meters**2/sec.)
base case
cell = lymphocyte

Figure 6.3.1

6.3.

* = c

inver

(see

P(est

Again

accur

6.3.2 The Effect of Varying Hd

TABLE 6.3.2 - The Effect of Varying Hd

	Hd E4 (m/sec)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	1.5	6.3 ± 0.075	0.002
	1.0	6.4 ± 0.076	0.002
	0.5	6.8 ± 0.078	0.002
	0.2	8.3 ± 0.060	0.001
*	0.148	9.3 ± 0.042	0.000
	0.1	11.4 ± 0.192	0.002
	0.05	21.8 ± 2.920	0.044
	0.03	61.0 ± 43.80	0.205

* = original base case

The result from this study show that the estimated permeability was inversely related to the mass transfer coefficient in a non-linear manner also (see Figure 6.3.2). For example, increasing H_d by a factor of 2 decreased P(est) 15%, while decreasing H_d by a factor of 2 increased P(est) 194%. Again, as was mentioned Section 6.2.2, to minimize the importance of accurately estimating H_d by increasing the bulk flow rate.

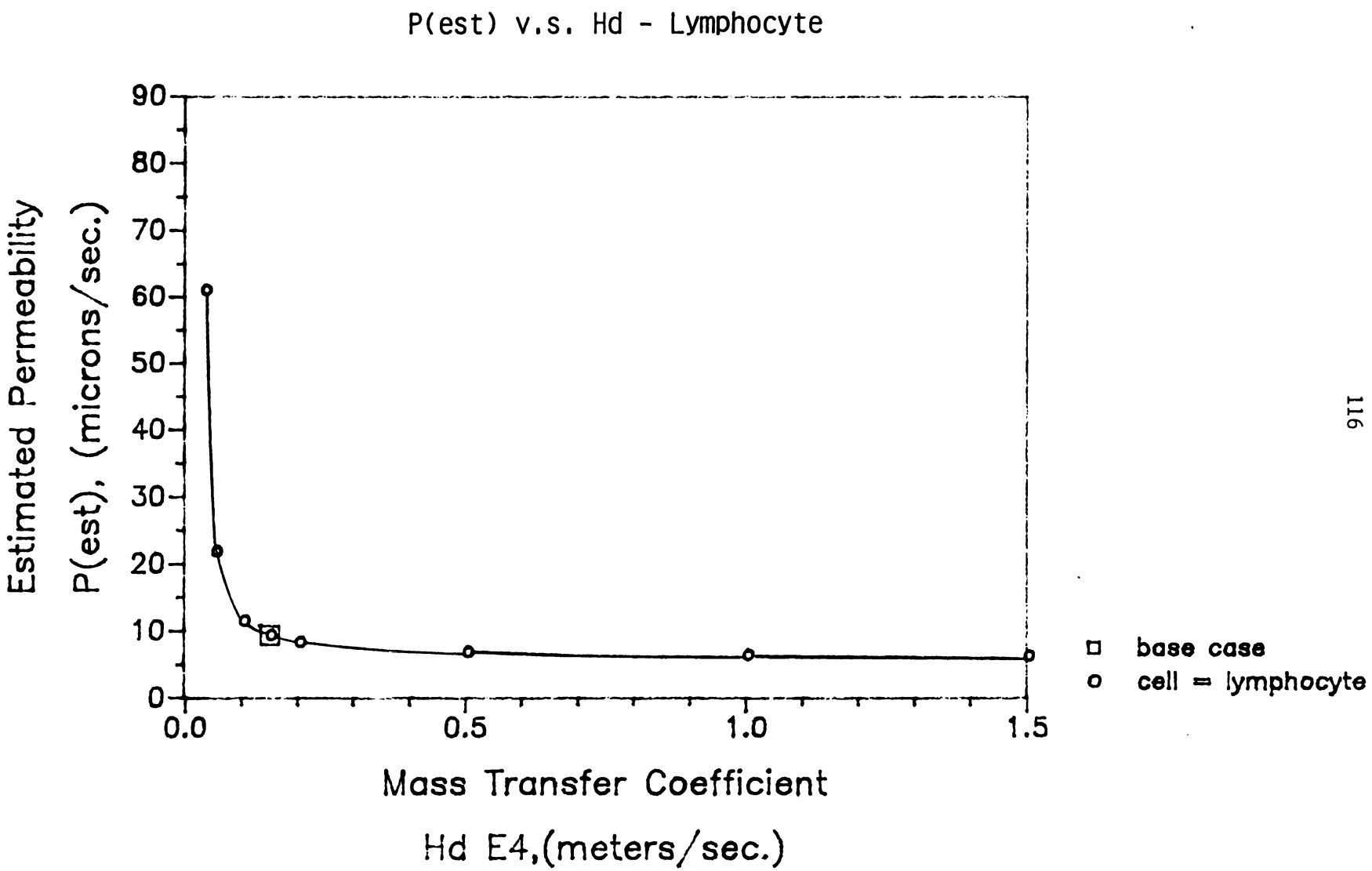

P(est) v.s. Hd - Lymphocyte
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
Mass Transfer Coefficient
Hd E4,(meters/sec.)
base case
cell = lymphocyte

Figure 6.3.2

6 - 3.3 The Effect of Varying RL2

TABLE 6.3.3 - The Effect of Varying RL2

	RL2 (μm)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	10	4.0 ± 0.039	0.002
	50	5.8 ± 0.097	0.005
	75	7.4 ± 0.112	0.003
*	100	9.3 ± 0.042	0.000
	150	12.9 ± 0.786	0.020
	200	15.7 ± 2.261	0.082
	250	17.3 ± 2.716	0.163
	300	18.1 ± 5.070	0.242

* — original base case

The results show the RL2 was proportionally related to P(est) in a slightly non-linear way. Recall from Section 6.2.3 the two extreme cases of a small RL2, which resulted in treating the sample region as a lumped system, and of a large RL2, which resulted in a infinite concentration gradient in the sample region. These two extreme cases cause the curve in Figure 6.3.3 to approach two asymptotes at about P(est)=4 μm/sec, for a small RL2, and about P(est)=20 μm/sec, for larger RL2. Again, the approximate range that was used when experiments were conducted was between 50 μm, which would decrease P(est) by 38% if RL2 had been underestimated, and 150 μm, which would increase P(est) by 39% if RL2 has been overestimated.

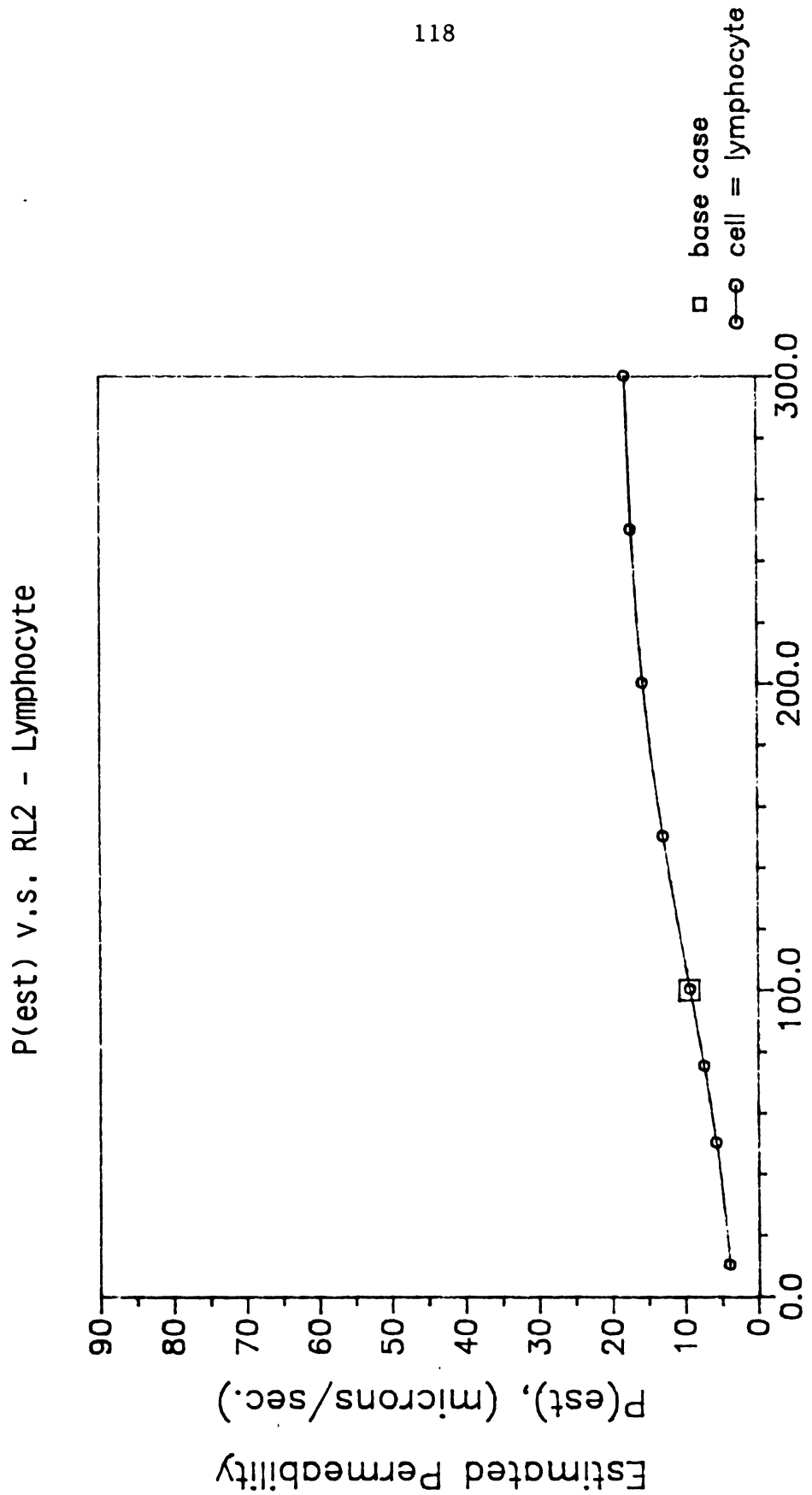

P(est) v.s. RL2 - Lymphocyte
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0.0
100.0
200.0
300.0
Sample Region Thickness
RL2, (microns)
base case
cell = lymphocyte

Figure 6.3.3

6.3.4 The Effect of Varying LIP

Note, investigating LIP for the lymphocyte case will not be necessary because the author was very certain that the position of the lymphocytes that were tested were always next to the dialysis membrane. Therefore the position was LIP = 5 for all five lymphocyte experiments for all five temperature ranges.

6.3.5 The Effect of Varying VINA

TABLE 6.3.5 - The Effect of Varying VINA

	VINA (%)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	15.0	3.9 ± 0.389	0.626
	20.0	4.9 ± 0.515	0.444
	25.0	6.3 ± 0.616	0.229
	30.0	7.9 ± 0.510	0.059
*	34.7	9.3 ± 0.042	0.000
	40.0	10.8 ± 1.307	0.081
	45.0	12.3 ± 3.702	0.310
	50.0	14.0 ± 8.100	0.688
	55.0	16.0 ± 16.20	1.211

* = original base case

The relationship that resulted between VINA and P(est) in this study was directly proportional and approximately linear (see Figure 6.3.4). It should be noted that the curve in Figure 5.2.2, which the normalized osmotically inactive cell volume obtained, appears to slightly non-linear. If one were to trace a spline curve through the points and extrapolate the curve to the y-axis the resulting $\hat{V}_b$ would approximately 45% which would increase the resulting permeability to 12.3 μm/sec. However, this $\hat{V}_b$ was not used in order to handle the resulting data the same as other investigators. To clarify this more experiments should be conducted.

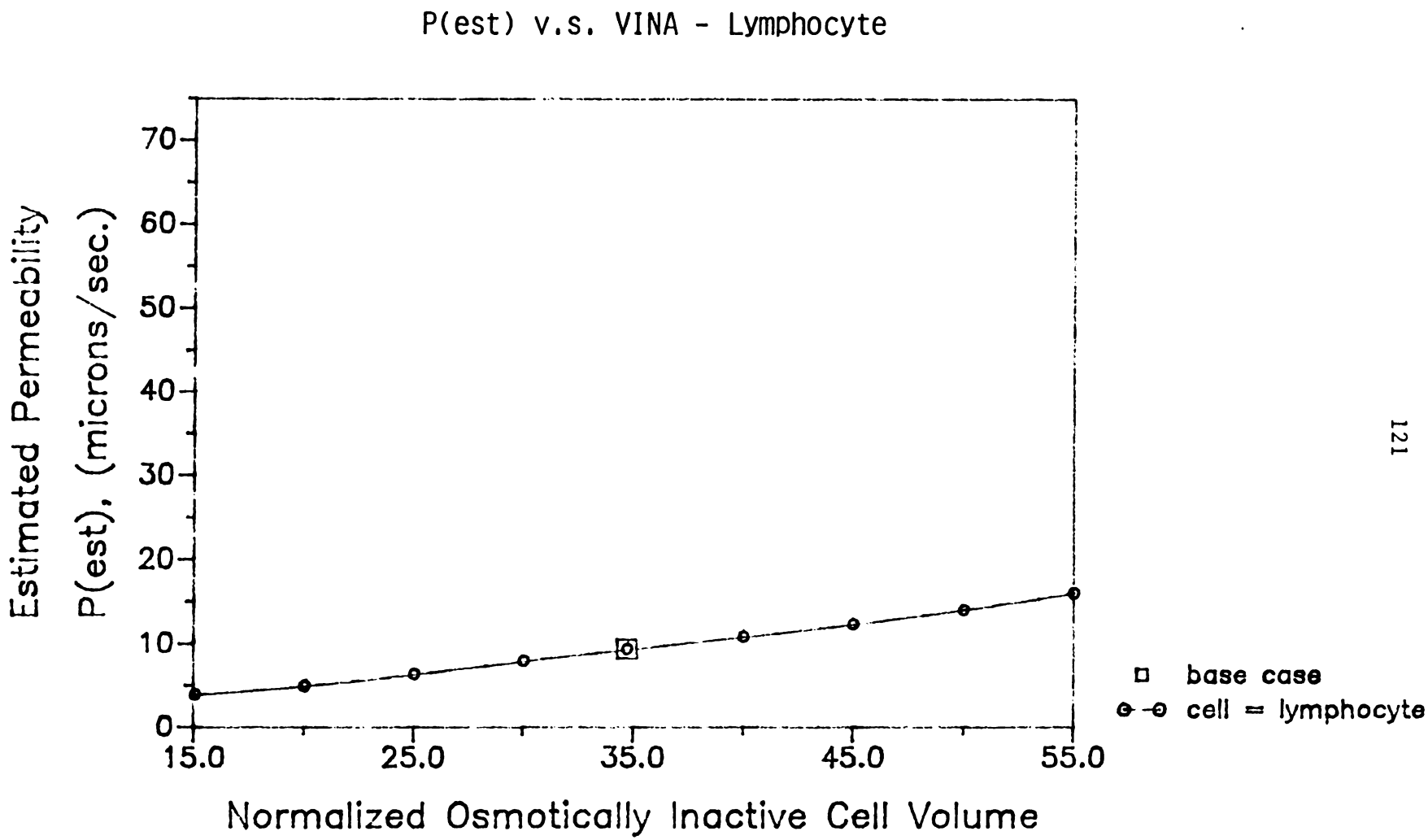

P(est) v.s. VINA - Lymphocyte
Estimated Permeability
P(est), (microns/sec.)
70
60
50
40
30
20
10
0
15.0
25.0
35.0
45.0
55.0
Normalized Osmotically Inactive Cell Volume
VINA, [V̂b = Vb/V(initial)]x100
base case
cell = lymphocyte

Figure 6.3.4

6.3.6 The Effect of Varying DELAY

TABLE 6.3.6 - The Effect of Varying Delay

	DELAY (sec)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
	0.0	7.3 ± 0.150	0.005
	1.0	7.8 ± 0.114	0.002
	2.0	8.5 ± 0.072	0.001
**	3.0	9.3 ± 0.043	0.000

** : The base case was modified here because during an actual experimental run there was a time delay (DELAY) when the hypertonic solution was started, (due to the time it took for the hypertonic solution to travel from the electronic solenoid valve to the bulk flow entrance port). Base upon the volumetric flow rate of the hypertonic solution, an average time delay was calculated to be about 2.7 sec. A time of 3.0 was chosen for the new base case to allow for a little extract delay in case the flow rate decreased.

The results from this study show that DELAY was directly proportional and approximately linearly related to P(est) (see Figure 6.3.5). Therefore, under estimating the delay time would result in under estimating the membrane permeability. For example, if the delay time of 3.0 seconds was not accounted for when the data was recorded, (which would imply a delay time of 0.0 seconds), P(est) would be under estimated by approximately 22%.

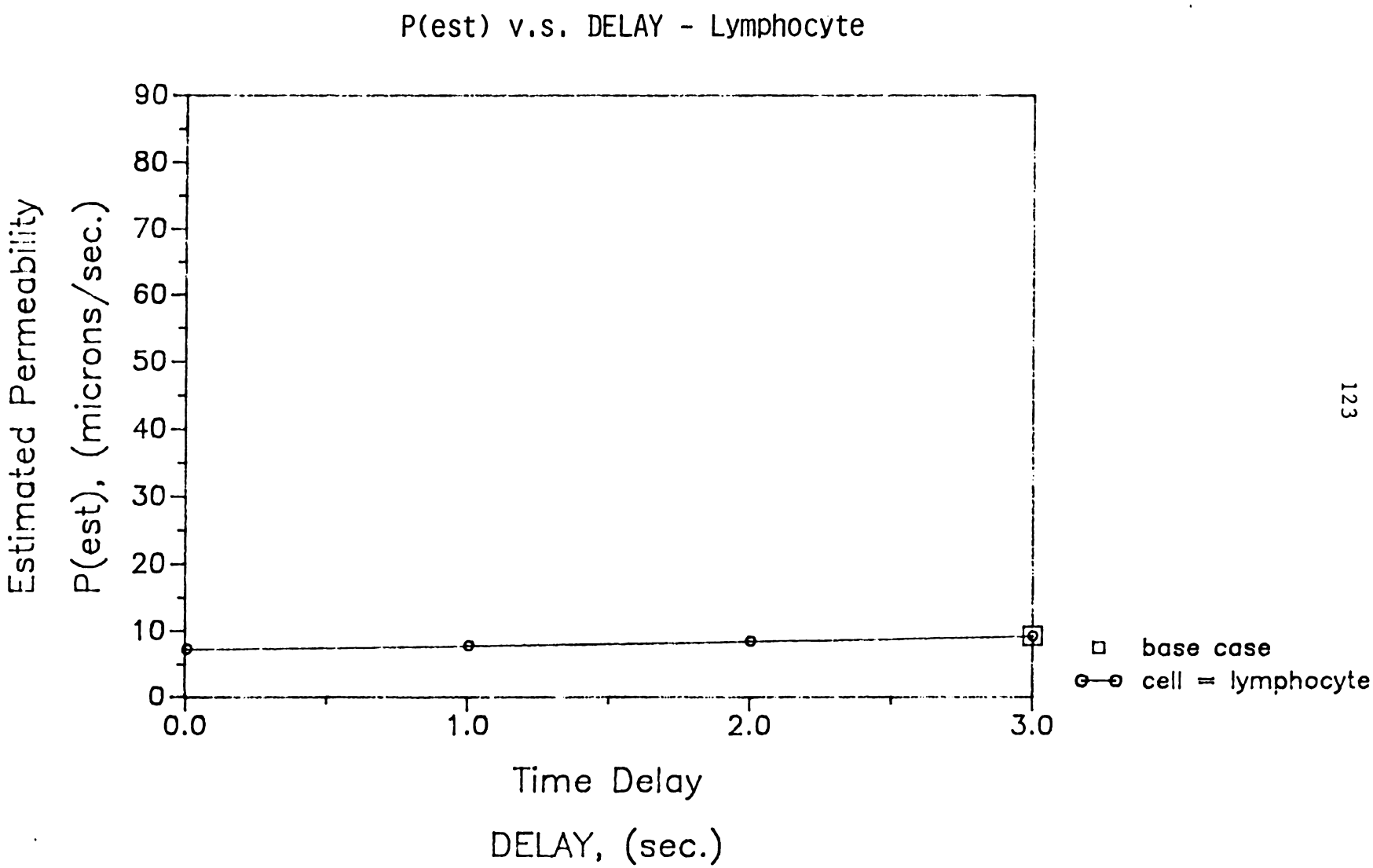

P(est) v.s. DELAY - Lymphocyte
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0.0
1.0
2.0
3.0
Time Delay
DELAY, (sec.)
base case
cell = lymphocyte

Figure 6.3.5

6.3.7 The Effect of Varying DR

TABLE 6.3.7 - The Effect of Varying DR

	DR (μm)	Permeability and Standard Deviation (μm/sec)	Minimum Sum
*	0.000	9.3 ± 0.042	0.000
	0.001	9.3 ± 0.042	0.000
	0.050	9.4 ± 0.091	0.001
	0.010	9.4 ± 0.180	0.003
	0.020	8.9 ± 0.373	0.014
	0.040	9.9 ± 0.784	0.055
	0.050	9.0 ± 1.030	0.085
	0.060	10.4 ± 1.270	0.123
	0.080	11.1 ± 1.880	0.218
	0.100	10.0 ± 2.650	0.338

* = original base case

The results from this study show that the estimated permeability, for the most part, was not greatly effected as the imposed randomness, DR, was increased, while the standard deviation increased, non-linearly, as DR was increased (see Figure 6.3.6). As mentioned in Section 6.2.7 an attempt was made to estimate the maximum possible inaccuracy obtained when measuring the cell radius (after an experimental run). This same approach was used to determine the likely range of uncertainty for the lymphocyte case also. The uncertainty in measuring the cell radius for this case was ±0.04 μm.

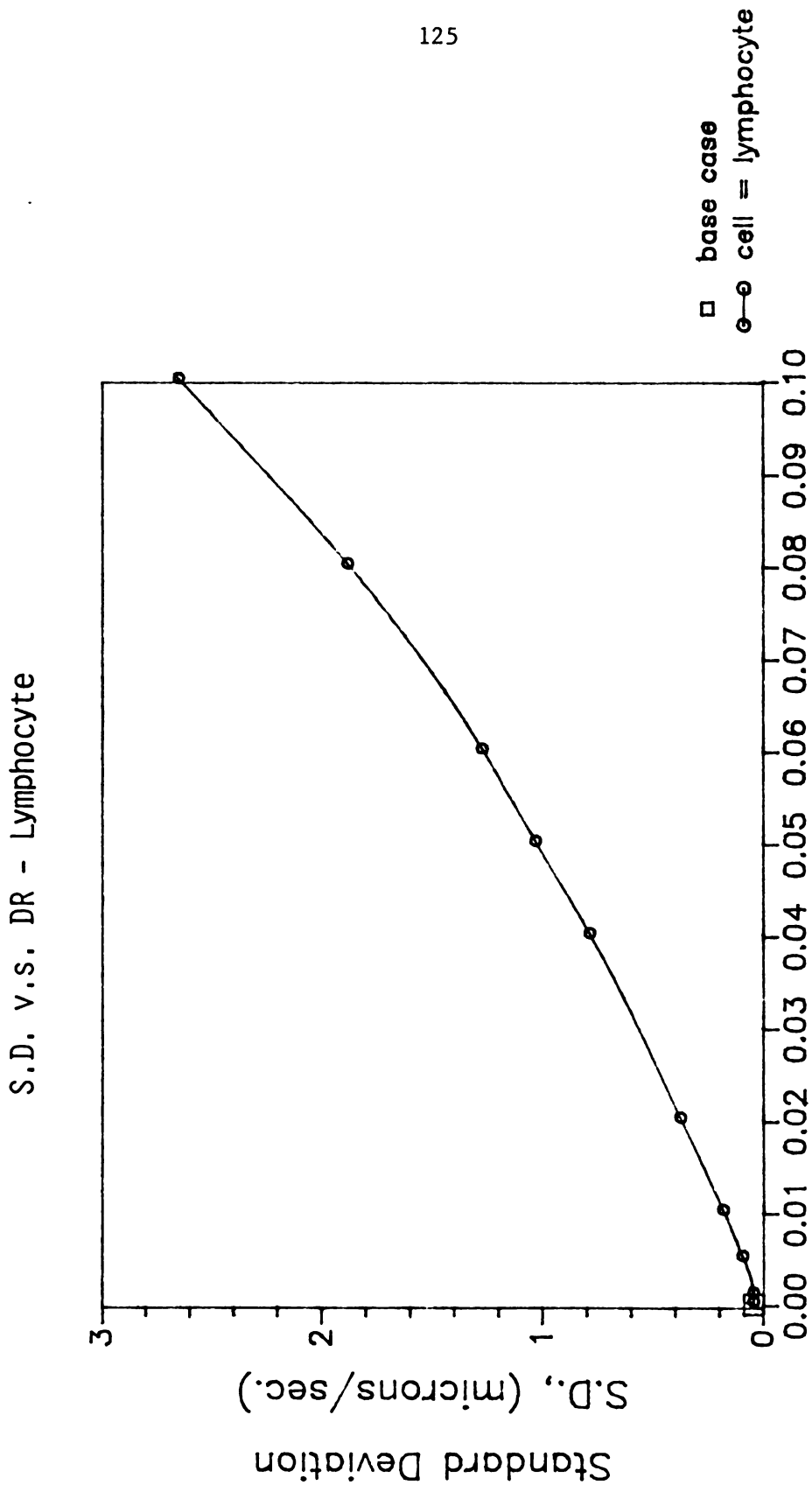

S.D. v.s. DR - Lymphocyte
Standard Deviation
S.D., (microns/sec.)
0
1
2
3
0.00 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.10
Imposed Randomness
DR, (microns)
base case
cell = lymphocyte

Figure 6.3.6

6.3.8 The Effect of the Number of Data Points

TABLE 6.3.8 - The Effect of the Number of Data Points

# of points	Permeability and Standard Deviation (μm/sec)	Minimum Sum
4	12.0 ± 1.76	0.003
6	12.0 ± 1.37	0.011
10	11.8 ± 1.29	0.014
15	11.3 ± 1.29	0.033
20	10.7 ± 1.02	0.044
25	10.0 ± 0.81	0.055
30	9.5 ± 0.65	0.060
35	9.3 ± 0.58	0.071
40	9.2 ± 0.55	0.084
45	9.1 ± 0.50	0.091
50	9.1 ± 0.47	0.103
100	9.2 ± 0.32	0.184

Note: All of the input parameters used for this case were the same as described for the base case except DR was set at 0.04 μm. This approach was based on the rational described in Section 6.2.8 and the uncertainty approximation made in Section 6.3.7. Also the number of data points used for each of the above cases were equally space with the 0 - 100 sec time range.

These results suggest that the estimated permeability was related to the number of data points recorded in a non-linear fashion. The estimated permeability started at about 12.0 μm/sec for a few points and decreased as the number of points was increased until the number of points reached about 30, where P(est) began steady at about 9.2 μm/sec (see Figure 6.3.7). Recall from section 4.5 that the number of data points recorded for each experimental run was 20 - 30.

Also from the above results, the standard deviation was related to the number of data points recorded in a decreasing non-linear manner, (as the number of points increased). Again as was mentioned in Section 6.2.8, if the

case where the number of data points equalled 4 was ignored, the standard deviation was then approximately linearly related to the inverse square root of the number of data points (see Figure 6.3.8), which was to be expected based on equation (2.3.10).

The relationship between the number of points and the minimum was approximately linear, which was also expected based on equation (2.3.3) (see Figure 6.3.9).

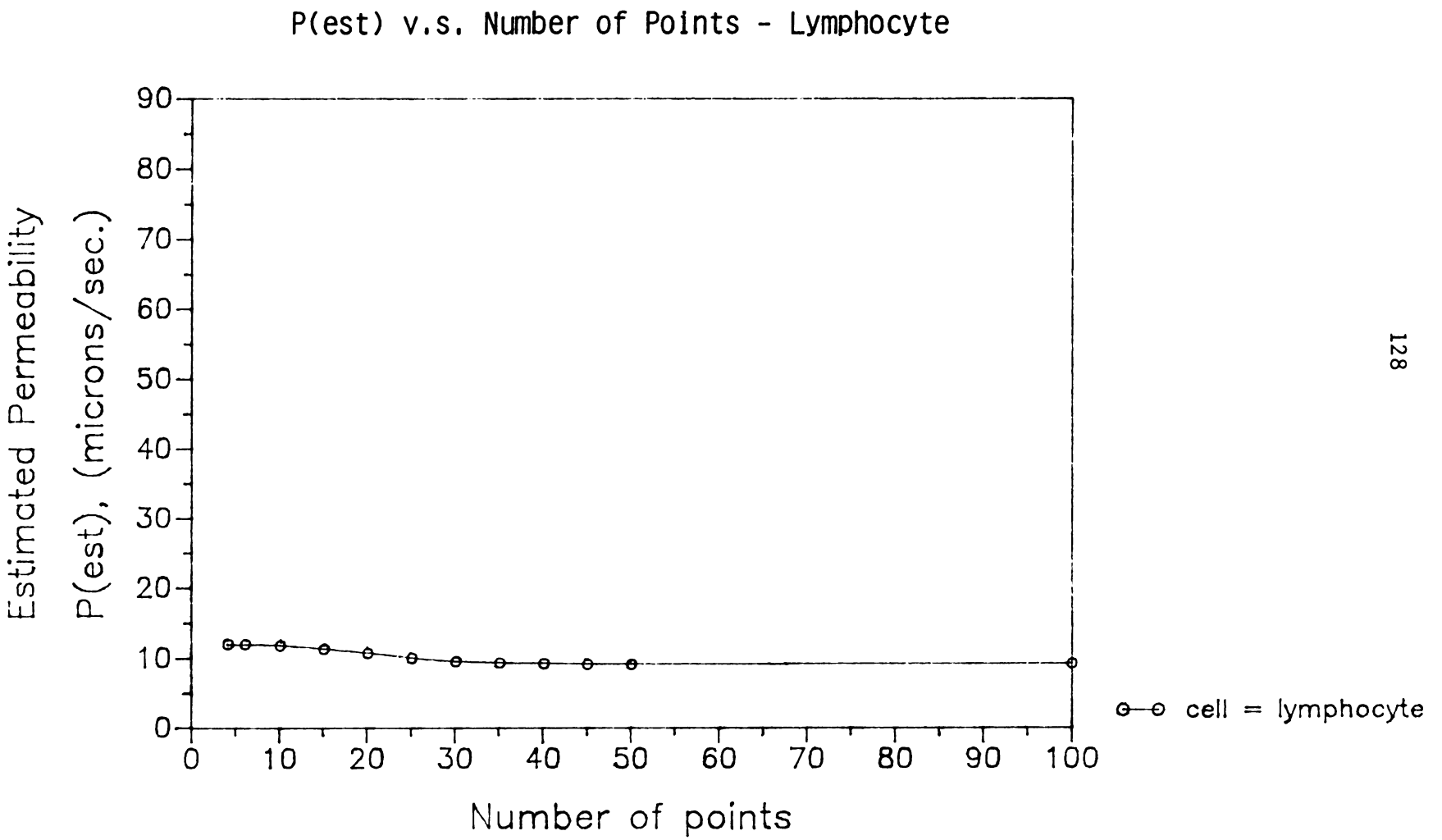

P(est) v.s. Number of Points - Lymphocyte
Estimated Permeability
P(est), (microns/sec.)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Number of points
cell = lymphocyte

Figure 6.3.7

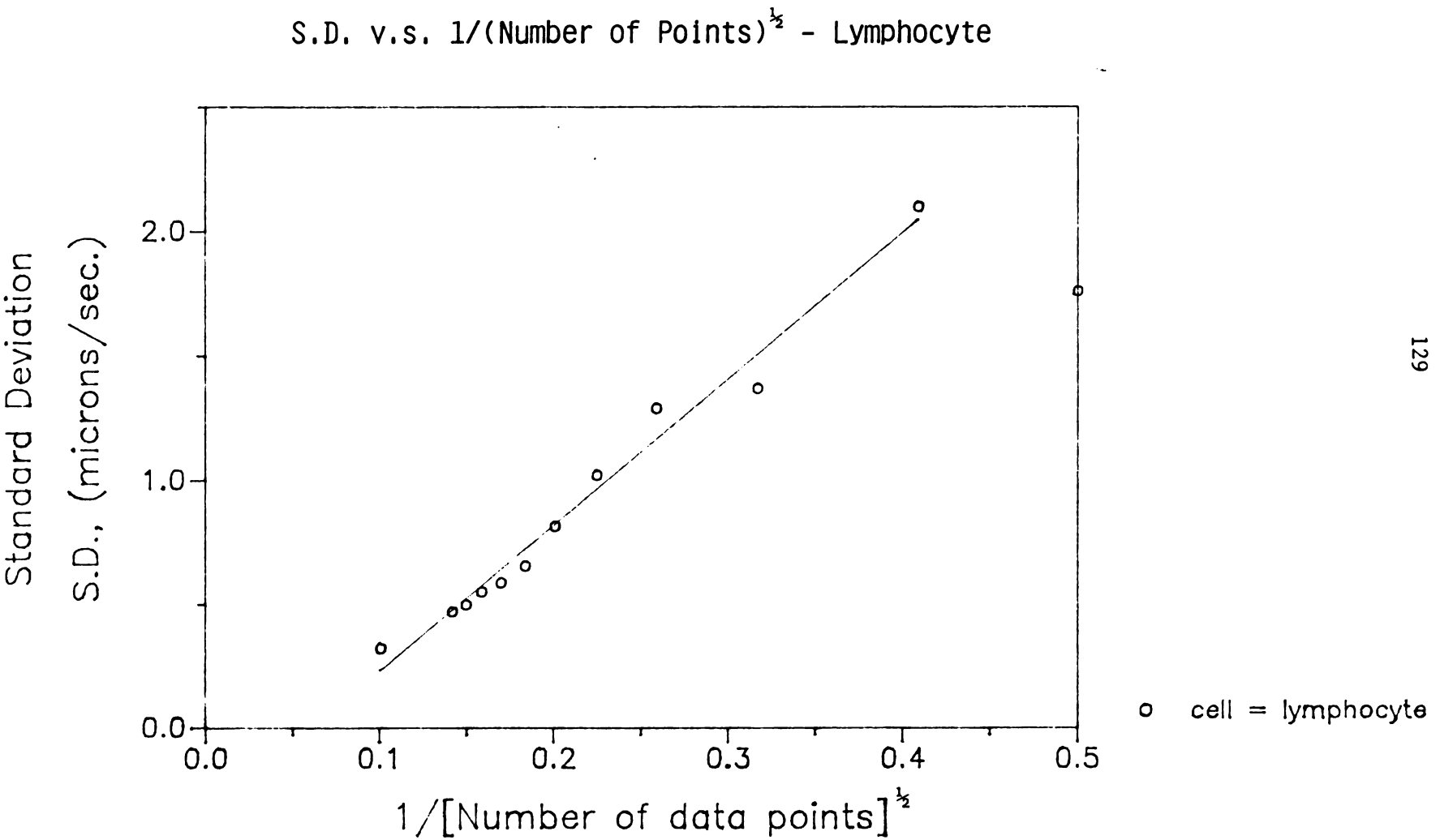

S.D. v.s. 1/(Number of Points)^½ - Lymphocyte
Standard Deviation
S.D., (microns/sec.)
2.0
1.0
0.0
0.0
0.1
0.2
0.3
0.4
0.5
1/[Number of data points]^½
o cell = lymphocyte

Figure 6.3.8

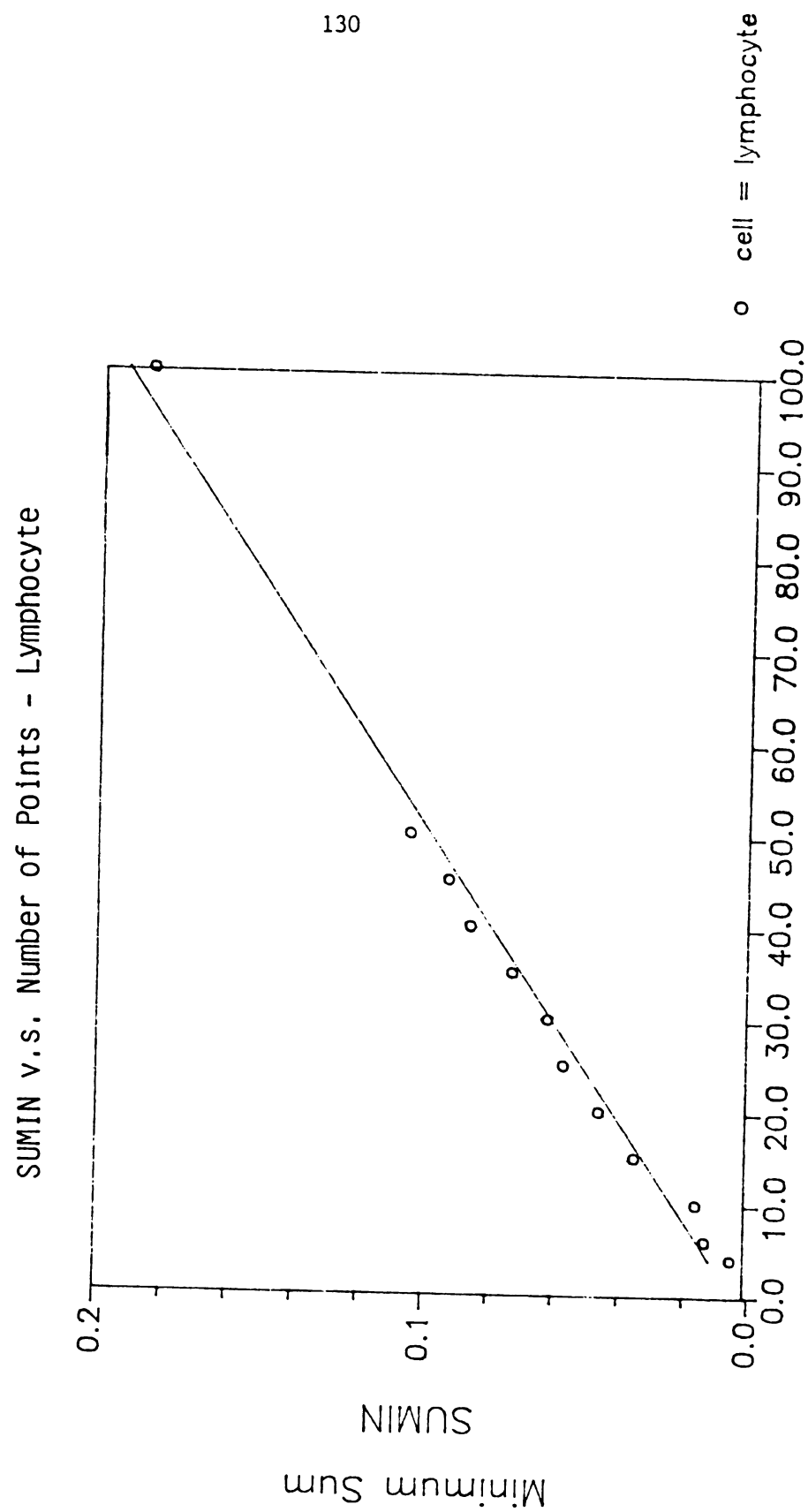

SUMIN v.s. Number of Points - Lymphocyte
o cell = lymphocyte
Minimum Sum
SUMIN
0.2
0.1
0.0
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0
Number of data points

Figure 6.3.9

6.3.9 The Effect of Varying Ea of the Dialysis Membrane on the Ea of the Cell

TABLE 6.3.9 - The Effect of Varying Ea of the Dialysis Membrane on the Ea of the Cell

	Ea, Dialysis Membrane (Kcal/mole)	Ea, Liposome Membrane (Kcal/mole)
	0.5	20.4
	1.0	20.1
	2.0	19.2
	4.0	17.5
*	6.53	15.1
	8.0	13.6
	10.0	11.7
	12.5	9.0
	15.0	6.2

* = original base case

The results for this study suggest the two membrane activation energies were inversely proportional and slightly non-linear in relation to one another (see Figure 6.3.10). Similarly, as mention in Section 6.2.9, if the manufacturer misstated the activation energy for the dialysis membrane then the resulting cell membrane activation energies would be effected.

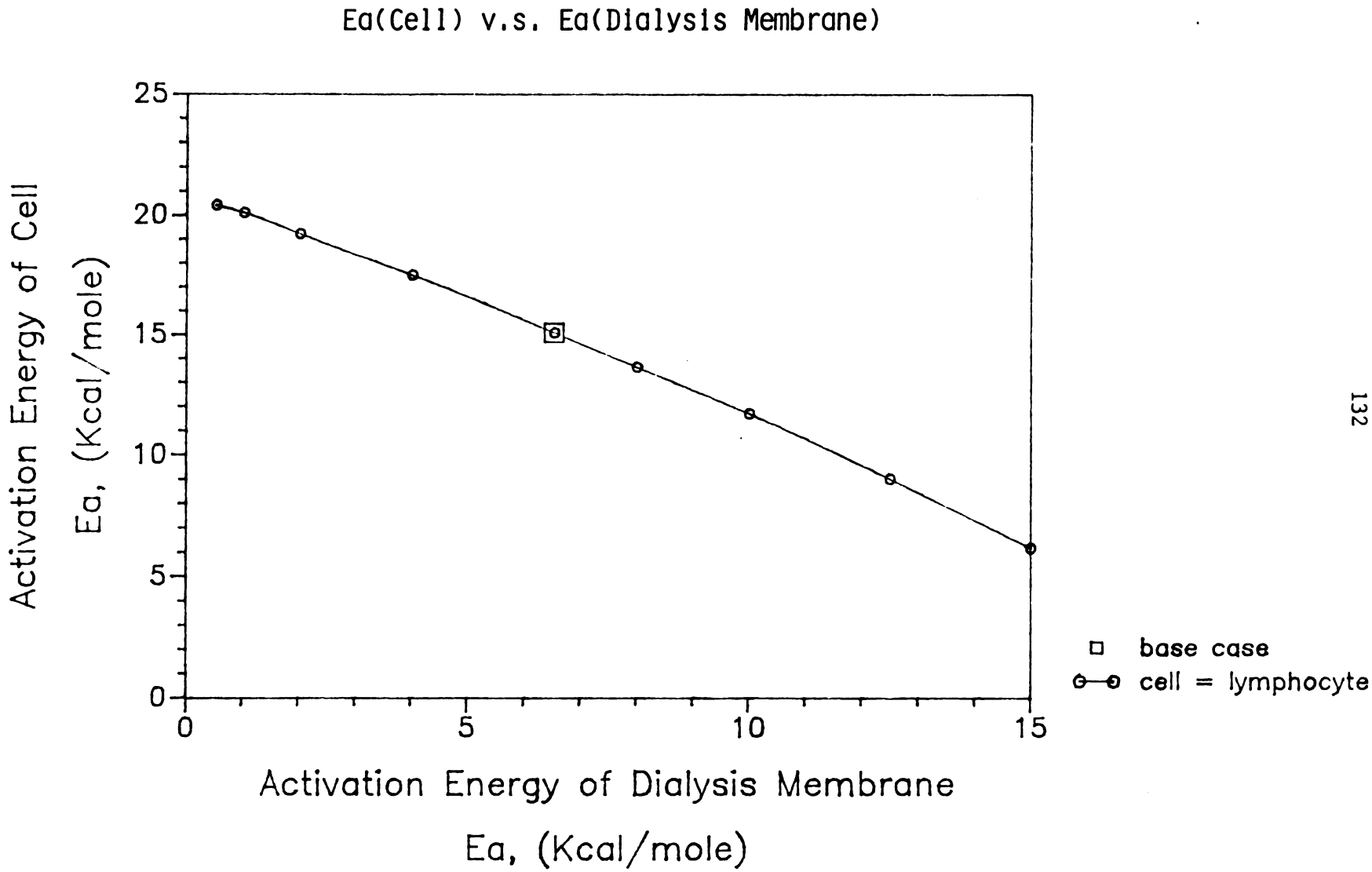

Ea(Cell) v.s. Ea(Dialysis Membrane)
Activation Energy of Cell
Ea, (Kcal/mole)
25
20
15
10
5
0
0
5
10
15
Activation Energy of Dialysis Membrane
Ea, (Kcal/mole)
base case
cell = lymphocyte

Figure 6.3.10

6.4 Discussion of Sensitivity Studies - Ranking Input Parameters

For the most part the sensitivity studies in Sections 6.2 and 6.3 produced similar results, when comparing the respective (general) shapes of the curves, (e.g. Figure 6.2.1 and 6.3.1, etc.). Each input parameter that was questioned effected the estimated membrane permeability to some degree. This section will rank the importance of correctly estimating input parameters, from most to least in terms of its "potential" to effect estimated membrane permeability (for the two cases studied).

Clearly the input parameters which could effect the estimated permeability the most are Hd and D_1 (see Figures 6.2.1, 6.2.2, 6.3.1 and 6.3.2). This can be explained by studying the resistance of the solute flow through the boundary layer, dialysis membrane and sample region, which can be written as 1/Hd, RL1/D_1 and RL2/D_2, respectively. (Recall that Hd was the convective mass transfer coefficient, RL1 was the thickness of the wetted dialysis membrane, D_1 was the solute diffusivity in the dialysis membrane, RL2 was the thickness of the sample region and D_2 was the solute diffusivity in free solution.) Calculating these resistances, 1/Hd=0.625, RL1/D_1=1.0 and RL2/D_2=0.625 (for the base case). Therefore, whenever Hd or D_1 was decreased its corresponding resistance became the dominant resistance to the solute flow and when ever Hd or D_1 was increased the resistance to solute flow in that area diminished.

The input parameter which probably had the next most influence on P(est) was RL2, the sample chamber thickness (see Figures 6.2.3 and 6.3.3). This can also be explained by studying the resistance to solute flow. As mentioned

above, when the resistance to solute flow was changed, P(est) would then be effected. More specifically, when RL2 was increased the resistance to solute flow was increased and when RL2 was decreased the resistance to solute flow was decreased, in the sample region.

Following RL2 would be VINA, the normalized osmotically inactive cell volume (see Figures 6.2.5 and 6.3.4). This parameter was determined by performing osmotic equilibrium experiments. It was observed that each cell that was tested had its own osmotically inactive volume, which wasn't too different for the average value used. The osmotically inactive cell volume that was used for the liposome and lymphocyte experiments was average based on a sample of the population.

The parameter with the next most influence, which wasn't a single input parameter, was the number of data points used to estimate the membrane permeability.

The last two input parameters that were studied were the time delay, DELAY, and the cell position in the diffusion chamber, LIP. Varying these parameters did not have much of an effect on P(est) (for these cases). However, the time delay could have a substantial effect on P(est) if the time duration of an experimental run is very short (less than about 15 seconds). LIP would only become important if the sample region thickness was large where a substantial concentration gradient could develop.

CHAPTER 7

Conclusions

Based upon the results of this work the following conclusions can be made:

1) The two preparation techniques used to prepare egg-lecithin liposomes and human lymphocytes can produce good yields, thus providing a good population from which a cell specimen can be chosen.

2) The microscope diffusion chamber system can effectively be used to produce reliable data for calculating the equilibrium osmotic response of liposomes and human lymphocytes because it is relatively simple to task a cell in some isotonic solution and expose it to a series of step-wise increases in concentration. (Note: the cell is allowed to come to an equilibrium volume after each step increase in concentration.) The cell system test in this work was human lymphocytes which yielded a normalized osmotically inactive cell volume of 34.7%. This normalized osmotically inactive volume was in good agreement with inactive volumes reported by other scientists. Therefore, the author believes the microscope diffusion chamber could be applied to other cell systems to determine their respective normalized osmotically inactive cell volume.

3) The microscope diffusion chamber system, in conjunction with the computer algorithm SENS, can be used to determine the dynamic non-

equilibrium osmotic response of egg-lecithin liposomes and human lymphocytes, (i.e. the cell membrane water permeability), at a specified temperature. The cell membrane water permeabilities determined for this work compared well with permeabilities published by other scientists. Specifically, the mean permeability calculated at 25°C for egg-lecithin liposomes was 40.2 μm/sec and for human lymphocytes was 9.3 μm/sec. Based on these results the author believes that the diffusion chamber, along with SENS, can be used to determine the membrane water permeability for other cell systems with similar membrane characteristics and could probably be applied to cell systems with even higher membrane permeabilities. However, there may be an upper limit, with respect to the permeability, at which the diffusion chamber may not produce reliable results.

4) Because the microscope diffusion chamber can be used at different temperatures, the data produced by the microscope diffusion chamber system can be used to determine the effect of temperature with respect to the membrane permeability, i.e. the activation energy. Based on the experiments conducted, for this work, it was discovered the cell membrane permeability for both liposomes and lymphocytes have a strong dependence on temperature. This temperature dependence was quantified into an activation energy. The activation energies calculated, for liposomes and lymphocytes, were in good agreement with activation energies published by other scientists, (8.9 Kcal/mole for egg-lecithin liposomes and 15.1 Kcal/mole for human lymphocytes). Based on these results the

author believes that the activation energy for other cell systems can successfully be determined.

5) Egg-lecithin liposomes represent a good model system for equilibrium and non-equilibrium osmotic studies because of the similar behavior observed as compared to using a living cell system, i.e. the osmotic shrinkage of liposomes is consistent with the irreversible thermodynamic model developed by Kedem and Katchalsky. As mentioned, the liposomes used for this work were egg-lecithin in composition. Liposomes of other compositions could be used in the diffusion chamber to determine the effect of the membrane composition with respect to the cell membrane permeability.
6) Human lymphocytes also behaved in a manner consistent with the irreversible thermodynamic model and represent a good "hearty" cell system to study, i.e. lymphocytes hold up well under adverse conditions like increases in concentration.
7) The original version of SENS developed by J. Tu was an excellent starting point for developing a user friendly program. The program can easily be used by a user who has a general working knowledge of the microscope diffusion chamber system.
8) The computer program SENS is a useful tool for setting up experimental conditions. For example, if an investigator wishes to know the approximate time duration of an experimental run, the preliminary experimental conditions can be entered and the normalized cell volume can be observed, (which will come to some equilibrium volume). At the point when the cell has reached 99% of

its new volume is the approximate time duration of an experimental run.

9) The computer program SENS can effectively be used to study the effect of under or over estimating an input parameter with respect to the resulting estimated membrane permeability. Thus a particularly "sensitive" parameter can be identified and procedures can be implemented to carefully estimate this parameter.

CHAPTER 8

Suggestions for Future Work

The following suggestions are made for future work regarding the design of the experimental system and the cell systems tested with the microscope diffusion chamber.

1) Redesign the pumping system used with the diffusion chamber system to allow for more solution bottles to be accessible at one time. The present design of the system only allows for only two solution bottles to be used at a time. If a third (or fourth, or fifth, etc.) solution were to be introduced, it would have to be changed manually. By having five or more solution bottles readily accessible the equilibrium osmotic response could more easily be studied, i.e. the normalized osmotically inactive volume could more easily be obtained.

2) Interface the switch which turns on the pumping system could also be interfaced with the timer so that only one switch is necessary to start an experiment. The present design of the microscope diffusion chamber system has two separate switches, one to start the hypertonic solution and one to start the timer, which take two hands to start. In the mean time the focusing knob on the microscope needs to have continual minor adjustments made. Having a single switch would make this process easier.

3) Incorporate electronically controlled valves to allow the solution lines leading from the solution bottle to the electronic solenoid valve to be preflushed. This would allow for more easily controlling the temperature of the in coming solution and any air bubbles in the solution lines to escape.

4) Make the bulk flow region thinner to allow for greater ease in focusing the microscope condenser, which would result in a sharper image project (and therefore video taped). The present design of the diffusion chamber is such that the microscope condenser has to be smashed into the plastic on the bottom of the diffusion chamber.

5) Modify to the diffusion chamber to allow more systematic control of the sample region thickness.

6) Develop a better method of recording the cell radius (or volume) history. This could be done by using an image analysis equipment which can more accurately calculate the dynamic volume change of the cell as it is exposed to the hypertonic solution.

7) Devise a method to verify the number of bilipid layers when liposomes are used. Presently the method used to determine the number of bilipid layers is based on the contrast of the liposome projected on the screen.

8) Incorporate into the modelling, of the membrane water permeability, the effect of internal and external solute concentration. The concentration dependence was not mentioned, or studied, in this work but has been shown by other investigators to have an effect.

9) Also incorporate into the modelling the estimation of the individual cell osmotically inactive volume, in addition to the

cell membrane water permeability. This would result in a more accurate estimate of the membrane permeability.

10) Do experiments to characterize the dialysis membrane permeability, thus verifying the results of the manufacturer. As was shown in the sensitivity studies the accurately knowing the solute diffusivity in the dialysis membrane, which is related to the membrane permeability by $Pmem*\Delta x=D_1$, is extremely important.

11) Devise a method to study the convective mass transfer coefficient and accurately determine its value. Also recall from the sensitivity studies that accurately knowing the convective mass transfer coefficient could be extremely important.

12) Apply the microscope diffusion chamber system to other cell systems, particularly cells with higher membrane water permeabilities, to determine if there are any problems in accurately estimating the membrane permeability. The microscope diffusion chamber system may produce misleading results if the cell has a high membrane water permeability. To further explain, if the dialysis membrane doesn't allow the passage of solute into the sample region fast enough, (in other words the dialysis membrane is too rate limiting with respect to solute transport), the resulting volume history may lead to an inaccurate membrane permeability.

APPENDICES

APPENDIX A

The Normalized Osmotically Inactive Volume

Van't Hoff was the first to study and develop the laws to osmotic equilibrium. His work was further expanded and applied to living cells by Boyle and Van't Hoff. The result has come to be known as the Boyle-Van't Hoff law, which has a form analogous to the perfect gas law (PV=nRT):

$$\pi V_w = \text{constant} \qquad \text{(A-1)}$$

where V_w represents the volume of solvent (water) and $\pi=RTC_s$ represents the osmotic pressure. This law states that if the intracellular solution can be considered ideal and if all of the solvent can be considered "free" or osmotically active then the osmotic pressure is inversely proportional to the osmotic pressure, or extracellular concentration. However, for many cell systems not all of the solvent can be considered free. In 1932 Lucké and McCutheon developed a relationship to compensate for the non-free solvent and anything else within the cell that can be considered non-solvent. This non-free solvent non-solvent volume is termed the "osmotically inactive cell volume".

The modified Boyle-Van't Hoff law, corrected for the osmotically inactive volume, may then be stated as

$$\pi(V_{cell} - V_b) = \pi^o(V^o_{cell} - V_b) \qquad \text{(A-3)}$$

where π is the osmotic pressure, (the extracellular and intracellular osmotic pressures are equal at equilibrium), V_{cell} is the total volume of the cell and V_b is the osmotically inactive volume. The superscript

values correspond to a reference initial states.[McGrath, Heat Trans...] Dividing (A-3) by the initial cell volume, V^o_{cell}, and rearranging, the modified Boyle-Van't Hoff law becomes

$$\hat{V}_{cell} = \frac{\pi^o}{\pi}(1 - \hat{V}_b) + \hat{V}_b \qquad (A\text{-}4)$$

where $\hat{V}_{cell} = V_{cell}/V^o_{cell}$ (the normalized cell volume) and $\hat{V}_b = V_b/V^o_{cell}$ (the normalized osmotically inactive cell volume). Therefore by plotting $\hat{V}_{cell}$ v.s. $1/\pi$ the normalized osmotically inactive volume can be found from the intercept when $1/\pi$ equals zero or from the slope $\pi^o(1 - \hat{V}_b)$ since both π^o is known.

Appendix B

Preliminary Testing and Set Up of Experimental Equipment

The magnification calibrations were necessary because a cell, which appeared on the video monitor, was not only magnified by the lenses in the microscope but was also magnified by the video camera and the video monitor. These calibrations were performed using a Petroff-Haussen Bacteria Counter. The counter had etches of a specific distance apart marked on the surface of the glass. After focusing the microscope, the length between the two etch marks projected on the video monitor was measured. The ratio of the measured projected image and the known specific distance on the bacteria counter resulted in a magnification factor, RMAG. The results of these calibrations are summarized in Table B.1. It should be noted here that the that SENS allowed the user to enter the radius history measured from the video monitor, providing RMAG was also entered.

In addition, before conducting any experiments the temperature distribution of the diffusion chamber was investigated. These tests were conducted to determine what temperature the controller (i.e. refrigerated circulating bath) needed to be set at in order to obtain the desired temperature at the cell chamber. The investigated temperature settings of the circulating bath were 0.0°C, 8.9°C, 21.1°C, 30.6°C, 35.2°C 50.8°C and 60.7°C. The resulting temperatures detected at the top fitting are summarized in Table B.2 and Figure B.1.

TABLE B.1 - Magnification Calibration

Camera	Objective Power	Optavar Power	μm/mark	Magnification, RMAG
Color	25	1.25	3.846	3175
Color	25	1.6	3.125	4064
Color	25	2.0	2.500	5080
Color	40	1.25	2.632	4683
Color	40	1.6	2.083	5994
Color	40	2.0	1.695	7492
Black/White	25	1.25	3.846	4750
Black/White	25	1.6	3.125	4064
Black/White	25	2.0	2.500	7600
Black/White	40	1.25	2.632	7005
Black/White	40	1.6	2.083	8968
Black/White	40	2.0	1.695	11209

TABLE B.2 - Temperature Bath Measurements

Room Temperature = 23°C
(Note: All temperature are recorded in °C)

Circulating Bath Temperature	Temperature of Top Fitting	Difference
0.0	4.6	4.6
8.9	12.6	3.7
21.1	21.9	0.8
23.0	23.0	0.0
30.6	29.8	-0.8
35.2	33.8	-1.4
50.8	48.0	-2.8
60.7	56.9	-3.8

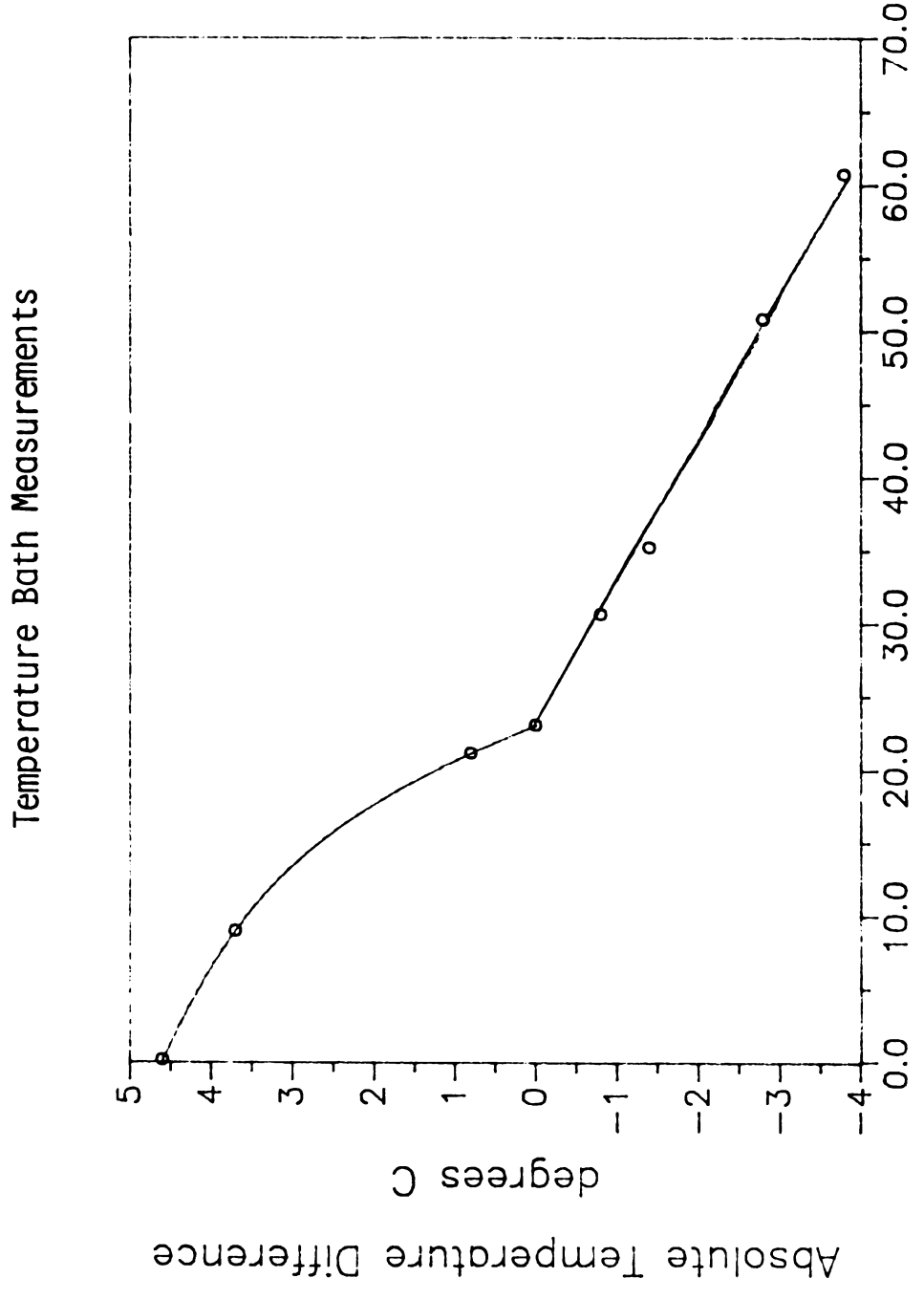

Temperature Bath Measurements
Absolute Temperature Difference
degrees C
5
4
3
2
1
0
-1
-2
-3
-4
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Circulating Bath Temperature
degrees C

Figure B.1

APPENDIX C

As mentioned in section 1.2 SENS was used to clarify previous results generated by M. Shabana using the microscope diffusion chamber. The cell system he used was unfertilized hamster ova. After Shabana gathered the experimental data he used a method devised be Terwilliger and Solomon [19] to calculate the membrane water permeability, P_{MS}[22]. To help clarify this results his data was reentered into SENS to generate a new permeability, P_{SENS}. The results are summarized in Table C.1.

TABLE C.1 - Summary of Shabana's Results

cell type: unfertilized hamster ova
initial concentration: 0.3 osmol (NaCl)
temperature: 25°C

Exp. #	Final Concentration (osmol)	P_{MS} (μm/sec)	P_{SENS} (μm/sec)
1	0.5	18.38	28.1 ± 1.6
2	0.5	21.26	51.3 ± 5.6
5	0.5	22.40	33.0 ± 3.4
6	0.5	20.75	42.8 ± 3.6
7	0.5	21.86	61.0 ± 13.0
3	0.8	20.35	28.1 ± 3.0
4	0.8	17.21	24.8 ± 1.7
8	0.8	16.96	15.2 ± 1.5
9	0.8	14.72	33.1 ± 2.0
10	0.8	18.64	36.4 ± 3.6
11	0.8	18.26	31.9 ± 1.8
12	1.5	17.68	35.3 ± 4.0
13	1.5	15.89	37.2 ± 5.6
14	1.5	15.36	22.3 ± 1.6
15	1.5	15.21	33.2 ± 4.5
16	1.76	17.05	29.3 ± 2.9
17	1.76	14.62	29.5 ± 2.4
18	1.76	?	25.1 ± 3.7
19	1.76	16.14	28.2 ± 3.1

APPENDIX D

SENS - The Prime Version Fortran Source Code

```
      PROGRAM SENS
C-----
C     THIS PROGRAM INCLUDES THE COMPUTER MODEL FOR THE
C     DIFFUSION CHAMBER AND THE PARAMETER ESTIMATION FOR FINDING
C     PERMEABILITY OF A CELL INSIDE THE CELL CHAMBER OF THE
C     DIFFUSION CHAMBER.
C
C     THIS PROGRAM CONSISTS OF 1 MAIN PROGRAM AND 8 SUBROUTINES
C     AND 4 FUNCTIONS.  THEY ALL ARE INSIDE THE FILES 'SENS',
C     'PAR.COUT', 'PLOT'.  THE INPUT FILENAME IS SPECIFIED BY THE USER
C     AND THE NUMERICAL OUTPUT FILENAMES ARE 'O_SENS.DAT' AND 'O_SMRY.DAT',
C     AND THE GRAPHICAL OUTPUT FILENAME IS 'G_PLOT'
C
C     THE INPUT FORM REQUIRED IS AS FOLLOW
C
C     RL1: THICKNESS OF DIALYSIS MEMBRANE (M)
C
C     RL2: THICKNESS OF CELL CHAMBER (M)
C
C     LIP: APPROXIMATE LOCATION OF THE LIPOSOME (FROM 5 TO 11)
C
C     D1: DIFFUSIVITY OF SOLUTE INSIDE DIALYSIS MEMBRANE (M*M/SEC)
C
C     D2: DIFFUSIVITY OF SOLUTE INSIDE CELL CHAMBER (M*M/SEC)
C
C     CINIT: INITIAL CONCENTRATION (OSM)
C
C     CINF: FINAL CONCENTRATION (OSM)
C
C     H: MASS TRANSFER COEFF. (APPROXIMATELY 10000*D2)
C
C     IPRINT: NUMERICAL DATA OUTPUT FREQUENCY. (EVERY IPRINT*DT
C            SEC. PRINTS THE CONC. DIST. ON OUTPUT FILE)
C
C     IF1: CONTROL THE OUTPUT OF CONC. CHANGE INSIDE THE CELL
C          CHAMBER
C
C     IF2: CONTROL THE OUTPUT OF SENSITIVITY COEFF. VERSUS TIME
C
C     IF3: CONTROL THE OUTPUT OF NORMALIZED VOLUME RESPONSE
C          VERSUS TIME
C
C     IF4: CONTROL THE OUTPUT OF SUM OF ERROR OF SQUARE VERSUS
C          PERMEABILITY
C
C          (THE VALUES FOR ABOVE INTEGER OPTIONS ARE 1-YES, 0-NO)
C
C     IRELPSE: OPTION FOR EXPERIMENTAL DATA INPUT 1=REAL-EXPERIMENT
C                                                 2=PSUESO-EXPERIMENT
C
C     IMICCEN: OPTION FOR ENTERING DATA IN 1=MICRONS  2=CENTIMETERS
C
```

```
C     TM0, TM1: SETTING THE TIME RANGE ON THE PLOTS (SEC.)
C
C     DT: TIME STEP FOR PROCEEDING THE CALCULATION (SEC.)
C         (NOTE: IF DT IS SET TOO LARGE, THE RESULT WILL FLUCTUATE.
C          IN THIS CASE, REDUCE THE SIZE OF DT AND TRY AGAIN.
C          THIS IS DUE TO THE UNSTABLE OF THE NUMERICAL METHOD.)
C
C     DELAY: TIME DELAY SUBTRACTED FROM TIME ARRAY TM(I)
C
C     CAB0, CAB1: WINDOW OF THE Y-DIRECTION ON FIRST GRAPH.
C                 (UNLESS NECESSARILY, SET THE VALUES AS 0. AND 5.)
C
C     PSEN: THE PERMEABILITY VALUE AT WHICH WE INVESTIGATE THE
C           SENSITIVITY COEFF. (P'S EFFECT ON R'S CHANGE)
C
C     SEN0, SEN1: SETTING THE RANGE FOR THE SENSITIVITY COEFF. PLOT
C
C     PTRU: THE PERMEABILITY VALUE WITH WHICH THE PROGRAM GENERATES
C           PSUDO-EXPERIMENTAL DATA (R(TM,PTRU)).
C
C     DT1: THE TIME STEP FOR THE PSUDO-EXPT'L DATA DURING TM0 TO
C          TM01. (SEC.)
C
C     TM01: THE PARTITION BETWEEN TWO DIFFERENT TIME STEPS RANGE.
C           (YOU CAN ASK THE PROGRAM TO GENERATES PSUDO-EXPT'L DATA
C            WITH TWO DIFFERENT INCREMENT IN TIME FOR TWO TIME
C            RANGE.)
C
C     DT2: THE TIME STEP FOR THE PSUDO-EXPT'L DATA DURING TM01
C          TO TM1. (SEC.)
C
C
C     RINIT: INITIAL RADIUS (CM)
C
C     VINA: INACTIVE VOLUME (%)
C
C     RMAG: THE MAGNIFICATION OF THE MICROSCOPE
C
C     DR: MAGNITUDE FOR THE PSEUDO-RANDOMNESS IMPOSED ON THE
C         PREDICTED RADIUS RESPONSE (CM)
C
C     P0,P1: PERMEABILITY RANGE FOR THE FOURTH PLOT (MICRON/SEC.)
C
C     DP: INCREMENT OF PERMEABILITY IN CALCULATING SUM OF ERROR OF
C         SQUARE FOR EACH P VALUE
C
C     SUM0, SUM1: RANGE FOR THE FOURTH PLOT
C
C
C     OUTPUT CONFIGURATION OF 'O_SENS.DAT':
C
C          (1) INPUT DATA
```

```
C          (2) PRINT CONC. DIST. OF THE SYSTEM AS A FUNCTION OF
C              TIME.
C          (3) SENSITIVITY COEFF. CORESPOND TO PSEN.
C          (4) THE ESTIMATED PERMEABILITY (LOCAL MINIMUM ON SUM
C              VERSUS P GRAPH)
C          (5) THE STANDARD DEVIATION OF THIS ESTIMATED P
C
C     OUTPUT CONFIGURATION OF 'O_SUMR.DAT':
C
C          (1) SUMMARY OF INPUT PARAMETERS AND DATA
C          (2) SUMMARY OF RESULTING PERMEABILITY, STANDARD DEVIATION
C              AND MINIMUM SUM
C
C      OUTPUT CONFIGURATION OF THE GRAPHICS FILE G_PLOT CAN BE
C      ONE OR MORE OF THE FOLLOWING:
C
C          (1) CONCENTRATION V.S. DIMENSIONLESS TIME
C          (2) SENSITIVITY COEFFICIENTS V.S. TIME
C          (3) NORMALIZED VOLUME V.S. TIME
C          (4) SUM OF SQUARE OF ERRORS V.S. PERMEABILITY
C
C-----
C     INITIALIZATION AND DECLARATIONS
C-----
      PARAMETER (N3=301,II=2,N1=301,N4=301,EP1=0.01)
      DIMENSION TMA(N3),TMB(N3),RA(N3),RB(N3),SEN(N3),SUM(N1),P(N1)
      DIMENSION VOLC(N3),VOLA(N3),TMS(N3),RS(N3),RC(N3),RSAVE(N3)
      CHARACTER*1 ICHANG, IGRAPH, IMORE, IAGAIN, IANOTH, ISAVE
      CHARACTER*1 IMISTAK, IFIRST, IQUIT, IDEL
      CHARACTER*11 XMICCEN,PROBLEM
      CHARACTER*10 NAMFIL,ISENS
      COMMON /C1/IPRINT,IF1,A1(II),A2(II),CAB0,CAB1
      COMMON /C2/RINIT,VINA,DT,TM1,COUT(N4)
      COMMON /C3/RL1,RL2,LIP,D1,D2,CINIT,CINF,H
      EXTERNAL FCT0,F
C-----
C     EXPLANATION TO THE USER WHAT THE PROGRAM DOES.
C-----
      IBACK = 0
      IRUNAG=0
      IOPNAG=0
      IMISTAK = 'N'
2000  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'WOULD YOU LIKE AN EXPLAINATION OF THIS PROGRAM, '
      WRITE(1,*)'(SENS), (Y/N)?'
      READ(1,'(A1)') IFIRST
      CALL IYESNO(IFIRST)
      IF(IFIRST.EQ.'N') GO TO 2002
      WRITE(1,*)
      WRITE(1,*)'   WELCOME TO THE PROGRAM SENS.  THIS PROGRAM WILL '
      WRITE(1,*)'ALLOW THE USER TO (1) ANALYZE THE DATA OBTAINED USING '
```

```
      WRITE(1,*)'THE MICROSCOPE DIFFUSION CHAMBER I.E. PARAMETER '
      WRITE(1,*)'ESTIMATION OF THE PERMEABILITY OF A CELL  OR  (2) RUN '
      WRITE(1,*)'A SIMULATION (PSEUDO) EXPERIMENT TO SEE WHAT MIGHT '
      WRITE(1,*)'TO A CELL UNDER SPECIFIED CONDITIONS.'
C-----
C     OPTION TO HAVE A LIST OF THE NECESSARY PARAMETERS SENT TO
C     'I_DATA.LST'
C-----
      WRITE(1,*)'   IF THIS IS THE FIRST TIME YOU HAVE USED THIS '
      WRITE(1,*)'PROGRAM AND YOU WANT TO ENTER DATA FROM A REAL '
      WRITE(1,*)'EXPERIMENT YOU MAY WANT TO OBTAIN A LIST OF THE '
      WRITE(1,*)'PARAMETERS AND DATA NECESSARY TO RUN THE PROGRAM.'
      WRITE(1,*)
      WRITE(1,*)'WOULD YOU LIKE TO DO THIS, (Y/N)?'
      READ(1,'(A1)') IFIRST
      CALL IYESNO(IFIRST)
      IF(IFIRST.EQ.'Y') THEN
          OPEN(13,FILE='I_DATA.LST')
          WRITE(1,*)
          WRITE(1,*)'THE LIST OF THE NECESSARY INPUT TO RUN THE PROGRAM'
          WRITE(1,*)'WILL BE IN FILE I_DATA.LST.  THE PROGRAM WILL'
          WRITE(1,*)'STOP NOW.  HAVE I_DATA.LST PRINT AT THE PRINTER.'
          WRITE(13,*)
          WRITE(13,*)'THE PARAMETES AND DATA NEEDED TO RUN THE PROGRAM'
          WRITE(13,*)'ARE:'
          WRITE(13,*)
          WRITE(13,*)'1) DIALYSIS MEMBRANE THICKNESS (RL1), MICRONS.'
          WRITE(13,*)'2) CELL CHAMBER THICKNESS (RL2), MICRONS.'
          WRITE(13,*)'3) DIFUSSIVITY OF SOLUTE IN MEMBRANE (D1), '
          WRITE(13,*)'                              - METERS*METERS/SEC.'
          WRITE(13,*)'4) DIFUSSIVITY OF SOLUTE IN FREE SOLUTION (D2),'
          WRITE(13,*)'                              - METERS*METERS/SEC.'
          WRITE(13,*)'5) MASS TRANSFER COEFFICIENT (H).'
          WRITE(13,*)'6) INITIAL CONCENTRATION (CINIT), OSMOLALITY.'
          WRITE(13,*)'7) FINAL CONCENTRATION (CINF), OSMOLALITY.'
          WRITE(13,*)'8) INACTIVE VOLUME (VINA), %'
          WRITE(13,*)'9) MAGNIFICATION FACTOR (RMAG).'
          WRITE(13,*)'10) TIME STEP (DT), SEC.'
          WRITE(13,*)'11) STARTING TIME (TM0), SEC.'
          WRITE(13,*)'12) ENDING TIME (TM1), SEC.'
          WRITE(13,*)'13) TIME DELAY (DELAY), SEC.'
          WRITE(13,*)'14) PERMEABILITY STEP (DP), MICRONS/SEC.'
          WRITE(13,*)'15) PERMEABILITY AT WHICH INVESTIGATE THE '
          WRITE(13,*)'    SENSITIVITY COEFFICIENTS (PSEN), MICRONS/SEC.'
          WRITE(13,*)'16) THE DATA POINTS: TIME (TMA(I)), SEC. AND '
          WRITE(13,*)'                     RADIUS (RA(I)), MICRONS OR'
          WRITE(13,*)'                                     CENTIMETERS.'
          WRITE(13,*)
          WRITE(13,*)'    (NOTE: YOU ONLY NEED THE DATA POINTS IF YOU'
          WRITE(13,*)'     ARE USING THE PARAMETER ESTIMATION OPTION,'
          WRITE(13,*)'     I.E. A REAL EXPERIMENT.)'
          CLOSE(13)
```

C-----
C
C-----

C-----
C
C-----

```
         GO TO 600
      ENDIF
C-----
C     EXPLAINING THE OPTION TO ENTER DATA USING KEYBOARD OR AN INPUT FILE
C-----
      WRITE(1,*)
      WRITE(1,*)'   THIS PROGRAM WILL ALLOW YOU TO ENTER THE DATA '
      WRITE(1,*)'USING THE TERMINAL/KEYBOARD OR A PRE-EXISTING INPUT'
      WRITE(1,*)'FILE SET UP BY THE USER.  AN EXAMPLE OF AN INPUT FILE'
      WRITE(1,*)'CAN BE SEEN BY QUITTING THIS PROGRAM AND PRINTING'
      WRITE(1,*)'I_SENS.EXP AT THE PRINTER.  DO YOU WISH TO QUIT AND '
      WRITE(1,*)'PRINT THE EXAMPLE (Y/N)?'
      READ(1,'(A1)') IQUIT
      CALL IYESNO(IQUIT)
C-----
C     IF IQUIT IS YES THE PROGRAM WILL GENERATE I_SENS.EXP AND QUIT.
C-----
      IF (IQUIT.EQ.'Y') THEN
         OPEN(12,FILE='I_SENS.EXP')
         WRITE(12,*)'THIS IS THE EXAMPLE INPUT FILE I_SENS.EXP FOR THE'
         WRITE(12,*)'PROGRAM SENS.FOR.  THE PROGRAM WILL READ THE DATA'
         WRITE(12,*)'ALINING THE VALUES UNDER THE LEFT MOST CHARACTER.'
         RL1 = 16.
         RL2 = 100.
         LIP = 9
         D1 = 5.21E-11
         D2 = 5.21E-10
         H = 5.21E-6
         CINIT = 0.02
         CINF = 0.04
         VINA = 6.
         RMAG =5080.
         DT = 5.0
         TM0 = 0.0
         TM1 = 500.0
         DELAY = 0.0
         DP = 2.
         P0 = 0.
         P1 = 100.
         PSEN = 40.
         RINIT = 7.7
         PTRU = 40.0
         DR = 0.01
         TM01 = 500.0
         DT1 = 5.
         DT2 = 5.0
         IRELPSE = 1
         IMICCEN = 2
         IF1 = 1
         IF2 = 1
         IF3 = 1
         IF4 = 1
```

```
         IPRINT = 10.
         CAB0 = 0.0
         CAB1 = 5.0
         SEN0 = -0.1
         SEN1 = 0.0
         VOL0 = 0.0
         VOL1 = 1.0
         SUM0 = 0.0
         SUM1 = 100.0
         P0 = 0.0
         P1 = 100.0
         ICOUNT =40
         RA(1) = 7.7
         TMA(1) = 0.0
         DO 1100 I = 2,40
             TMA(I) = TMA(I-1) + 10.
             RA(I) = RA(I-1) -0.05
1100     CONTINUE
         GO TO 331
      ENDIF
C-----
C     EXPLAINING THE INPUT AND OUTPUT OPTIONS
C-----
      WRITE(1,*)
      WRITE(1,*)'   THE PROGRAM WILL ALSO ALLOW THE USER TO VIEW THE'
      WRITE(1,*)'RESULTS BY 1) TABLES AND/OR 2) GRAPHICALLY.  THE'
      WRITE(1,*)'TABLES GENERATED CAN BE FOUND IN A FILE CALLED'
      WRITE(1,*)'"O_SENS.DAT".  A SUMMARY OF THE INPUT PARAMETERS AND'
      WRITE(1,*)'DATA CAN BE FOUND IN "O_SMRY.DAT".  THE GRAPHICAL '
      WRITE(1,*)'OUTPUT WILL BE DISPLAYED ON THE SCREEN AND STORED'
      WRITE(1,*)'IN A GRAPHICS FILE G_PLOT.'
      WRITE(1,*)'THE USER MUST THEN USE "PRINTX" TO GENERATE A'
      WRITE(1,*)'PRINTED COPY, (NOTE: THE FIRST GRAPHICS FILE WILL'
      WRITE(1,*)'START ON PAGE 2 !!).  THE PLOTS THAT CAN BE GENERATED'
      WRITE(1,*)'ARE:'
      WRITE(1,*)
      WRITE(1,*)'  1) CONCENTRATION V.S. DIMENSIONLESS TIME'
      WRITE(1,*)'  2) SENSITIVITY COEFFICIENTS V.S. TIME'
      WRITE(1,*)'  3) NORMALIZED VOLUME V.S. TIME'
      WRITE(1,*)'  4) SUM OF SQUARE OF ERRORS V.S. PERMEABILITY'
      WRITE(1,*)
      WRITE(1,*)'YOU ARE NOW READY TO START THE PROGRAM.'
2002  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'DO YOU WISH TO ENTER THE DATA USING (1) THE TERMINAL'
      WRITE(1,*)'OR (2) A PRE-EXISTING INPUT FILE, (ENTER 1 OR 2)?'
      WRITE(1,*)
      READ(1,*,ERR=2000) ITERINP
      CALL IONETWO(ITERINP)
      IF(ITERINP.EQ.2) THEN
          WRITE(1,*)
          WRITE(1,*)'PLEASE ENTER THE NAME OF THE INPUT FILE TO BE '
```

```
          WRITE(1,*)'USED, (ENTER NO MORE THAN 10 CHARACTERS).'
          READ(1,'(A10)') ISENS
          CALL CHANNAM(ISENS)
          WRITE(1,*)
          WRITE(1,*)'OKAY, THE PROGRAM IS CRUNCHING.'
          GO TO 2999
       ENDIF
C
       IPRINT = 10
C-----
C      PROMPTING THE USER TO ENTER THE REQUIRED DATA AND PARAMETERS
C      NEEDED TO RUN THE PROGRAM.
C-----
2005   CONTINUE
       WRITE(1,*)
       WRITE(1,*)'DO YOU WISH TO (1) ENTER DATA FROM A REAL EXPERIMENT'
       WRITE(1,*)'OR (2) USE THE PROGRAM FOR A SIMULATION (PSEUDO-'
       WRITE(1,*)'EXPERMINT), (ENTER 1 OR 2)?'
       WRITE(1,*)
       READ(1,*,ERR=2005) IRELPSE
       CALL IONETWO(IRELPSE)
2100   CONTINUE
       WRITE(1,*)'ENTERING THE PHYSICAL PARAMETERS OF THE SYSTEM:'
       WRITE(1,*)
       WRITE(1,*)'PLEASE ENTER THICKNESS OF THE DIALYSIS MEMBRANE, '
       WRITE(1,*)'(MICRONS).'
       WRITE(1,*)
       READ(1,*,ERR=2100) RL1
       WRITE(1,*)
2105   CONTINUE
       WRITE(1,*)'PLEASE ENTER THE THICKNESS OF THE CELL CHAMBER,'
       WRITE(1,*)'(MICRONS).'
       WRITE(1,*)
       READ(1,*,ERR=2105) RL2
       WRITE(1,*)
2106   CONTINUE
       WRITE(1,*)'PLEASE ENTER THE CELL POSITION, (5-11).'
       WRITE(1,*)'(SEE THE DIAGRAM BELOW FOR BETTER UNDERSTANDING.)'
       WRITE(1,*)
       WRITE(1,*)'    DIALYSIS MEMBRANE             CELL CHAMBER'
       WRITE(1,*)'  |                    |                          |'
       WRITE(1,*)'B |                    |                          |'
       WRITE(1,*)'U |                    |                          |'
       WRITE(1,*)'L |                    |                          |'
       WRITE(1,*)'K |                    |                          |'
       WRITE(1,*)'  1    2    3    4    5    6    7    8    9    10   11'
       WRITE(1,*)'F |                    |                          |'
       WRITE(1,*)'L |                    |                          |'
       WRITE(1,*)'O |                    |                          |'
       WRITE(1,*)'W |                    |                          |'
       WRITE(1,*)'  |                    |                          |'
       WRITE(1,*)
```

```
      READ(1,*,ERR=2106) LIP
      WRITE(1,*)
2110  CONTINUE
      WRITE(1,*)'PLEASE ENTER THE DIFFUSIVITY OF THE SOLUTE INSIDE'
      WRITE(1,*)'THE CELL CHAMBER, (METERS*METERS/SEC.), D2.'
      WRITE(1,*)
      READ(1,*,ERR=2110) D2
      WRITE(1,*)
2120  CONTINUE
      WRITE(1,*)'DO YOU WISH TO ENTER (1) SEPARATE VALUES FOR THE'
      WRITE(1,*)'DIFFUSIVITY OF THE SOLUTE INSIDE THE DIALYSIS'
      WRITE(1,*)'MEMBRANE (METERS*METERS/SEC.), D1, AND THE MASS'
      WRITE(1,*)'TRANSFER COEFFICIENT, H, OR (2) USE  PRESET'
      WRITE(1,*)'VALUES OF D1=D2/10 AND H=10000*D2?'
      WRITE(1,*)
      READ(1,*,ERR=2120) ISEPPRE
      CALL IONETWO(ISEPPRE)
      IF(ISEPPRE.EQ.1) THEN
2130  CONTINUE
         WRITE(1,*)'PLEASE ENTER D1 (METERS*METERS/SEC.).'
         WRITE(1,*)
         READ(1,*,ERR=2130) D1
         WRITE(1,*)
2140     CONTINUE
         WRITE(1,*)'PLEASE ENTER H, (METERS*METERS/SEC.)'
         WRITE(1,*)
         READ(1,*,ERR=2140) H
      ELSE IF (ISEPPRE.EQ.2) THEN
         D1 = D2/10.0
         H = 10000.*D2
      ENDIF
2150  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'PLEASE ENTER THE INITIAL AND FINAL CONCENTRATION, '
      WRITE(1,*)'CINIT CINF, (OSMOLALITY).  (ENTER BOTH VALUES AND'
      WRITE(1,*)'SEPARATE WITH A SPACE.)'
      WRITE(1,*)
      READ(1,*,ERR=2150) CINIT, CINF
2160  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'PLEASE ENTER THE INACTIVE VOLUME (%).'
      WRITE(1,*)
      READ(1,*,ERR=2160) VINA
      WRITE(1,*)
2170  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'PLEASE ENTER THE MAGNIFICATION FACTOR.  (USE 5080.0 '

      WRITE(1,*)'EXACT CELL SIZE ENTER 1.0.)'
      WRITE(1,*)
      READ(1,*,ERR=2170) RMAG
      WRITE(1,*)
```

```
2180  CONTINUE
      WRITE(1,*)
      WRITE(1,2190) RL1,RL2,LIP,D2,D1,H,CINIT,CINF,VINA,RMAG
2190  FORMAT(1X,'THE VALUES ENTERED SO FAR ARE:',/,
     . 1X,'1) DIALYSIS MEMBRANE THICKNESS = ',E11.3,' MICRONS',/,
     . 1X,'2) CELL CHAMBER THICKNESS = ',E11.3,' MICRONS',/,
     . 1X,'3) LIPOSOME POSITION (5-11) = ',I2,/,
     . 1X,'4) DIFFUSIVITY IN CELL CHAMBER = ',E11.3,' M*M/SEC.',/,
     . 1X,'5) DIFFUSIVITY IN DIALYSIS MEMBRANE = ',E11.3,' M*M/SEC.',/,
     . 1X,'6) MASS TRANSFER COEFFICIENT = ',E11.3,'   ',/,
     . 1X,'7) INITIAL CONCENTRATION = ',F7.3,' OSMOLALITY',/,
     . 1X,'8) FINAL CONCENTRATION = ',F7.3,' OSMOLALITY',/,
     . 1X,'9) INACTIVE VOLUME % = ',F5.2,/,
     . 1X,'10) MAGNIFICATION FACTOR = ',F7.1,/)
      WRITE(1,*)'DO YOU WISH TO CHANGE ANY OF THEM, (Y/N)?'
      READ(1,'(A1)') ICHANG
      CALL IYESNO(ICHANG)
      IF(ICHANG.EQ.'Y') THEN
2210     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'PLEASE ENTER THE NUMBER BESIDE THE PARAMETER'
         WRITE(1,*)'YOU WISH TO CHANGE, (1-10). '
         READ(1,*,ERR=2210) NCHANG
2215     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'PLEASE ENTER THE NEW VALUE.'
         IF(NCHANG.EQ.1) THEN
             READ(1,*,ERR=2215) RL1
         ELSE IF(NCHANG.EQ.2) THEN
             READ(1,*,ERR=2215) RL2
         ELSE IF(NCHANG.EQ.3) THEN
            READ(1,*,ERR=2215) LIP
         ELSE IF(NCHANG.EQ.4) THEN
            READ(1,*,ERR=2215) D2
         ELSE IF(NCHANG.EQ.5) THEN
            READ(1,*,ERR=2215) D1
         ELSE IF(NCHANG.EQ.6) THEN
            READ(1,*,ERR=2215) H
         ELSE IF(NCHANG.EQ.7) THEN
            READ(1,*,ERR=2215) CINIT
         ELSE IF(NCHANG.EQ.8) THEN
            READ(1,*,ERR=2215) CINF
         ELSE IF(NCHANG.EQ.9) THEN
            READ(1,*,ERR=2215) VINA
         ELSE IF(NCHANG.EQ.10) THEN
            READ(1,*,ERR=2215) RMAG
         ELSE IF(NCHANG.LE.0.OR.NCHANG.GE.11) THEN
             CALL INCORRES
            GO TO 2210
         ENDIF
         GO TO 2180
      ENDIF
```

```
      IF(IRUNAG.GE.1.OR.IMISTAK.EQ.'Y') GO TO 2235
      WRITE(1,*)
      WRITE(1,*)'IN ORDER FOR THE PROGRAM TO RUN THE USER'
      WRITE(1,*)'MUST ALSO ENTER THE FOLLOWING DATA:'
2220  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'THE TIME RANGE OF THE EXPERIMENT, TM0  TM1, (SEC.),'
      WRITE(1,*)'(NOTE:  PLEASE MAKE SURE TM1 IS GREATER THAN THE '
      WRITE(1,*)'TIME OF THE LAST DATA POINT TO BE ENTERED AND ENTER '
      WRITE(1,*)'BOTH VALUES WITH A SPACE BETWEEN THEM.)'
      READ(1,*,ERR=2220) TM0, TM1
2223  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'THE TIME STEP, DT, (SEC.).  (NOTE:  THIS TIME STEP IS'
      WRITE(1,*)'USED FOR NUMERICAL INTEGRATION - CHOOSE DT SUCH THAT'
      WRITE(1,*)'DT .GE. (TM1-TM0)/301  , TO PREVENT ARRAY OVERFLOW.)'
      READ(1,*,ERR=2223) DT
2225  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'THE PERMEABILITY STEP, DP, (MICRONS/SEC.) (NOTE: '
      WRITE(1,*)'CHOOSE DP SUCH THAT DP .GE. (THE MAGINTUE OF THE'
      WRITE(1,*)'PERMEABILITY RANGE UNDER INVESTIGATION)/301 TO'
      WRITE(1,*)'PREVENT ARRAY OVERFLOW.)'
      READ(1,*,ERR=2225) DP
2230  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'THE PERMEABILITY RANGE UNDER INVESTIGATION, P0 P1,'
      WRITE(1,*)'(MICRONS/SEC.).  (NOTE: ENTER BOTH VALUES WITH A'
      WRITE(1,*)'SPACE BETWEEN THEM.)'
      READ(1,*,ERR=2230) P0, P1
2231  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'THE PERMEABILITY VALUE AT WHICH THE SENSITIVITY'
      WRITE(1,*)'COEFFICIENT WILL BE EVALUATED, PSEN, (MICRONS/SEC.) '
      READ(1,*,ERR=2230) PSEN
2235  CONTINUE
      WRITE(1,*)
      WRITE(1,2240) DT,TM0,TM1,DP,P0,P1,PSEN
2240  FORMAT(1X,'THE VALUES ENTERED ARE:',/,
     .  1X,'1) TIME STEP = ',F7.2,' SEC.',/,
     .  1X,'2) TIME RANGE = ',F8.1,' SEC.',' TO ',F8.1,' SEC.',/,
     .  1X,'3) PERMEABILITY STEP = ',F7.2,' MICRONS/SEC.',/,
     .  1X,'4) PERMEABILITY RANGE = ',F7.2,' MICRONS/SEC.',' TO '
     .     ,F7.2,' MICRONS/SEC.',/,
     .  1X,'5) INVESTIGATING PERMEABILITY = ',F7.2,' MICRONS/SEC.',/)
      WRITE(1,*)'DO YOU WISH TO CHANGE ANY OF THEM, (Y/N)?'
      READ(1,'(A1)') ICHANG
      CALL IYESNO(ICHANG)
      IF(ICHANG.EQ.'Y')THEN
2245    CONTINUE
        WRITE(1,*)
        WRITE(1,*)'PLEASE ENTER THE NUMBER BESIDE THE PARAMETER'
```

```
         WRITE(1,*)'YOU WISH TO CHANGE, (1-5). '
         READ(1,*,ERR=2245) NCHANG
2246     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'PLEASE ENTER THE NEW VALUE(S). '
         READ(1,*,ERR=2246)
         IF(NCHANG.EQ.1) THEN
            READ(1,*,ERR=2246) DT
         ELSE IF(NCHANG.EQ.2) THEN
            WRITE(1,*)'(BOTH TM0 AND TM1 - SEPARATE WITH A SPACE)'
            READ (1,*,ERR=2246) TM0, TM1
         ELSE IF(NCHANG.EQ.3) THEN
            READ(1,*,ERR=2246) DP
         ELSE IF(NCHANG.EQ.4) THEN
            WRITE(1,*)'(BOTH P0 AND P1 - SEPARATE WITH A SPACE)'
            READ(1,*,ERR=2246) P0, P1
         ELSE IF(NCHANG.EQ.5) THEN
            READ(1,*,ERR=2246) PSEN
         ELSE IF(NCHANG.LE.0.OR.NCHANG.GE.6) THEN
             CALL INCORRES
             GO TO 2246
         ENDIF
        GO TO 2235
      ENDIF
C
      IF(IRELPSE.EQ.1) THEN
         IF(IRUNAG.GE.1.OR.IMISTAK.EQ.'Y') THEN
2248        CONTINUE
            WRITE(1,*)
            WRITE(1,*)'DO YOU WISH TO (1) ENTER ALL NEW DATA POINTS'
            WRITE(1,*)'OR (2) REVIEW THE PREVIOUS DATA POINTS,'
            WRITE(1,*)'(Y/N)?'
            READ(1,*,ERR=2248) INR
            CALL IONETWO(INR)
            IF(INR.EQ.2) GO TO 2261
            IF(INR.EQ.1) THEN
               I=0
               WRITE(1,*)
               GO TO 2250
            ENDIF
         ENDIF
         I = 0
         WRITE(1,*)
         WRITE(1,*)'SINCE YOU HAVE CHOSEN THE REAL-EXPERIMENTAL'
         WRITE(1,*)'OPTION YOU MUST NOW ENTER THE DATA POINTS.'
2250     CONTINUE
         WRITE(1,*)'BEFORE ENTERING THE DATA, IS THERE A TIME'
         WRITE(1,*)'DELAY THAT YOU WOULD LIKE TO HAVE SUBTRACTED'
         WRITE(1,*)'FROM THE TIME ARRAY YOU WILL BE ENTERING,'
         WRITE(1,*)'(Y/N)?'
         READ(1,'(A1)') IDEL
         CALL IYESNO(IDEL)
```

```
      IF(IDEL.EQ.'N') THEN
         DELAY = 0.0
      ELSE IF(IDEL.EQ.'Y') THEN
2251     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'PLEASE ENTER THE TIME DELAY TO BE SUBTRACTED,'
         WRITE(1,*)'(SEC.)'
         READ(1,*,ERR=2251) DELAY
         WRITE(1,2252) DELAY
2252     FORMAT(/,' THE TIME DELAY THAT WILL BE SUBTRACTED IS ',
     .       F7.4,' SEC. ',/,/,' DO YOU WISH TO CHANGE IT, (Y/N)?')
         READ(1,'(A1)') ICHANG
         CALL IYESNO(ICHANG)
         IF(ICHANG.EQ.'Y') GO TO 2251
      ENDIF
      WRITE(1,*)
      WRITE(1,*)'DO YOU WISH TO ENTER THE RADIUS USING UNITS'
      WRITE(1,*)'OF (1) MICRONS OR (2) CENTIMETERS?'
2253  READ(1,*,ERR=2253) IMICCEN
      CALL IONETWO(IMICCEN)
      WRITE(1,*)
      WRITE(1,*)'PLEASE ENTER THE DATA POINTS,'
      IF(IMICCEN.EQ.1)THEN
         WRITE(1,*)'TIME(I) (SEC.)  RADIUS(I) (MICRONS)'
      ELSE IF(IMICCEN.EQ.2) THEN
         WRITE(1,*)'TIME(I) (SEC.)  RADIUS(I) (CENTIMETERS)'
      ENDIF
      WRITE(1,*)'(ENTER BOTH VALUES AND SEPARATE WITH A SPACE.)'
      WRITE(1,*)'(NOTE:  ENTER 0.0 0.0 FOR THE LAST DATA POINT.) '
      WRITE(1,*)
2255  I = I+1
      WRITE(1,2256) I
2256  FORMAT(1X,'ENTER POINT ',I3)
2257  READ(1,*,ERR=2257) TMA(I), RA(I)
      IF((TMA(I).GT.0.).AND.(RA(I).GT.0.)) TMA(I)=TMA(I)-DELAY
      IF(I.EQ.1) RAMAX = RA(I)
      IF(I.GE.2.AND.RA(I).GT.RAMAX) RAMAX = RA(I)
      IF((TMA(I).GT.0.).OR.(RA(I).GT.0.)) GO TO 2255
      ICOUNT = I-1
      IF(TM1.LE.TMA(ICOUNT)) THEN
          WRITE(1,*)
          WRITE(1,*)'PLEASE MAKE SURE THE TIME, LIMIT TM1 IS'
          WRITE(1,*)'LARGER THAN THE TIME OF THE LAST DATA POINT.'
      WRITE(1,*)'IF YOU DO NOT CHANGE THIS THE PROGRAM WILL STOP.'
          WRITE(1,*)'(RELAX YOU WILL HAVE A CHANCE TO CHANGE IT.)'
          WRITE(1,*)
      ENDIF
2260  CONTINUE
      WRITE(1,*)'THE NEXT TABLE WILL SHOW YOU THE POINTS YOU HAVE'
      WRITE(1,*)'JUST ENTERED.  IF YOU HAVE ENTERED MORE THAN 20'
      WRITE(1,*)'POINTS THE TABLE WILL STOP SPOOLING EVERY 20 POINTS'
      WRITE(1,*)'TO ALLOW YOU TO REVIEW THE POINTS ENTERED.  MAKE'
```

```
      WRITE(1,*)'A NOTE OF WHICH POINT YOU WISH TO CHANGE OR'
      WRITE(1,*)'INSERT AND PRESS [RETURN] TO CONTINUE NOTE, YOU'
      WRITE(1,*)'YOU WILL ONLY BE ABLE TO CHANGE ONE POINT AT'
      WRITE(1,*)'A TIME.'
      WRITE(1,*)'(NOW PRESS [RETURN] TO CONTINUE.)'
      READ(1,'(A1)') ICHANG
2261  CONTINUE
      WRITE(1,*)
      WRITE(1,*)'THE DATA POINTS YOU HAVE ENTERED ARE: '
      WRITE(1,2259) DELAY
2259  FORMAT(' (INCLUDING THE SUBTRACTED TIME DELAY OF ',
     .       F10.4,' SEC.)')
      WRITE(1,*)
      WRITE(1,*)'  J       TIME(J)      RADIUS(J)'
      IF(IMICCEN.EQ.1) THEN
      WRITE(1,*)'           (SEC.)      (MICRONS)'
      ELSE IF(IMICCEN.EQ.2) THEN
      WRITE(1,*)'          (SEC.)       (CENTIMETERS)'
      ENDIF
      DO 2265 J=1,ICOUNT+1
          WRITE(1,2263) J, TMA(J), RA(J)
2263      FORMAT(1X,I3,5X,F8.2,5X,F8.2)
          IF((J/20)*20.EQ.J) THEN
             WRITE(1,*)
             WRITE(1,*)'PRESS [RETURN] TO CONTINUE.'
             READ(1,'(A1)') ICHANG
          ENDIF
2265  CONTINUE
      IF(IBACK.EQ.1) GO TO 2281
      WRITE(1,*)
      WRITE(1,*)'DO YOU WISH TO CHANGE OR INSERT ANY OF THE POINTS,'
      WRITE(1,*)'(Y/N)?'
      READ(1,'(A1)') ICHANG
      CALL IYESNO(ICHANG)
      IF(ICHANG.EQ.'Y') THEN
2266       CONTINUE
           WRITE(1,*)
          WRITE(1,*)'ENTER (1) TO CHANGE AND (2) TO INSERT.'
          READ(1,*,ERR=2266) ICHAINS
          CALL IONETWO(ICHAINS)
        IF(ICHAINS.EQ.1) THEN
2267       CONTINUE
           WRITE(1,*)
           WRITE(1,*)'PLEASE ENTER THE INDEX NUMBER  J'
           READ(1,*,ERR=2267) JI
           IF(JI.LE.0.OR.JI.GE.I+1) THEN
               CALL INCORRES
               GO TO 2267
            ENDIF
           WRITE(1,*)
           WRITE(1,*)'PLEASE ENTER THE NEW VALUES FOR '
           WRITE(1,*)'TIME(J) AND RADIUS(J). '
```

```
2270          READ(1,*,ERR=2270) TMA(JI), RA(JI)
              GO TO 2261
            ELSE IF(ICHAINS.EQ.2) THEN
2272           CONTINUE
              WRITE(1,*)
              WRITE(1,*)'ENTER THE INDEX NUMBER J YOU WISH TO '
              WRITE(1,*)'CHANGE, (OR PUSH DOWN).'
              READ(1,*,ERR=2272) JI
              IF(JI.LE.0.OR.JI.GE.I+1) THEN
                  CALL INCORRES
                  GO TO 2272
              ENDIF
              I = I+1
              ICOUNT = I-1
              DO 2278 J=JI,I-1
                 TMA(I+JI-J) = TMA((I-1)+JI-J)
                 RA(I+JI-J) = RA((I-1)+JI-J)
2278          CONTINUE
              WRITE(1,*)
              WRITE(1,*)'PLEASE ENTER THE NEW VALUES FOR'
              WRITE(1,*)'TIME(J) AND RADIUS(J).'
2280          READ(1,*,ERR=2280) TMA(JI), RA(JI)
              GO TO 2261
            ENDIF
          ENDIF
2281      CONTINUE
          IF(IRUNAG.GT.0) THEN
              WRITE(1,*)
              WRITE(1,*)'DO YOU WISH TO HAVE A TIME DELAY SUBTRACTED'
              WRITE(1,*)'FROM THE DATA POINTS, (Y/N)?'
              READ(1,'(A1)') IDEL
              CALL IYESNO(IDEL)
              IF(IDEL.EQ.'Y') THEN
2282              CONTINUE
                  WRITE(1,*)
                  WRITE(1,*)'PLEASE ENTER THE DELAY TO BE SUBTRACTED.'
                  READ(1,*,ERR=2282) DELAY
                  WRITE(1,*)
                  WRITE(1,2283) DELAY
2283              FORMAT(' THE DELAY ENTERED IS ',F10.4,' (SEC.)',/,
     .             ' DO YOU WISH TO CHANGE IT, (Y/N)?')
                  READ(1,'(A1)') ICHANG
                  CALL IYESNO(ICHANG)
                  IF(ICHANG.EQ.'Y') GO TO 2282
              ELSE IF(IDEL.EQ.'N') THEN
                  DELAY = 0.0
                  GO TO 2341
              ENDIF
              DO 2284 J=1,ICOUNT
                  TMA(J) = TMA(J) - DELAY
2284          CONTINUE
              IBACK = 1
```

```
            GO TO 2261
         ENDIF
         IBACK = 0
         RINIT = RA(1)
      ELSE IF(IRELPSE.EQ.2) THEN
         IF(IRUNAG.GE.1.OR.IMISTAK.EQ.'Y') GO TO 2319
         WRITE(1,*)
         WRITE(1,*)'SINCE YOU HAVE CHOSEN THE PSEUDO-EXPERIMENTAL'
         WRITE(1,*)'OPTION, THE FOLLOWING PARAMETERS MUST ALSO'
         WRITE(1,*)'BE ENTERED.'
         WRITE(1,*)
2300     CONTINUE
         WRITE(1,*)'THE PERMEABILITY VALUE WITH WHICH THE PROGRAM'
         WRITE(1,*)'GENERATES PSEUDO-EXPERIMENTAL DATA, PTRU, '
         WRITE(1,*)'(MICRONS/SEC.).'
         READ(1,*,ERR=2300) PTRU
2303     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'DO YOU WISH TO ENTER THE INITIAL RADIUS USING UNITS'
         WRITE(1,*)'OF (1) MICRONS OR (2) CENTIMETERS, (ENTER 1 OR 2)?'
         READ(1,*,ERR=2303) IMICCEN
         CALL IONETWO(IMICCEN)
2305     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'PLEASE ENTER THE INITIAL RADIUS OF THE CELL, RINIT,'
         IF(IMICCEN.EQ.1) WRITE(1,*)'(MICRONS).'
         IF(IMICCEN.EQ.2) WRITE(1,*)'(CENTIMETERS).'
         READ(1,*,ERR=2305) RINIT
2310     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'SINCE THIS IS A SIMULATION THE DATA POINTS '
         WRITE(1,*)'GENERATED WILL HAVE A RADIUS HISTORY THAT WILL'
         WRITE(1,*)'LOOK EXACTLY LIKE THAT OF THEORY, THEREFORE'
         WRITE(1,*)'THE PROGRAM ALLOWS THE USER TO IMPOSE A PSEUDO-'
         WRITE(1,*)'RANDOMNESS FACTOR, DR, ON THE DATA.  DR CAN BE'
         WRITE(1,*)'THOUGHT OF AS THE MAGNITUDE OF THE VARIATION'
         WRITE(1,*)'IN MEASURING THE RADIUS OF THE CELL.'
         WRITE(1,*)'PLEASE ENTER DR NOW.'
         IF(IMICCEN.EQ.1) WRITE(1,*)'(MICRONS).'
         IF(IMICCEN.EQ.2) WRITE(1,*)'(CENTIMETERS).'
         READ(1,*,ERR=2310) DR
         WRITE(1,*)
         WRITE(1,*)'WHEN USING THIS OPTION THE USER CAN CHANGE'
         WRITE(1,*)'THE TIME STEP IN TWO REGIONS TO STUDY THE '
         WRITE(1,*)'EFFECT OF DATA SPACING OF PARAMETER ESTIMATION.'
         WRITE(1,*)'DO YOU WISH TO DO THIS, (Y/N)? '
         READ(1,'(A1)') IMORE
         CALL IYESNO(IMORE)
         IF(IMORE.EQ.'N') THEN
            TM01 = TM1
            DT1 = DT
            DT2 = DT
```

```
         ELSE IF(IMORE.EQ.'Y') THEN
2311        CONTINUE
            WRITE(1,*)
            WRITE(1,*)'PLEASE ENTER THE FIRST TIME STEP, DT1, (SEC.). '
            READ(1,*,ERR=2311) DT1
2312        CONTINUE
            WRITE(1,*)
            WRITE(1,*)'PLEASE ENTER THE SECOND TIME STEP, DT2, (SEC.) '
            READ(1,*,ERR=2312) DT2
2313        CONTINUE
            WRITE(1,*)
           WRITE(1,*)'PLEASE ENTER THE INTERMEDIATE TIME LIMIT, TM01,'
            WRITE(1,*)'(SEC.) FOR THE FIRST TIME INTERVAL.  (TM01  '
            WRITE(1,*)'TO TM1 IS ASSUMED TO BE THE SECOND TIME  '
            WRITE(1,*)'INTERVAL.)'
            READ(1,*,ERR=2313) TM01
         ENDIF
C
2319     CONTINUE
         IF(IMICCEN.EQ.1) XMICCEN ='MICRONS'
         IF(IMICCEN.EQ.2) XMICCEN ='CENTIMETERS'
         WRITE(1,*)
         WRITE(1,2320) PTRU,RINIT,XMICCEN,DR,XMICCEN,DT1,DT2,TM01
2320     FORMAT(1X,'THE VALUES ENTERED FOR THE PSEUDO-EXPERIMENTAL'
     .   ,' OPTION ARE:',/,
     . 1X,'1) PSEUDO-PERMEABILITY = ',F8.1,' MICRONS/SEC.',/,
     . 1X,'2) INITIAL CELL RADIUS = ',E11.3,1X,A11,/,
     . 1X,'3) RADIUS RANDOMNESS = ',E11.4,1X,A11,/,
     . 1X,'4) FIRST TIME STEP = ',F7.2,' SEC.',/,
     . 1X,'5) SECOND TIME STEP = ',F7.2,' SEC.',/,
     . 1X,'6) INTERMEDIATE TIME LIMIT = ',F8.1,' SEC.',/)
         WRITE(1,*)'DO YOU WISH TO CHANGE ANY OF THEM, (Y/N)?'
         READ(1,'(A1)') ICHANG
         CALL IYESNO(ICHANG)
         IF(ICHANG.EQ.'Y') THEN
2330        CONTINUE
            WRITE(1,*)
            WRITE(1,*)'PLEASE ENTER THE NUMBER BESIDE THE PARAMETER'
            WRITE(1,*)'YOU WISH TO CHANGE, (1-5). '
            READ(1,*,ERR=2330) NCHANG
2340        CONTINUE
            WRITE(1,*)
            WRITE(1,*)'PLEASE ENTER THE NEW VALUE. '
            IF(NCHANG.EQ.1) THEN
                READ(1,*,ERR=2340) PTRU
            ELSE IF(NCHANG.EQ.2) THEN
                READ(1,*,ERR=2340) RINIT
            ELSE IF(NCHANG.EQ.3) THEN
                READ(1,*,ERR=2340) DR
            ELSE IF(NCHANG.EQ.4) THEN
                READ(1,*,ERR=2340) DT1
            ELSE IF(NCHANG.EQ.5) THEN
```

```
                READ(1,*,ERR=2340) DT2
            ELSE IF(NCHANG.EQ.6) THEN
                READ(1,*,ERR=2340) TM01
            ELSE IF(NCHANG.LE.0.OR.NCHANG.GE.7) THEN
                CALL INCORRES
                GO TO 2330
            ENDIF
           GO TO 2319
         ENDIF
      ENDIF
C
2341  CONTINUE
      IF(IRUNAG.EQ.0) THEN
         IF1 = 0
         IF2 = 0
         IF3 = 0
         IF4 = 0
         CAB0 = 0.0
         CAB1 = 5.0
         SEN0 = -0.1
         SEN1 = 0.0
         SUM0 = 0.0
         SUM1 = 100.0
      ELSE IF(IRUNAG.GT.0) THEN
         WRITE(1,*)
         WRITE(1,*)'DO YOU WISH TO CHANGE ANY OF THE LIMITS OF THE'
         WRITE(1,*)'GRAPHICAL OPTIONS YOU HAVE CHOOSEN AND/OR '
         WRITE(1,*)'WOULD YOU LIKE TO CHOOSE ANOTHER OPTION, (Y/N)?'
         READ(1,'(A1)') ICHANG
         CALL IYESNO(ICHANG)
         IF(ICHANG.EQ.'N') GO TO 3999
         IF(ICHANG.EQ.'Y') THEN
            WRITE(1,*)
            WRITE(1,*)'THE PROGRAM WILL NOW ALLOW TO CHANGE OR '
            WRITE(1,*)'CHOOSE ANOTHER OPTION BY SELECTING ONE'
            WRITE(1,*)'OF THE FOLLOWING OPTIONS.'
            GO TO 2345
         ENDIF
      ENDIF
      WRITE(1,*)'DO YOU WISH TO VIEW THE OUTPUT IN GRAPHICAL '
      WRITE(1,*)'FORM, (Y/N)?'
      READ(1,'(A1)') IGRAPH
      CALL IYESNO(IGRAPH)
      IF(IGRAPH.EQ.'Y') THEN
2345     CONTINUE
         WRITE(1,*)
         WRITE(1,*)'THE OUTPUT CAN BE VIEWED IN THE FOLLOWING WAYS:'
         WRITE(1,*)
         WRITE(1,*)'1) CONCENTRATION V.S. DIMENSIONLESS TIME'
         WRITE(1,*)'2) SENSITIVITY COEFFICIENT V.S. TIME'
         WRITE(1,*)'3) NORMALIZED VOLUME V.S. TIME'
         WRITE(1,*)'4) SUM OF ERRORS OF SQUARES V.S. PERMEABILITY'
```

```
      WRITE(1,*)
      IF(IRUNAG.EQ.0) THEN
        WRITE(1,*)'WHICH GRAPHICAL OPTION WOULD YOU LIKE (1-4),'
      ELSE IF(IRUNAG.GT.0) THEN
        WRITE(1,*)'WHICH GRAPHICAL OPTION WOULD YOU LIKE TO'
        WRITE(1,*)'SELECT OR CHANGE LIMITS ON, (1-4)'
      ENDIF
      WRITE(1,*)'(PLEASE CHOOSE ONE GRAPH AT A TIME).'
2347  CONTINUE
      READ(1,*,ERR=2347) IOPTGR
      IF(IOPTGR.LE.0.OR.IOPTGR.GE.5) THEN
          CALL INCORRES
          GO TO 2345
      ENDIF
      WRITE(1,*)'(NOTE: PLEASE ENTER BOTH LOWER AND UPPER LIMITS'
      WRITE(1,*)' WITH A SPACE SEPARATING THE VALUES.)'
      IF(IOPTGR.EQ.1) THEN
          IF1 = 1
2348      CONTINUE
          WRITE(1,*)
     WRITE(1,*)'PLEASE ENTER THE CONCENTRATION LIMITS, CAB0 CAB1. '
     WRITE(1,*)'(UNLESS NECESSARY TO CHANGE THESE SET THE VALUES'
     WRITE(1,*)'AS  0.0  AND 5.0.)'
          READ(1,*,ERR=2348) CAB0, CAB1
          CALL CHANLIM(CAB0,CAB1)
      ELSE IF(IOPTGR.EQ.2) THEN
          IF2 = 1
2350      CONTINUE
          WRITE(1,*)
     WRITE(1,*)'PLEASE ENTER THE SENSITIVITY COEFFICIENT LIMITS, '
          WRITE(1,*)'(SUGGESTED LIMITS ARE -0.1 AND 0.0)'
          WRITE(1,*)'SEN0 SEN1.'
          READ(1,*,ERR=2350) SEN0, SEN1
         CALL CHANLIM(SEN0,SEN1)
      ELSE IF(IOPTGR.EQ.3) THEN
          IF3 = 1
2360      CONTINUE
          WRITE(1,*)
          WRITE(1,*)'PLEASE ENTER THE LIMITS FOR THE NORMALIZED'
          WRITE(1,*)'VOLUME, VOL0 VOL1.  (UNLESS NECESSARY TO '
          WRITE(1,*)'CHANGE THESE, SET THE VALUES AS 0.0 AND 1.0).'
          READ(1,*,ERR=2360) VOL0, VOL1
          CALL CHANLIM(VOL0,VOL1)
      ELSE IF(IOPTGR.EQ.4) THEN
          IF4 = 1
2370      CONTINUE
          WRITE(1,*)
     WRITE(1,*)'PLEASE ENTER THE SUM OF ERRORS LIMITS, SUM0 SUM1. '
          WRITE(1,*)'(SUGGESTED LIMITS 0.0 AND 20.0)'
          READ(1,*,ERR=2370) SUM0, SUM1
          CALL CHANLIM(SUM0,SUM1)
      ENDIF
```

```
         WRITE(1,*)
         WRITE(1,*)'DO YOU WISH TO CHOOSE ANOTHER GRAPH, (Y/N)? '
         READ(1,'(A1)') IANOTH
         CALL IYESNO(IANOTH)
         IF(IANOTH.EQ.'Y') GO TO 2345
      ENDIF
2400  CONTINUE
      WRITE(1,*)'HAVE YOU MADE ANY MISTAKES THAT YOU WOULD LIKE '
      WRITE(1,*)'ANOTHER CRACK AT ENTERING/CHANGING THE PARAMETERS '
      WRITE(1,*)'OR DATA AGAIN, (Y/N)?'
      READ(1,'(A1)') IMISTAK
      CALL IYESNO(IMISTAK)
      IF(IMISTAK.EQ.'Y') GO TO 2180
      WRITE(1,*)
      WRITE(1,*)'OKAY, THE PROGRAM IS NOW CRUNCHING.'
      GO TO 3999
C-----
C     OPTION ITERINP = 2: ENTERING THE INPUT FILE
C-----
2999  CONTINUE
      OPEN(11,FILE=ISENS)
      READ(11,'(A11)') PROBLEM
      READ(11,3100) RL1,RL2,LIP
3100  FORMAT(///,1X,F11.6,F11.6,I2)
      READ(11,3110) D1,D2,H
3110  FORMAT(//,1X,E11.2,E11.2,E11.2)
      READ(11,3120) CINIT, CINF
3120  FORMAT(//,1X,F11.6,F11.6)
      READ(11,3125) VINA,RMAG
3125  FORMAT(//,1X,E11.6,E11.6)
      READ(11,3130) DT,TM0,TM1,DELAY
3130  FORMAT(//,1X,F11.6,F11.6,F11.6,F11.6)
      READ(11,3140) DP,PSEN
3140  FORMAT(//,1X,F11.6,F11.6)
      READ(11,3145) RINIT
3145  FORMAT(//,1X,F11.6)
      READ(11,3150) PTRU,DR,TM01,DT1,DT2
3150  FORMAT(///,1X,F11.6,F11.6,F11.6,F11.6,F10.6)
      READ(11,3160) IRELPSE,IMICCEN
3160  FORMAT(///,1X,I9,I9)
      READ(11,3170) IF1,IF2,IF3,IF4,IPRINT
3170  FORMAT(///,1X,I11,I11,I11,I11,I2,/)
      READ(11,3180) CAB0,CAB1
3180  FORMAT(//,1X,F11.6,F11.6)
      READ(11,3180) SEN0,SEN1
      READ(11,3180) VOL0,VOL1
      READ(11,3190) P0,P1,SUM0,SUM1
3190  FORMAT(//,1X,F11.6,F11.6,F11.6,F11.6,///)
C
      IF(IMICCEN.EQ.1) XMICCEN='MICRONS'
      IF(IMICCEN.EQ.2) XMICCEN='CENTIMETERS'
C
```

```
C-----
C     WRITING THE INPUT DATA TO THE OUTPUT FILE O_SENS.DAT'
C-----
      WRITE(1,3195) ISENS
3195  FORMAT(/,' THE INPUT FILE ',A10,' HAS BEEN ENTERED.')
3999  CONTINUE
      OPEN(10,FILE='O_SENS.DAT')
      WRITE(10,4000) ISENS
4000  FORMAT(1X,'********************  THE INPUT FILE ',A10,
     .       '  **********************',/)
      WRITE(10,4005)
4005  FORMAT(/,'RL1,RL2,LIP ARE',/)
      WRITE(10,*) RL1,RL2,LIP
      WRITE(10,4010)
4010  FORMAT(/,'D1,D2,H ARE',/)
      WRITE(10,*) D1, D2, H
      WRITE(10,4020)
4020  FORMAT(/,'CINIT,CINF, ARE:',/)
      WRITE(10,*)CINIT,CINF
      WRITE(10,4030)
4030  FORMAT(/,'VINA,RMAG ARE:',/)
      WRITE(10,*)VINA,RMAG
      WRITE(10,4040)
4040  FORMAT(/,'DT,TM0,TM1,DELAY ARE:',/)
      WRITE(10,*) DT,TM0,TM1,DELAY
      WRITE(10,4050)
4050  FORMAT(/,'DP,PSEN ARE:',/)
      WRITE(10,*) DP, PSEN
      WRITE(10,4060)
4060  FORMAT(/,'RINIT IS:',/)
      WRITE(10,*) RINIT
      WRITE(10,4070)
4070  FORMAT(/,'PTRU,DR,TM01,DT1,DT2 ARE:',/)
      WRITE(10,*) PTRU,DR,TM01,DT1,DT2
      WRITE(10,4080)
4080  FORMAT(/,'IRELPSE,IMICCEN ARE:',/)
      WRITE(10,*) IRELPSE, IMICCEN
      WRITE(10,4090)
4090  FORMAT(/,'IF1,IF2,IF3,IF4,IPRINT ARE:',/)
      WRITE(10,*) IF1,IF2,IF3,IF4,IPRINT
      WRITE(10,4100)
4100  FORMAT(/,'CAB0,CAB1 ARE:',/)
      WRITE(10,*) CAB0, CAB1
      WRITE(10,4110)
4110  FORMAT(/,'SEN0,SEN1 ARE:',/)
      WRITE(10,*) SEN0,SEN1
      WRITE(10,4120)
4120  FORMAT(/,'VOL0,VOL1 ARE:',/)
      WRITE(10,*) VOL0,VOL1
      WRITE(10,4130)
4130  FORMAT(/,'P0,P1,SUM0,SUM1 ARE:',/)
      WRITE(10,*) P0,P1,SUM0,SUM1
```

```
C
C-----
C     USING SUBROUTINE MBCON TO PREDICT THE CONCENTRATION CHANGE
C     INSIDE THE CELL CHAMBER
C-----
      CALL MBCON(IOPNAG)
C-----
C     INITIALIZATION OF THE TIME VARIABLES AND RINIT
C-----
      IT=INT((TM1-TM0)/DT)+1
      DO 100 I=1,IT
         TMS(I)=TM0+(I-1)*DT
         TMB(I)=TMS(I)
100    CONTINUE
      IF(IMICCEN.EQ.1) THEN
         RINIT = RINIT/RMAG
      ELSE IF(IMICCEN.EQ.2) THEN
         RINIT = RINIT*10000./RMAG
      ENDIF
C-----
C     CALCULATE R(TM,P) AND R(TM,P+DP)
C-----
      CALL RGKT(TMS,RS,IT,DT,RINIT,PSEN)
      CALL RGKT(TMB,RB,IT,DT,RINIT,PSEN+EP1*PSEN)
C-----
C     CALCULATE SENSITIVITY COEFF. (SEN)
C-----
      WRITE(10,4175)
4175  FORMAT(//,1X,'*********  DIMENSIONLESS SENSIVITITY ',
     .          'COEFFICIENT VERSUS TIME  ********')
      WRITE(10,4180)
4180  FORMAT(/,6X,'TIME(I)',7X,'SEN(I)',/)
      DO 4190 J=1,IT
         SEN(J)=(RB(J)-RS(J))*PSEN/RINIT/(EP1*PSEN)
         WRITE(10,*)TMS(J),SEN(J)
4190    CONTINUE
C-----
C     PLOTTING SENSITIVITY VERSUS TIME (IF2=0 STOP THE OUTPUT)
C-----
      IF(IF2.EQ.0)GO TO 4230
      CALL NEWPAG
      CALL PLOT(TM0,TM1,10,SEN0,SEN1,10,TMS,SEN,IT,FCT0,5,2)
      CALL ANMODE
      CALL HOME
      WRITE(1,*)
      WRITE(1,*)'PRESS [RETURN] TO CONTINUE.'
      READ(1,'(A1)') IMORE
4230  CONTINUE
      WRITE(10,*)
      WRITE(10,4235)
4235  FORMAT(1X,'************************  RADIUS VERSUS TIME',
     .        '  ************************')
```

```
C-----
C     READING THE EXPERIMENTAL DATA AND CONVERTING TO MICRONS
C-----
      IF (IRELPSE.EQ.1.AND.ITERINP.EQ.2) THEN
         I=0
         WRITE(10,4240)
4240     FORMAT(/,6X,'TM(I)',10X,'R(I)',/)
4250     I=I+1
         READ(11,*)TMA(I),RA(I)
         RSAVE(I) = RA(I)
         IF(RA(I).GT.0.)  TMA(I) = TMA(I)-DELAY
         WRITE(10,4253)TMA(I),RA(I)
         IF(IMICCEN.EQ.1) THEN
            RA(I) = RA(I)/RMAG
         ELSE IF(IMICCEN.EQ.2) THEN
            RA(I) = RA(I)*10000./RMAG
         ENDIF
         IF(I.EQ.1) RAMAX = RA(I)
         IF(I.GE.2) THEN
              IF(RA(I).GT.RAMAX) RAMAX = RA(I)
         ENDIF
         IF ((TMA(I).GT.0.).OR.(RA(I).GT.0.)) GO TO 4250
         ICOUNT=I-1
         IF (TM1.LE.TMA(ICOUNT)) THEN
            WRITE(1,*)
            WRITE(1,*)'PLEASE MAKE SURE TM1 IS LARGER THAN THE TIME'
            WRITE(1,*)'OF THE LAST DATA POINT, AND RUN IT AGAIN.'
            GO TO 6000
         END IF
C-----
C     CONVERTING INPUT DATA, ENTERED BY THE TERMINAL, TO MICRONS
C-----
      ELSE IF(IRELPSE.EQ.1.AND.ITERINP.EQ.1) THEN
         WRITE(10,4240)
         DO 4255 K=1,ICOUNT+1
            RSAVE(K) = RA(K)
            WRITE(10,4253) TMA(K),RA(K)
4253        FORMAT(5X,F7.2,5X,E11.4)
4255     CONTINUE
         IF(IMICCEN.EQ.1)THEN
             DO 4260 K=1,ICOUNT
                 RA(K) = RA(K)/RMAG
4260         CONTINUE
             RAMAX = RAMAX/RMAG
         ELSE IF(IMICCEN.EQ.2) THEN
             DO 4270 K=1,ICOUNT
                 RA(K) = RA(K)*10000./RMAG
4270         CONTINUE
             RAMAX = RAMAX*10000./RMAG
         ENDIF
C-----
C     GENERATING THE SIMULATED (PSEUDO) EXPERIMENTAL DATA
```

```
C-----
      ELSE IF(IRELPSE.EQ.2) THEN
         ICOUNT = ((TM01-TM0)/DT1+(TM1-TM01)/DT2+1)
         CALL RGKT(TMS,RB,IT,DT,RINIT,PTRU)
         DO 4280 J=1,IT
              TMA(J) = TMS(J)
              RC(J) = RB(J)
4280     CONTINUE
         TMA(1)=TM0
         DO 4290 J=2,ICOUNT
            IF (TMA(J-1).LT.TM01) THEN
               TMA(J)=TMA(J-1)+DT1
            ELSE IF (TMA(J-1).GE.TM01) THEN
               TMA(J)=TMA(J-1)+DT2
            END IF
4290     CONTINUE
         IF(IMICCEN.EQ.1) THEN
             DR = DR/RMAG
         ELSE IF(IMICCEN.EQ.2) THEN
             DR = DR*10000./RMAG
         ENDIF
         RAMAXC = RC(1)
         DO 4292 J=2,IT
             IF(RC(J).GT.RAMAXC) RAMAXC = RC(J)
4292     CONTINUE
         RAMAX = RB(1)
         DO 4300 J=1,ICOUNT
            ITM=INT(TMA(J)/DT)+1
            IF (ITM.GE.IT) THEN
               RA(J)=RB(ITM)+DR*RANND()
            ELSE
               DRA=(RB(ITM+1)-RB(ITM))*(TMA(J)-(ITM-1)*DT)/DT
               RA(J)=RB(ITM)+DRA+DR*RANND()
            END IF
            IF(RA(J).GT.RAMAX) RAMAX = RA(J)
4300     CONTINUE
         WRITE(10,*)
      WRITE(10,4301)
4301  FORMAT(//,1X,'***** SIMULATED RADIUS VERSUS TIME  ',
     .          '**********',/)
         DO 4302 J=1,ICOUNT+1
             IF(IMICCEN.EQ.1) RSAVE(J)=RA(J)*RMAG
             IF(IMICCEN.EQ.2) RSAVE(J)=RA(J)*RMAG/10000.
4302     CONTINUE
         DO 4305 J=1,ICOUNT
              WRITE(10,4253) TMA(J),RA(J)
4305     CONTINUE
      END IF
C-----
C     CALCULATING THE NORMALIZED VOLUME
C-----
      DO 4315 I=1,ICOUNT
```

```
         VOLA(I)=(RA(I)/RAMAX)**3
4315  CONTINUE
      IF(IRELPSE.EQ.2) THEN
         DO 4316 J=1,IT
            VOLC(J) =(RC(J)/RAMAXC)**3
4316     CONTINUE
      ENDIF
C-----
C     CALCULATE SUM OF ERROR OF SQUARE FOR P VALUES FROM
C     P0 TO P1
C-----
      IP=(P1-P0)/DP+1
      DO 4330 I=1,IP
         P(I)=P0+(I-1)*DP
         CALL RGKT(TMB,RB,IT,DT,RINIT,P(I))
         SUM(I)=0.
         DO 4320 J=1,ICOUNT
            ITM=INT(TMA(J)/DT)+1
            IF (ITM.GE.IT) THEN
               RN=RB(ITM)
            ELSE
               DRN=(RB(ITM+1)-RB(ITM))*(TMA(J)-(ITM-1)*DT)/DT
               RN=RB(ITM)+DRN
            END IF
            SUM(I)=SUM(I)+(RN-RA(J))**2
4320     CONTINUE
4330  CONTINUE
C----
C
C----
      WRITE(10,4332)
4332  FORMAT(///,1X,'***********  SUM OF SQUARES OF ERRORS ',
     .             'VERSUS PERMEABILITY  *************')
      WRITE(10,4335)
4335  FORMAT(/,'     P(J)        SUM(J)',/)
      DO 4338 J=1,IP
         WRITE(10,4336) P(J),SUM(J)
4336      FORMAT(1X,F8.2,6X,E10.4)
4338   CONTINUE
C
      SUMIN=SUM(1)
      PEST = P(1)
      DO 4340 I=2,IP
         IF (SUMIN.GT.SUM(I)) THEN
            SUMIN=SUM(I)
            PEST=P(I)
         END IF
4340  CONTINUE
      CALL RGKT(TMB,RC,IT,DT,RINIT,PEST)
      CALL RGKT(TMB,RB,IT,DT,RINIT,PEST+EP1*PEST)
      DO 4350 J=1,IT
         SEN(J)=(RB(J)-RC(J))/(EP1*PEST)
```

```
4350   CONTINUE
      DO 4360 J=1,ICOUNT
         ITM=INT(TMA(J)/DT)+1
         IF (ITM.GE.IT) THEN
            SEN(J)=SEN(ITM)
         ELSE
            DSEN=(SEN(ITM+1)-SEN(ITM))*(TMA(J)-(ITM-1)*DT)/DT
            SEN(J)=SEN(ITM)+DSEN
         END IF
4360  CONTINUE
      SENSUM=0.
      DO 4365 I=1,ICOUNT
         SENSUM=SENSUM+SEN(I)**2
4365  CONTINUE
C----
C     DUMPING SUMMARY INPUT PARAMETERS, INPUT DATA AND RESULTING
C     PEST, SDP, SUMIN TO 'O_SMRY.DAT'
C----
      OPEN(14,FILE='O_SMRY.DAT')
      WRITE(14,*)
      WRITE(14,4366)
4366  FORMAT(//,'**********************  THIS IS FILE O_SMRY.DAT'
     .         '  **********************',//)
      WRITE(14,4367)
4367  FORMAT(/,'THE INPUT PARAMETERS AND DATA WERE:',//)
      WRITE(14,2190)RL1,RL2,LIP,D2,D1,H,CINIT,CINF,VINA,RMAG
      WRITE(14,*)
      WRITE(14,2240)DT,TM0,TM1,DP,P0,P1,PSEN
      IF(IRELPSE.EQ.1) THEN
        WRITE(14,*)
        WRITE(14,*)'THE DATA POINTS ENTERED WERE:'
        WRITE(14,2259) DELAY
        WRITE(14,*)
        WRITE(14,*)'  J       TIME(J)      RADIUS(J)'
        IF(IMICCEN.EQ.1) THEN
        WRITE(14,*)'           (SEC.)      (MICRONS)'
        ELSEIF(IMICCEN.EQ.2) THEN
        WRITE(14,*)'           (SEC.)      (CENTIMETERS)'
        ENDIF
        DO 4368 J=1,ICOUNT+1
            WRITE(14,2263) J,TMA(J),RA(J)
4368    CONTINUE
      ELSE IF(IRELPSE.EQ.2) THEN
        WRITE(14,*)
        WRITE(14,2320)PTRU,RINIT,XMICCEN,DR,XMICCEN,DT1,DT2,TM01
      ENDIF
C
C----
C     THE ESTIMATED PERMEABILITY
C----
      WRITE(10,4375)
      WRITE(14,4375)
```

```
4375  FORMAT(///,'********************  THE RESULTING '
     .           'PERMEABILITY  ********************',/)
      SDP=SQRT(SUMIN/(ICOUNT-1)/SENSUM)
      WRITE(10,4380)PEST
      WRITE(14,4380)PEST
4380  FORMAT(/,'THE LOCAL MINIMUN OCCURS AT P =',F8.3,' MICRONS/SEC.')
      WRITE(10,4390)SDP
      WRITE(14,4390)SDP
4390  FORMAT(/,'THE STANDARD DEVIATION OF ESTIMATED P IS  ',E8.3)
      WRITE(10,4395)SUMIN
      WRITE(14,4395)SUMIN
4395  FORMAT(/,'THE MINIMUM VALUE OF SUM IS',F8.3)
C
      CLOSE(14)
C
      IF(IRELPSE.EQ.1) THEN
          DO 4396 J=1,IT
             IF(J.EQ.1) RAMAXC = RC(1)
             IF(J.GE.2.AND.RC(J).GT.RAMAXC) RAMAXC=RC(J)
4396      CONTINUE
          DO 4397 J=1,IT
             VOLC(J) = (RC(J)/RAMAXC)**3
4397      CONTINUE
      ENDIF
C
      IF(IF3.EQ.0) GO TO 4398
C-----
C     PLOTTING NORMALIZED VOLUME CHART (IF3=0 STOP THE OUTPUT)
C-----
      CALL NEWPAG
      CALL PLOT(TM0,TM1,10,VOL0,VOL1,10,TMA,VOLA,ICOUNT,FCT0,1,3)
      CALL PLOT(TM0,TM1,10,VOL0,VOL1,10,TMS,VOLC,IT,FCT0,5,3)
      CALL ANMODE
      CALL HOME
      WRITE(1,*)
      WRITE(1,*)'PRESS [RETURN] TO CONTINUE.'
      READ(1,'(A1)') IMORE
C
4398  CONTINUE
C
      IF(IF4.EQ.0)GO TO 6000
C-----
C     PLOTTING SUM OF SQUARE OF ERRORS (IF4=0 STOP THE OUTPUT)
C-----
      CALL NEWPAG
      CALL PLOT(P0,P1,10,SUM0,SUM1,10,P,SUM,IP,FCT0,5,4)
      CALL ANMODE
      CALL HOME
      WRITE(1,*)
      WRITE(1,*)'PRESS [RETURN] TO CONTINUE.'
      READ(1,'(A1)') IMORE
6000  CONTINUE
```

```
      WRITE(1,6100) PEST,SDP,SUMIN
6100  FORMAT(' ESTIMATED PERMEABILITY = ',F8.3,
     .       ' STANDARD DEVIATION = ',E8.3,
     .       ' MINIMUM SUM SQUARES = ',F8.3)
      WRITE(1,*)
      WRITE(1,*)'PRESS [RETURN] TO CONTINUE'
      READ(1,'(A1)') IMORE
C-----
C     RESETTING THE RA(I) = RSAVE(I) AND RINIT, RAMAX TO ORIGINAL VALUESS
C-----
      IF(IMICCEN.EQ.1) THEN
         RINIT = RINIT * RMAG
         RAMAX = RAMAX * RMAG
         IF(IRELPSE.EQ.2) DR = DR *RMAG
         DO 280 K = 1, ICOUNT+1
             RA(K) = RSAVE(K)
280      CONTINUE
      ELSE IF(IMICCEN.EQ.2) THEN
         RINIT = RINIT *RMAG/10000.
         RAMAX = RAMAX *RMAG/10000.
         IF(IRELPSE.EQ.2) DR = DR*RMAG/10000.
         DO 290 K=1,ICOUNT+1
             RA(K) = RSAVE(K)
290      CONTINUE
      ENDIF
C-----
C     OPTION TO SAVE INPUT FILE
C-----
      DELAY = 0.0
      WRITE(1,*)
      WRITE(1,*)'DO YOU WISH TO SAVE THE INPUT DATA IN A FILE,'
      WRITE(1,*)'(Y/N)?'
      READ(1,'(A1)') ISAVE
      CALL IYESNO(ISAVE)
      IF(ISAVE.EQ.'Y') THEN
320       CONTINUE
         WRITE(1,*)
         WRITE(1,*)'WHAT WOULD YOU LIKE TO NAME THIS FILE,'
         WRITE(1,*)'(ENTER NO MORE THAN 10 CHARATERS)?'
         READ(1,'(A10)',ERR=320) NAMFIL
         OPEN(12,FILE=NAMFIL)
         WRITE(12,325)
325      FORMAT(1X,'THIS IS THE FILE YOU HAD SAVED.  YOU CAN '
     .    ,'USE THIS FILE AS',/,' AN INPUT FILE IF YOU DESIRE BY'
     .   ,' ACCESSING THIS FILE',/,' WHEN YOU ARE PROMPTED FOR'
     .   ,' THE NAME OF AN INPUT FILE.')
331      CONTINUE
         WRITE(12,*)'RL1         RL2         LIP'
         WRITE(12,332) RL1,RL2,LIP
332      FORMAT(1X,F5.2,6X,F7.2,4X,I2,/)
         WRITE(12,*)'D1          D2          H'
         WRITE(12,333) D1,D2,H
```

```
333      FORMAT(1X,E9.3,2X,E9.3,2X,E9.3,/)
         WRITE(12,*)'CINIT      CINF'
         WRITE(12,334) CINIT,CINF
334      FORMAT(1X,F8.3,3X,F8.3,/)
         WRITE(12,*)'VINA       RMAG'
         WRITE(12,335) VINA,RMAG
335      FORMAT(1X,F5.2,6X,F9.2,/)
         WRITE(12,*)'DT         TM0          TM1         DELAY'
         WRITE(12,336) DT,TM0,TM1,DELAY
336      FORMAT(1X,F6.3,5X,F7.2,4X,F7.2,4X,F7.2,/)
         WRITE(12,*)'DP         PSEN'
         WRITE(12,337) DP,PSEN
337      FORMAT(1X,F5.2,6X,F6.2,/)
         WRITE(12,*)'RINIT'
         WRITE(12,338) RINIT
338      FORMAT(1X,E10.4,/)
         WRITE(12,339)
339      FORMAT(1X,'THE PARAMETERS FOR THE LINE BELOW ARE FOR ',
     .  'SIMULATION OPTION:')
         WRITE(12,*)'PTRU       DR          TM01        DT1        DT2'
         WRITE(12,340) PTRU,DR,TM01,DT1,DT2
340      FORMAT(1X,F6.2,5X,F7.3,4X,F7.2,4X,F6.3,5X,F6.3,/)
         WRITE(12,341)
341      FORMAT(1X,'THESE PARAMETERS BELOW ARE FOR DATA INPUT '
     .   ,'CONTROL:')
         WRITE(12,*)'IRELPSE    IMICCEN'
         WRITE(12,342) IRELPSE, IMICCEN
342      FORMAT(2X,I1,9X,I1,/)
         WRITE(12,*)'THESE PARAMETERS ARE FOR DATA OUTPUT CONTROL:'
      WRITE(12,*)'IF1         IF2         IF3         IF4         IPRINT'
         WRITE(12,345) IF1,IF2,IF3,IF4,IPRINT
345      FORMAT(1X,I1,10X,I1,10X,I1,10X,I1,10X,I2,/)
         WRITE(12,346)
346      FORMAT(1X,'THESE PARAMETERS ARE FOR GRAPHICAL OUTPUT ',
     .   'CONTROL:')
         WRITE(12,*)'CAB0       CAB1'
         WRITE(12,347) CAB0,CAB1
347      FORMAT(1X,F5.2,6X,F7.2,/)
         WRITE(12,*)'SEN0       SEN1'
         WRITE(12,347) SEN0, SEN1
         WRITE(12,*)'VOL0       VOL1'
         WRITE(12,347) VOL0,VOL1
         WRITE(12,*)'P0         P1          SUM0        SUM1'
         WRITE(12,348) P0,P1,SUM0,SUM1
348      FORMAT(1X,F6.2,5X,F6.2,5X,F5.2,6X,F6.2,5X,/)
         WRITE(12,349)
349      FORMAT(1X,'THE LAST GROUP BELOW IS THE DATA POINTS:')
         WRITE(12,*)'  TMA(I)          RA(I)'
         DO 360 I =1,ICOUNT
            WRITE(12,350) TMA(I), RA(I)
350         FORMAT(1X,F9.4,5X,F7.2)
360      CONTINUE
```

```
         WRITE(12,*)'  0.0            0.0'
         CLOSE(12)
         IF(IQUIT.EQ.'Y') GO TO 600
         WRITE(1,362) NAMFIL
362      FORMAT('OKAY, THE FILE ',A10,' HAS BEEN SAVED.')
      ENDIF
      CLOSE(11)
C-----
C     OPTION TO RUN THE PROGRAM AGAIN
C-----
      WRITE(1,*)
      WRITE(1,*)'DO YOU WISH TO RUN THE PROGRAM AGAIN, (Y/N)?'
      READ(1,'(A1)') IAGAIN
      CALL IYESNO(IAGAIN)
      IOPNAG = IOPNAG + 1
      IF(IAGAIN.EQ.'Y') THEN
500       CONTINUE
C-----
C     OPTION TO ENTER NEW DATA OR REVIEW OLD DATA
C-----
          WRITE(1,*)
          WRITE(1,*)'DO YOU WISH TO (1) ENTER ALL NEW DATA OR '
          WRITE(1,*)'(2) USE AND REVIEW THE DATA ALREADY ENTERED,'
          WRITE(1,*)'(ENTER 1 OR 2)?'
          READ(1,*,ERR=500) INEWREV
          CALL IONETWO(INEWREV)
          DELAY = 0.0
          IBACK = 0
          IF(INEWREV.EQ.1) THEN
              IRUNAG = 0
              GO TO 2000
          ELSE IF(INEWREV.EQ.2) THEN
              ITERINP = 1
              IRUNAG = IRUNAG + 1
              GO TO 2180
          ENDIF
      ENDIF
C-----
C     STOPPING THE PROGRAM
C-----
600       WRITE(1,*)
          WRITE(1,*)'OKAY, PROGRAM DONE.'
          CALL CLOSTK(I)
          CLOSE(10)
          CALL EXIT
      END
C-----
C     SUBROUTINE RGKT(X,Y,N,DX,YO,P)
C
C     USE RUNGE-KUTTA METHOD TO SOLVE ORDINARY DIFFERENTIAL
C     EQUATION
C
```

```
C     X: INDEPENDENT VARIABE
C
C     Y: DEPENDENT VARIABLE
C
C     N: DIMENSION OF X(N) AND Y(N)
C
C     DX: INCREMENT OF X
C
C     YO: INITIAL CONDITION OF Y
C
C     P: PARAMETER
C
C     F: THE SUPLLIED FUNCTION.  (DY/DX=F(X,Y))
C-----
      SUBROUTINE RGKT(X,Y,N,DX,YO,P)
      DIMENSION X(N),Y(N)
      Y(1)=YO
      DO 1 I=1,N-1
         RK1=DX*F(X(I),Y(I),P)
         RK2=DX*F(X(I)+DX/2.,Y(I)+RK1/2.,P)
         RK3=DX*F(X(I)+DX/2.,Y(I)+RK2/2.,P)
         RK4=DX*F(X(I)+DX,Y(I)+RK3,P)
         Y(I+1)=Y(I)+(RK1+2*RK2+2*RK3+RK4)/6.
1     CONTINUE
      RETURN
      END
C-----
C     FUNCTION F(X,Y,Z)
C
C     X: INDEPENDENT VARIABLE
C
C     Y: DEPENDENT VARIABLE
C
C     Z: PARAMETER
C-----
      FUNCTION F(X,Y,Z)
      PARAMETER N4=301,PI=3.14159
      COMMON /C2/RINIT,VINA,DT,TM1,COUT(N4)
      COMMON /C3/RL1,RL2,LIP,D1,D2,CINIT,CINF,H
      V=0.018
      I=INT(X/DT)+1
      CO=COUT(I)+(COUT(I+1)-COUT(I))*(X-(I-1)*DT)/DT
      VO=4.*PI*RINIT**3/3.
      VIN=VINA*VO/100.
      F=-Z*V*(CO-CINIT*(VO-VIN)/(4.*PI*Y**3/3.-VIN))
      RETURN
      END
C-----
C     FUNCTION RANND()
C
C     RANDOM VARIABLE GENERATOR
C     NORMAL DISTRIBUTION WITH STANDARD DEVIATION EQUAL TO 1.
```

```
C-----
      FUNCTION RANND()
      DOUBLE PRECISION RANDOM
      R=RANDOM()
      A0=2.30753
      A1=0.27061
      B1=0.99299
      B2=0.04481
      IF (R-0.5) 10,10,20
10    AK=1.
      GO TO 30
20    AK=-1.
      R=R-0.5
30    T=SQRT(ALOG(1./(R*R)))
      E=T-(A0+A1*T)/(1.+B1*T+B2*T*T)
      RANND=AK*E
      RETURN
      END
C
      SUBROUTINE IONETWO(ITEST)
10    CONTINUE
      IF(ITEST.LE.0.OR.ITEST.GE.3) THEN
         WRITE(1,*)
         WRITE(1,*)'**  INCORRECT RESPONSE **'
         WRITE(1,*)'PLEASE ENTER  1  OR  2'
         WRITE(1,*)
         READ(1,*) ITEST
         GO TO 10
      ENDIF
      RETURN
      END
C
      SUBROUTINE IYESNO(ITEST)
      CHARACTER *1 ITEST
10    CONTINUE
      IF(ITEST.NE.'Y'.AND.ITEST.NE.'N') THEN
         WRITE(1,*)
         WRITE(1,*)'**  INCORRECT RESPONSE **'
         WRITE(1,*)'PLEASE ENTER  "Y"  OR  "N"'
         WRITE(1,*)
         READ(1,'(A1)') ITEST
         GO TO 10
      ENDIF
      RETURN
      END
C
      SUBROUTINE CHANLIM(Z0,Z1)
      CHARACTER *1 ICHANGE
10    CONTINUE
      WRITE(1,*)
      WRITE(1,*)'THE VALUES ENTERED ARE:  '
      WRITE(1,*)
```

```
      WRITE(1,*) Z0, Z1
      WRITE(1,*)
      WRITE(1,*)' DO YOU WISH TO CHANGE THEM,(Y/N)? '
      READ(1,'(A1)') ICHANG
      CALL IYESNO(ICHANG)
      IF(ICHANG.EQ.'Y') THEN
         WRITE(1,*)
20       CONTINUE
         WRITE(1,*)'PLEASE ENTER THE NEW VALUES. '
         WRITE(1,*)
         READ(1,*,ERR=20) Z0,Z1
         GO TO 10
      ENDIF
      RETURN
      END
      SUBROUTINE INCORRES
      WRITE(1,*)
      WRITE(1,*)'**  INCORRECT RESPONSE  **'
      RETURN
      END
C
      SUBROUTINE CHANNAM(FILNAM)
      CHARACTER*1 ICHANG
      CHARACTER*10 FILNAM
33    CONTINUE
      WRITE(1,*)
      WRITE(1,35) FILNAM
35    FORMAT(1X,'THE FILE NAME ENTERED IS ',A10)
      WRITE(1,*)
      WRITE(1,*)'DO YOU WISH TO CHANGE IT, (Y/N)?'
      READ(1,'(A1)') ICHANG
      CALL IYESNO(ICHANG)
      IF(ICHANG.EQ.'Y') THEN
          WRITE(1,*)
36        CONTINUE
          WRITE(1,*)'PLEASE ENTER THE NEW NAME. '
          WRITE(1,*)
          READ(1,'(A10)',ERR=36) FILNAM
          GO TO 33
      ENDIF
      RETURN
      END
```

```
      SUBROUTINE MBCON(IOPNAG)
C-----
C     JOB: 1. PREDICT THE CONCENTRATION CHANGE INSIDE THE CELL
C .           CHAMBER
C          2. PLOTTING CONCENTRATION VERSUS TIME CHART
C-----
      PARAMETER (N1=4,N2=6,II=2,N4=301)
      REAL L1,L2,M
      CHARACTER*1 ICONT
      DIMENSION CN(N1+N2+1),CO(N1+N2+1),CE(N1+N2+1,N1+N2+1),
     &          W(N1+N2+2,N1+N2+2),CONC(N1+N2+1),CA(25),CB(25),
     &          DC(25),TM(25),X(II,II),Y1(II),Y2(II),W1(II+1,II+1)
      COMMON /C1/IPRINT,IF1,A1(II),A2(II),CAB0,CAB1
      COMMON /C2/RINIT,VINA,DT,TM1,COUT(N4)
      COMMON /C3/RL1,RL2,LIP,D1,D2,CINIT,CINF,H
      EXTERNAL FCT1,FCT2
C-----
C     INITIALIZATION
C-----
      L1=RL1*1.0E-6
      L2=RL2*1.0E-6
      TMAX=TM1
      DX1=L1/N1
      DX2=L2/N2
      RX=DX2/DX1
      P1=DT*D1/DX1/DX1
      P2=DT*D2/DX2/DX2
      B1=H*DX1/D1
      M=2./(1+RX)
      DO 1 I=1,N1+N2+1
         CN(I)=0.
         CO(I)=0.
         DO 1 J=1,N1+N2+1
            CE(I,J)=0.
1     CONTINUE
      ICOUNT=0
C-----
C     USING THE BACKWARD DIFFERENCE METHOD TO CALCULATE THE
C     CONCENTRATION INSIDE THE CELL CHAMBER
C-----
      WRITE(10,151)
151   FORMAT(///,1X,'************************  THE CONCENTRATION',
     .           ' HISTORY  ************************')
C-----
C        INPUT VALUES TO THE COEFFICIENT MATRIX
C-----
      CE(1,1)=1+2*P1+2*P1*B1
      CE(1,2)=-2*P1
      DO 2 I=2,N1
         CE(I,I-1)=-P1
         CE(I,I)=1+2*P1
         CE(I,I+1)=-P1
```

```
2     CONTINUE
      CE(N1+1,N1)=-P1*M
      CE(N1+1,N1+1)=1+P1*M+P1*M*(D2/D1)/RX
      CE(N1+1,N1+2)=-P1*M*(D2/D1)/RX
      DO 3 I=N1+2,N1+N2
         CE(I,I-1)=-P2
         CE(I,I)=1+2*P2
         CE(I,I+1)=-P2
3     CONTINUE
      CE(N1+N2+1,N1+N2)=-2*P2
      CE(N1+N2+1,N1+N2+1)=1+2*P2
6     CO(1)=CO(1)+2*B1*P1
C-----
C     CALCULATE THE COEFFICIENT MATRIX
C-----
      CALL LINEQ(CN,CO,CE,W,N1+N2+1,N1+N2+2,I)
C-----
C     PUT CN INTO CO FOR NEXT CALCULATION
C-----
      ICOUNT=ICOUNT+1
      DO 4 I=1,N1+N2+1
         CO(I)=CN(I)
4     CONTINUE
      COUT(ICOUNT)=CN(LIP)*(CINF-CINIT)+CINIT
C     COUT(ICOUNT)=CINF+(CINIT-CINF)*EXP(-(ICOUNT-1)*DT/19.6)
C-----
C     CHECK TO SEE WHETHER IT IS TIME TO OUTPUT THE DATA
C-----
      IF (ICOUNT/IPRINT*IPRINT.EQ.ICOUNT) THEN
         WRITE(10,101)ICOUNT*DT
101      FORMAT(/,'CONCENTRATION DIST. AT TIME=',F10.4,'SEC. IS',/)
         DO 5 I=1,N1+N2+1
            CONC(I)=CN(I)*(CINF-CINIT)+CINIT
5        CONTINUE
      WRITE(10,*)
      WRITE(10,*)'    DIALYSIS MEMBRANE          CELL CHAMBER'
      WRITE(10,*)'  |                    |                              |'
      WRITE(10,*)'B |                    |                              |'
      WRITE(10,*)'U |                    |                              |'
      WRITE(10,*)'L |                    |                              |'
      WRITE(10,*)'K |                    |                              |'
      WRITE(10,*)'  |                    |                              |'
      WRITE(10,*)'  1         3         5         7         9        11'
      WRITE(10,201) CONC(1),CONC(3),CONC(5),CONC(7),CONC(9),CONC(11)
201   FORMAT(1X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3)
      WRITE(10,*)'  |                    |                              |'
      WRITE(10,*)'  |    2         4     |     6         8        10    |'
      WRITE(10,202) CONC(2),CONC(4),CONC(6),CONC(8),CONC(10)
202   FORMAT(3X,E9.3,2X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3)
      WRITE(10,*)'F |                    |                              |'
      WRITE(10,*)'L |                    |                              |'
      WRITE(10,*)'O |                    |                              |'
```

```
      WRITE(10,*)'W |                    |                              |'
      WRITE(10,*)
      WRITE(10,*)
         IP=ICOUNT/IPRINT
         IF (CN(N1+1).GE.1.) CN(N1+1)=1.-1.E-6
         IF (CN(N1+N2+1).GE.1.) CN(N1+N2+1)=1.-1.E-6
         CA(IP)=-LOG(1-CN(N1+1))
         CB(IP)=-LOG(1-CN(N1+N2+1))
         DC(IP)=CONC(N1+1)-CONC(N1+N2+1)
         TM(IP)=ICOUNT*DT/TMAX
      END IF
C-----
C     CHECK TO SEE WHETHER IT IS TIME TO STOP THE EXECUTION
C-----
      IF (ICOUNT*DT.LT.TMAX+DT) GO TO 6
C-----
C     FINDING THE BEST LINEAR FIT FOR THE CONCENTRATION VERSUS
C     TIME POINTS
C-----
      DO 10 I=1,2
         X(I,2)=0.
         Y1(I)=0.
         Y2(I)=0.
10    CONTINUE
      DO 11 I=1,IP
         X(1,2)=X(1,2)+TM(I)
         X(2,2)=X(2,2)+TM(I)**2
         Y1(1)=Y1(1)+CA(I)
         Y1(2)=Y1(2)+CA(I)*TM(I)
         Y2(1)=Y2(1)+CB(I)
         Y2(2)=Y2(2)+CB(I)*TM(I)
11    CONTINUE
      X(2,1)=X(1,2)
      X(1,1)=IP
      CALL LINEQ(A1,Y1,X,W1,2,3,I)
      CALL LINEQ(A2,Y2,X,W1,2,3,I)
      WRITE(10,103)A1(1),A1(2)
103   FORMAT(/,' THE EQUATION FOR THE LINEAR BEST FIT FOR THE',/,
     .       ' CONCENTRATION VERSUS TIME IS,',/,
     .       ' Y = ',F6.3,' + ',F6.3,' *X',/)
      WRITE(10,103)A2(1),A2(2)
      IF(IOPNAG.EQ.0) CALL PLOTINIT
      IF (IF1.EQ.0) GO TO 12
C-----
C     PLOTTING THE CONCENTRATION VERSUS TIME CHART
C     (IF1=0 STOP THE OUTPUT)
C-----
      CALL PLOT(0.,1.,10,CAB0,CAB1,10,TM,CA,IP,FCT1,1,1)
      CALL PLOT(0.,1.,10,CAB0,CAB1,10,TM,CB,IP,FCT2,2,0)
      CALL ANMODE
      CALL HOME
      WRITE(1,*)
```

```
      WRITE(1,*)'PRESS [RETURN] TO CONTINUE.'
      READ(1,'(A1)') ICONT
12    RETURN
      END
C-----
C     FUNCTIONS FOR THE INPUT OF THE SUBROUTINE 'PLOT'
C-----
      FUNCTION FCT1(X)
      PARAMETER II=2
      COMMON /C1/IPRINT,IF1,A1(II),A2(II),CAB0,CAB1
      FCT1=A1(1)+A1(2)*X
      RETURN
      END
      FUNCTION FCT2(X)
      PARAMETER II=2
      COMMON /C1/IPRINT,IF1,A1(II),A2(II),CAB0,CAB1
      FCT2=A2(1)+A2(2)*X
      RETURN
      END
```

```
C-----
C     OPEN THE GRAPHIC FILE
C-----
      SUBROUTINE PLOTINIT
      CALL INITT(480)
      CALL OPENTK('G_PLOT',I)
      RETURN
      END
C-----
C     SUBROUTINE PLOT(X0,X1,NX,Y0,Y1,NY,X,Y,N,FCT,IMARK,IFX)
C
C     THIS SUBROUTINE PLOT THE GRAPH WITH WINDOW X0,X1,Y0,Y1.
C
C     X0, X1: RANGE ON X-AXIS
C
C     Y0, Y1: RANGE ON Y-AXIS
C
C     NX: NUMBER OF SCALE MARK ON X-AXIS
C
C     NY: NUMBER OF SCALE MARK ON Y-AXIS
C
C     X, Y: THE SUPPLIED DATA POINTS TO BE PLOTTED ON THE GRAPH
C
C     N: TOTAL NUMBER OF DATA POINTS
C
C     FCT: SUPPLIED FUNCTION TO COMPARE WITH THE DATA POINTS
C          (MIGHT BE THE EXACT SOLUTION CURVE)
C
C     IMARK: SELECT THE KIND OF SYMBOL TO MARK THE DATA POINTS.
C
C            1. SQUARE
C            2. TRIANGLE (POINTS UPWARD)
C            3. TRIANGLE (POINTS DOWNWARD)
C            4. DIAMAND SHAPE
C            5. CONTINUOUS CURVE
C
C      IFX: GRAPH TO BE PLOTTED.
C
C          1.  CONCENTRATION  V.S.  DIMENSIONLESS TIME
C          2.  SENSITIVITY COEFFICIENT  V.S.  TIME
C          3.  NORMALIZED VOLUME  V.S.  TIME
C          4.  SUM OF THE SQUARES OF THE ERRORS  V.S.  PERMEABILITY
C
C-----
      SUBROUTINE PLOT(X0,X1,NX,Y0,Y1,NY,X,Y,N,FCT,IMARK,IFX)
      PARAMETER IN=1,EP1=1E-15
      DIMENSION X(N),Y(N)
      CHARACTER*10 LABEL(2,21)
      CHARACTER*24 YTITLE
      CHARACTER*1 A
      RNX=NX
      RNY=NY
```

```
      RGX=X1-X0
      RGY=Y1-Y0
      IF (ABS(RGX).LT.EP1.OR.ABS(RGY).LT.EP1) THEN
         WRITE(1,102)
102      FORMAT(/,'THE SIZE OF THE WINDOW IS ZERO',/)
         GO TO 11
      END IF
      CALL DWINDO(0.5*(X1+X0)-RGX,0.5*(X1+X0)+RGX,0.5*(Y1+Y0)
     &-RGY,0.5*(Y1+Y0)+RGY)
C-----
C     DRAW HORIZONTAL AND VERTICAL GRID TICKS
C
C        NOTICE TYPE CONVERSION IN THE STATEMENTS INVOLVING
C        RNX AND RNY
C-----
      CALL MOVEA(X0,Y0)
      CALL DRAWA(X1,Y0)
      DO 1 I=2,NX+1
         P=(I-1.)*RGX/RNX+X0
         CALL MOVEA(P,Y0)
         CALL DRAWA(P,Y0+RGY/20)
1     CONTINUE
      CALL MOVEA(X0,Y0)
      CALL DRAWA(X0,Y1)
      DO 2 I=2,NY+1
         Q=(I-1.)*RGY/RNY+Y0
         CALL MOVEA(X0,Q)
         CALL DRAWA(X0+RGX/20,Q)
2     CONTINUE
C-----
C     WRITING CHARACTERS
C-----
      DO 3 I=1,NX+1,2
         P=(I-1.)*RGX/RNX+X0-RGX/15.
         Q=(I-1.)*RGX/RNX+X0
         CALL MOVEA(P,Y0-RGY/9.)
         WRITE(LABEL(1,I),101) Q
         CALL CHARTK(LABEL(1,I),0.7)
3     CONTINUE
      DO 4 I=1,NY+1,2
         P=(I-1.)*RGY/RNY+Y0
         CALL MOVEA(X0-RGX/6.,P)
         WRITE(LABEL(2,I),101) P
101      FORMAT(E8.2)
         CALL CHARTK(LABEL(2,I),0.7)
4     CONTINUE
C
C-----
C     LABELLING THE AXES
C-----
      CALL MOVEA(X0+(RGX*0.25),Y0-3.*(RGY/10.))
      IF(IFX.EQ.1) THEN
```

```
         CALL CHARTK('DIMENSIONLESS TIME',0.85)
      ELSE IF(IFX.EQ.2.OR.IFX.EQ.3) THEN
         CALL CHARTK('TIME (SEC.)',0.85)
      ELSE IF(IFX.EQ.4) THEN
         CALL CHARTK('PERMEABILITY (UM/SEC.)',0.85)
      ENDIF
      IF(IFX.EQ.1) THEN
         YTITLE = 'CONCENTRATION'
         NCHAR = 13
      ELSE IF(IFX.EQ.2) THEN
         YTITLE = 'SENSITIVITY COEFFICIENT'
         NCHAR = 23
      ELSE IF(IFX.EQ.3) THEN
         YTITLE = 'NORMALIZED VOLUME'
         NCHAR = 17
      ELSE IF(IFX.EQ.4) THEN
         YTITLE = 'SUM OF SQUARES OF ERRORS'
         NCHAR = 24
      ENDIF
C
      IF(IFX.EQ.1.OR.IFX.EQ.2.OR.IFX.EQ.3.OR.IFX.EQ.4) THEN
         DO 200 I=1,NCHAR
             AINDEX = I-1
             YYY = (Y0+RGY)-(AINDEX*RGY*0.0533)
            CALL MOVEA((X0-0.3*RGX),YYY)
            A = YTITLE(I:I)
            CALL CHARTK(A,0.85)
200      CONTINUE
      ENDIF
C-----
C     PLOTTING THE CURVES
C-----
      CALL MOVEA(X0,FCT(X0))
      DO 5 I=1,IN*N
         XF=X0+I*RGX/IN/N
         CALL DRAWA(XF,FCT(XF))
5     CONTINUE
      IF (IMARK.EQ.1) THEN
         DO 6 I=1,N
            CALL SQUARE(X(I),Y(I),RGX/60,RGY/60)
6        CONTINUE
      ELSE IF (IMARK.EQ.2) THEN
           DO 7 I=1,N
              CALL TRI(X(I),Y(I),RGX/60,RGY/60)
7          CONTINUE
      ELSE IF (IMARK.EQ.3) THEN
           DO 8 I=1,N
              CALL TRI2(X(I),Y(I),RGX/60,RGY/60)
8          CONTINUE
      ELSE IF (IMARK.EQ.4) THEN
           DO 9 I=1,N
              CALL DIAMAND(X(I),Y(I),RGX/60,RGY/60)
```

```
9          CONTINUE
      ELSE IF (IMARK.EQ.5) THEN
           CALL MOVEA(X0,Y0)
           DO 10 I=1,N
              CALL DRAWA(X(I),Y(I))
10         CONTINUE
      END IF
C
      CALL MOVEA(X0,0.5*(Y1+Y0)+RGY)
      CALL DRAWA(X0+0.0000000001,0.5*(Y1+Y0)+RGY)
C
11    RETURN
      END
C-----
C     SUBROUTINE SQUARE(X,Y,DX,DY)
C
C     X, Y: POSITION TO PLACE THIS MARK
C
C     DX, DY: SIZE OF THIS MARK
C-----
      SUBROUTINE SQUARE(X,Y,DX,DY)
      CALL MOVEA(X-DX/2,Y-DY/2)
      CALL DRAWA(X-DX/2,Y+DY/2)
      CALL DRAWA(X+DX/2,Y+DY/2)
      CALL DRAWA(X+DX/2,Y-DY/2)
      CALL DRAWA(X-DX/2,Y-DY/2)
      RETURN
      END
C-----
C     SUBROUTINE TRI(X,Y,DX,DY)
C-----
      SUBROUTINE TRI(X,Y,DX,DY)
      CALL MOVEA(X-DX/2,Y-DY/2)
      CALL DRAWA(X,Y+DY/2)
      CALL DRAWA(X+DX/2,Y-DY/2)
      CALL DRAWA(X-DX/2,Y-DY/2)
      RETURN
      END
C-----
C     SUBROUTINE TRI2(X,Y,DX,DY)
C-----
      SUBROUTINE TRI2(X,Y,DX,DY)
      CALL MOVEA(X-DX/2,Y+DY/2)
      CALL DRAWA(X,Y-DY/2)
      CALL DRAWA(X+DX/2,Y+DY/2)
      CALL DRAWA(X-DX/2,Y+DY/2)
      RETURN
      END
C-----
C     SUBROUTINE DIANAMD(X,Y,DX,DY)
C-----
      SUBROUTINE DIAMAND(X,Y,DX,DY)
```

```
      CALL MOVEA(X,Y+DY/2)
      CALL DRAWA(X-DX/2,Y)
      CALL DRAWA(X,Y-DY/2)
      CALL DRAWA(X+DX/2,Y)
      CALL DRAWA(X,Y+DY/2)
      RETURN
      END
C-----
C     FUNCTION FCT0(X)
C
C     TRIVIAL CURVE, IT PLOTS A STRAIGHT LINE AT Y=0.
C-----
      FUNCTION FCT0(X)
      FCT0=0.
      RETURN
      END
```

APPENDIX E

SENS - The IBM PC Version Fortran Source Code

```
^H                                                                    Page
1

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
      1       PROGRAM SENS
      2 C$DEBUG
      3 C-----
      4 C     THIS PROGRAM INCLUDES THE COMPUTER MODEL FOR THE
      5 C     DIFFUSION CHAMBER AND THE PARAMETER ESTIMATION FOR FINDING
      6 C     PERMEABILITY OF A CELL INSIDE THE CELL CHAMBER OF THE
      7 C     DIFFUSION CHAMBER.
      8 C
      9 C     THIS PROGRAM CONSISTS OF 1 MAIN PROGRAM, 5 SUBROUTINES
     10 C     AND 2 FUNCTIONS.  THEY ALL ARE INSIDE THE FILES 'SENS.FOR',
     11 C     'MBCON.FOR'.
     12 C
     13 C     THE INPUT DATA CAN BE ENTERED ONE OF TWO WAYS, VIA THE
     14 C     TERMINAL/KEYBOARD OR BY USING A PRE-EXISTING INPUT FILE SET
     15 C     UP BY THE USER; FOR EXAMPLE 'I_SENS.DAT'.
     16 C
     17 C     THE OUTPUT CAN BE VIEWED BY (1) LOOKING AT THE TABLES GENERATED,
     18 C     WHICH RESIDE IN FILE 'O_SENS.DAT' AND 'O_SMRY.DAT', AND/OR (2)
     19 C     HAVING THE PROGRAM GENERATE FILES TO BE USED IN CONJUNCTION WITH
     20 C     PLOTIT TO VIEW THE OUTPUT GRAPHICALLY.
     21 C
     22 C     THE INPUT DATA REQUIRED IS AS FOLLOWS:
     23 C
     24 C     RL1: THICKNESS OF DIALYSIS MEMBRANE (M)
     25 C
     26 C     RL2: THICKNESS OF CELL CHAMBER (M)
     27 C
     28 C     LIP: APPROXIMATE LOCATION OF THE LIPOSOME (FROM 5 TO 11)
     29 C
     30 C     D1: DIFFUSIVITY OF SOLUTE INSIDE DIALYSIS MEMBRANE (M*M/SEC)
     31 C
     32 C     D2: DIFFUSIVITY OF SOLUTE INSIDE CELL CHAMBER (M*M/SEC)
     33 C
     34 C     CINIT: INITIAL CONCENTRATION (OSM)
     35 C
     36 C     CINF: FINAL CONCENTRATION (OSM)
     37 C
     38 C     H: MASS TRANSFER COEFF. (APPROXIMATELY 10000*D2)
     39 C
     40 C     IPRINT: NUMERICAL DATA OUTPUT FREQUENCY. (EVERY IPRINT*DT
     41 C            SEC. PRINTS THE CONC. DIST. ON OUTPUT FILE)
     42 C
     43 C     IRELPSE: OPTION FOR EXPERIMENTAL DATA INPUT 1=REAL-EXPERIMENT
     44 C                                                 2=PSUESO-EXPERIMENT
     45 C
     46 C     IMICCEN: OPTION FOR ENTERING DATA IN 1=MICRONS  2=CENTIMETERS
     47 C
```

```
     48 C     TM0, TM1: SETTING THE TIME RANGE ON THE PLOTS (SEC.)
     49 C
     50 C     DT: TIME STEP FOR PROCEEDING THE CALCULATION (SEC.)
     51 C         (NOTE: IF DT IS SET TOO LARGE, THE RESULT WILL FLUCTUATE.
     52 C          IN THIS CASE, REDUCE THE SIZE OF DT AND TRY AGAIN.
     53 C          THIS IS DUE TO THE UNSTABLE OF THE NUMERICAL METHOD.)
     54 C
     55 C     DELALY: TIME DELAY SUBTRACTED FROM TIME ARRAY TM(I).
     56 C
^H                                                                    Page
2
                                                                   07-20-87
                                                                   19:28:31
D Line# 1      7                          Microsoft FORTRAN77 V3.31 August 1985
     57 C     PSEN: THE PERMEABILITY VALUE AT WHICH WE INVESTIGATE THE
     58 C           SENSITIVITY COEFF. (P'S EFFECT ON R'S CHANGE)
     59 C
     60 C     PTRU: THE PERMEABILITY VALUE WITH WHICH THE PROGRAM GENERATES
     61 C           PSUDO-EXPERIMENTAL DATA (R(TM,PTRU)).
     62 C
     63 C     DT1: THE TIME STEP FOR THE PSUDO-EXPT'L DATA DURING TM0 TO
     64 C          TM01. (SEC.)
     65 C
     66 C     TM01: THE PARTITION BETWEEN TWO DIFFERENT TIME STEPS RANGE.
     67 C           (YOU CAN ASK THE PROGRAM TO GENERATES PSUDO-EXPT'L DATA
     68 C            WITH TWO DIFFERENT INCREMENT IN TIME FOR TWO TIME
     69 C            RANGE.)
     70 C
     71 C     DT2: THE TIME STEP FOR THE PSUDO-EXPT'L DATA DURING TM01
     72 C          TO TM1. (SEC.)
     73 C
     74 C
     75 C     RINIT: INITIAL RADIUS (MICRONS OR CM)
     76 C
     77 C     VINA: INACTIVE VOLUME (%)
     78 C
     79 C     RMAG: THE MAGNIFICATION OF THE MICROSCOPE
     80 C
     81 C     DR: MAGNITUDE FOR THE PSEUDO-RANDOMNESS IMPOSED ON THE
     82 C         PREDICTED RADIUS RESPONSE (MICRONS OR CM)
     83 C
     84 C     P0, P1: PERMEABILITY RANGE UNDER INVESTIGATION (MICRONS/SEC.)
     85 C
     86 C     DP: INCREMENT OF PERMEABILITY IN CALCULATING SUM OF ERROR OF
     87 C         SQUARE FOR EACH P VALUE
     88 C
     89 C     CON1:  NAME OF THE FILE CONTAINING  CA  V.S.
     90 C            DIMENSIONLESS TIME
     91 C
     92 C     CON2:  NAME OF THE FILE CONTAINING  CB  V.S.
     93 C            DIMENSEIONLESS TIME
     94 C
```

```
     95 C     SENC:  NAME OF THE FILE CONTAINING THE SENSITIVITY COEFFICIENTS V.
     96 C                                                                  TIME
     97 C
     98 C     IF IRELPSE = 1
     99 C
    100 C           VOL1:  NAME OF THE FILE CONTAINING THE NORMALIZED VOLUME V.
    101 C                  TIME (REAL EXPERIMENTAL DATA).
    102 C           VOL2:  NAME OF THE FILE CONTAINING THE NORMALIZED VOLUME V.
    103 C                  TIME (THEORETICAL CURVE BASED OF THE ESTIMATED
    104 C                  PERMEABILITY WHICH IS CALCULATED).
    105 C
    106 C     IF IRELPSE = 2
    107 C
    108 C           VOL1:  NAME OF THE FILE CONTAINING THE NORMALIZED VOLUME V.
    109 C                  TIME (WITH AN IMPOSED RANDOMNESS), BASED ON THE TRUE
    110 C                  PERMEABILITY ENTERED, PTRU.
    111 C           VOL2:  NAME OF THE FILE CONTAINING THE NORMALIZED VOLUME V.
    112 C                  TIME (NO RANDOMNESS), ALSO BASED ON PTRU.
^H                                                                        Page
3

                                                                      07-20-87
                                                                      19:28:31
D Line# 1    7                          Microsoft FORTRAN77 V3.31 August 1985
    113 C
    114 C     SUMR:  NAME OF THE FILE CONTAINING THE SUM OF THE SQUARE OF THE ER
    115 C            V.S. PERMEABILITY.
    116 C
    117 C     OUTPUT CONFIGURATION OF 'O_SENS.DAT':
    118 C
    119 C          (1) INPUT DATA
    120 C          (2) PRINT CONC. DIST. OF THE SYSTEM AS A FUNCTION OF
    121 C              TIME.
    122 C          (3) SENSITIVITY COEFF. CORRESPOND TO PSEN.
    123 C          (4) THE ESTIMATED PERMEABILITY (LOCAL MINIMUN ON SUM
    124 C              VERSUS P GRAPH)
    125 C          (5) THE STANDARD DEVIATION OF THIS ESTIMATED P
    126 C
    127 C     OUTPUT CONFIGURATION OF 'O_SMRY.DAT':
    128 C
    129 C          (1) SUMMARY OF INPUT PARAMETERS AND DATA
    130 C          (2) SUMMARY OF RESULTING PERMEABILITY, STANDARD DEVIATION
    131 C              AND MINIMUM SUM
    132 C
    133 C     THE OUTPUT FILES THAT CAN BE CREATED BY THE PROGRAM IF THE
    134 C     USER DESIRES.
    135 C
    136 C          THE FILES CREATED WILL BE COMPATABLE TO USE WITH
    137 C          PLOTIT USING FREE FORMAT.
    138 C
    139 C          (1) CONCENTRATION V.S. DIMENSIONLESS TIME
    140 C          (2) SENSITIVITY COEFFICIENTS V.S. TIME
    141 C          (3) NORMALIZED VOLUME V.S. TIME
```

```
    142 C          (4) SUM OF SQUARE OF ERRORS V.S. PERMEABILITY
    143 C
    144 C-----
    145 C     INITIALIZATION AND DECLARATIONS
    146 C-----
    147 $INCLUDE: 'IMSL'
      1 $LARGE: DMY327
      2       DIMENSION DMY327(1)
    148       PARAMETER (N3=301,II=2,N1=301,N4=301,EP1=0.01,N5=25)
    149       DIMENSION TMA(N3),TMB(N3),RA(N3),RB(N3),SEN(N3),SUM(N1),P(N1)
    150       DIMENSION VOLC(N3),VOLA(N3),TMS(N3),RS(N3),RC(N3),RSAVE(N3)
    151       DIMENSION CA(N5),CB(N5)
    152       CHARACTER*1 ICHANG, IGRAPH, IMORE, IAGAIN, IANOTH, ISAVE
    153       CHARACTER*1 IMISTAK, IFIRST, IQUIT, IDEL
    154       CHARACTER*11 XMICCEN,PROBLEM
    155       CHARACTER*10 NAMFIL,CON1,SENC,VOL1,SUMR,CON2,VOL2,ISENS
    156       COMMON /C1/IPRINT,IF1,A1(II),A2(II)
    157       COMMON /C2/RINIT,VINA,DT,TM1,COUT(N4)
    158       COMMON /C3/RL1,RL2,LIP,D1,D2,CINIT,CINF,H
    159       EXTERNAL F
    160       XSEED =566387.0
    161       ISC = 0
    162       IBACK = 0
    163       IRUNAG=0
    164       IOPNAG=0
    165       IMISTAK = 'N'
    166 C-----
^H                                                                    Page
4

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    167 C     EXPLANATION TO THE USER WHAT THE PROGRAM DOES.
    168 C-----
    169 2000  CONTINUE
    170       WRITE(*,*)
    171       WRITE(*,*)'WOULD YOU LIKE AN EXPLANATION OF THIS PROGRAM, '
    172       WRITE(*,*)'(SENS), (Y/N)?'
    173       READ(*,'(A1)') IFIRST
    174       IF(IFIRST.EQ.'N') GO TO 1200
    175       WRITE(*,*)
    176       WRITE(*,*)'   WELCOME TO THE PROGRAM SENS.  THIS PROGRAM WILL '
    177       WRITE(*,*)'ALLOW THE USER TO (1) ANALYZE THE DATA OBTAINED USING '
    178       WRITE(*,*)'THE MICROSCOPE DIFFUSION CHAMBER I.E. PARAMETER '
    179       WRITE(*,*)'ESTIMATION OF THE PERMEABILITY OF A CELL  OR  (2) RUN '
    180       WRITE(*,*)'A SIMULATION (PSEUDO) EXPERIMENT TO SEE WHAT MIGHT '
    181       WRITE(*,*)'TO A CELL UNDER SPECIFIED CONDITIONS.'
    182 C-----
    183 C     OPTION TO HAVE A LIST OF THE NECESSARY PARAMETERS SENT TO
    184 C     'I_DATA.LST'
    185 C-----
    186       WRITE(*,*)'   IF THIS IS THE FIRST TIME YOU HAVE USED THIS '
```

```
    187       WRITE(*,*)'PROGRAM AND YOU WANT TO ENTER DATA FROM A REAL '
    188       WRITE(*,*)'EXPERIMENT YOU MAY WANT TO OBTAIN A LIST OF THE '
    189       WRITE(*,*)'PARAMETERS AND DATA NECESSARY TO RUN THE PROGRAM.'
    190       WRITE(*,*)
    191       WRITE(*,*)'WOULD YOU LIKE TO DO THIS, (Y/N)?'
    192       READ(*,'(A1)') IFIRST
    193       CALL IYESNO(IFIRST)
    194       IF(IFIRST.EQ.'Y') THEN
    195           OPEN(13,FILE='I_DATA.LST',STATUS='NEW')
    196           WRITE(*,*)
    197           WRITE(*,*)'THE LIST OF THE NECESSARY INPUT TO RUN THE PROGRAM'
    198           WRITE(*,*)'WILL BE IN FILE "I_DATA.LST".  THE PROGRAM WILL'
    199           WRITE(*,*)'STOP NOW.  HAVE I_DATA.LST PRINT AT THE PRINTER.'
    200           WRITE(13,*)
    201           WRITE(13,*)'THE PARAMETES AND DATA NEEDED TO RUN THE PROGRAM'
    202           WRITE(13,*)'ARE:'
    203           WRITE(13,*)
    204           WRITE(13,*)'1) DIALYSIS MEMBRANE THICKNESS (RL1), MICRONS.'
    205           WRITE(13,*)'2) CELL CHAMBER THICKNESS (RL2), MICRONS.'
    206           WRITE(13,*)'3) CELL POSITION IN THE CELL CHAMBER (5-11).'
    207           WRITE(13,*)'4) DIFUSSIVITY OF SOLUTE IN MEMBRANE (D1), '
    208           WRITE(13,*)'                                  - METERS*METERS/SEC.'
    209           WRITE(13,*)'5) DIFUSSIVITY OF SOLUTE IN FREE SOLUTION (D2),'
    210           WRITE(13,*)'                                  - METERS*METERS/SEC.'
    211           WRITE(13,*)'6) MASS TRANSFER COEFFICIENT (H).'
    212           WRITE(13,*)'                                  - METERS/SEC.'
    213           WRITE(13,*)'7) INITIAL CONCENTRATION (CINIT), OSMOLALITY.'
    214           WRITE(13,*)'8) FINAL CONCENTRATION (CINF), OSMOLALITY.'
    215           WRITE(13,*)'9) INACTIVE VOLUME (VINA), %'
    216           WRITE(13,*)'10) MAGNIFICATION FACTOR (RMAG).'
    217           WRITE(13,*)'11) TIME STEP (DT), SEC.'
    218           WRITE(13,*)'12) STARTING TIME (TM0), SEC.'
    219           WRITE(13,*)'13) ENDING TIME (TM1), SEC.'
    220           WRITE(13,*)'14) TIME DELAY (DELAY), SEC., (0.0 IF NO DELAY)'
    221           WRITE(13,*)'15) PERMEABILITY STEP (DP), MICRONS/SEC.'
    222           WRITE(13,*)'16) THE LIMITS OF THE PERMEABILITY RANGE UNDER ,'
^H                                                                      Page
5

                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    223           WRITE(13,*)'    INVESTIGATION (P0 TO P1), MICRONS/SEC.'
    224           WRITE(13,*)'17) PERMEABILITY AT WHICH INVESTIGATE THE '
    225           WRITE(13,*)'    SENSITIVITY COEFFICIENTS (PSEN), MICRONS/SEC.'
    226           WRITE(13,*)'18) THE DATA POINTS: TIME (TMA(I)), SEC. AND '
    227           WRITE(13,*)'                     RADIUS (RA(I)), MICRONS OR'
    228           WRITE(13,*)'                                     CENTIMETERS.'
    229           WRITE(13,*)
    230           WRITE(13,*)'     (NOTE: YOU ONLY NEED THE DATA POINTS IF YOU'
    231           WRITE(13,*)'      ARE USEING THE PARAMETER ESTIMATION OPTION,'
    232           WRITE(13,*)'      I.E. A REAL EXPERIMENT.)'
    233           CLOSE(13,STATUS='KEEP')
```

```
    234           GO TO 600
    235         ENDIF
    236 C-----
    237 C       EXPLAINING THE OPTION TO ENTER DATA USING KEYBOARD OR AN INPUT
    238 C       FILE (WHICH IS SPECIFIED BY THE USER).
    239 C-----
    240         WRITE(*,*)
    241         WRITE(*,*)'   THIS PROGRAM WILL ALLOW YOU TO ENTER THE DATA'
    242         WRITE(*,*)'USING THE TERMINAL/KEYBOARD OR A PRE-EXISTING INPUT'
    243         WRITE(*,*)'FILE SET UP BY THE USER.  AN EXAMPLE OF AN INPUT FILE'
    244         WRITE(*,*)'CAN BE SEEN BY QUITTING THIS PROGRAM AND PRINTING '
    245         WRITE(*,*)'I_SENS.EXP AT THE PRINTER.  DO YOU WISH TO QUIT AND '
    246         WRITE(*,*)'PRINT THE EXAMPLE, (Y/N)?'
    247         READ(*,'(A1)') IQUIT
    248         CALL IYESNO(IQUIT)
    249 C-----
    250 C       IF IQUIT IT YES THE PROGRAM WILL GENERATE I_SENS.EXP AND QUIT.
    251 C-----
    252         IEXP =0
    253         IF (IQUIT.EQ.'Y') THEN
    254            OPEN(12,FILE='I_SENS.EXP',STATUS='NEW')
    255            WRITE(12,*)'THIS IS THE EXAMPLE INPUT FILE I_SENS.EXP FOR THE '
    256            WRITE(12,*)'PROGRAM SENS.FOR.  THE PROGRAM WILL READ THE DATA'
    257            WRITE(12,*)'ALINING THE VALUE UNDER THE LEFT MOST CHARACTER.'
    258            RL1=16.
    259            RL2 = 100.
    260            LIP = 9
    261            D1 = 5.21E-11
    262            D2 = 5.21E-10
    263            H = 5.21E-6
    264            CINIT = 0.02
    265            CINF = 0.04
    266            VINA = 6.
    267            RMAG = 5080.
    268            DT = 5.0
    269            TM0 = 0.
    270            TM1 =500.
    271            DELAY =0.0
    272            DP = 2.
    273            P0 = 0.
    274            P1 = 100.
    275            PSEN = 40.
    276            RINIT = 7.7
    277            PTRU = 40.
    278            DR = 0.01
^H                                                                    Page
6

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    279            TM01 = 500.
    280            DT1 = 5.0
```

```
      DT2 = 5.0
      IRELPSE = 1
      IMICCEN = 2
      IF1 = 1
      IF2 = 1
      IF3 = 1
      IF4 = 1
      IPRINT = 10
      CON1 ='O_CON1.DAT'
      CON2 ='O_CON2.DAT'
      SENC ='O_SENC.DAT'
      VOL1 ='O_VOL1.DAT'
      VOL2 ='O_VOL2.DAT'
      SUMR ='O_SUMR.DAT'
      ICOUNT = 40
      RA(1) = 7.7
      TMA(1) = 0.0
      DO 1100 I = 2,40
1       TMA(I) = TMA(I-1) + 10.
1       RA(I) = RA(I-1) - 0.05
1  1100 CONTINUE
      IEXP = 1
      ISAVE = 'Y'
      GO TO 319
    ENDIF
C-----
C     EXPLAINING THE INPUT AND OUTPUT OPTIONS
C-----
    WRITE(*,*)
    WRITE(*,*)'   THE PROGRAM WILL ALSO ALLOW THE USER TO VIEW THE'
    WRITE(*,*)'RESULTS BY 1) TABLES AND/OR 2) HAVING FILES CREATED'
    WRITE(*,*)'WHICH THE USER USES "PLOTIT" TO GENERATE GRAPHICAL'
    WRITE(*,*)'OUTPUT.  THE TABLES GENERATED CAN BE FOUND IN A FILE'
    WRITE(*,*)'CALLED "O_SENS.DAT".  A SUMMARY OF THE INPUT '
    WRITE(*,*)'PARAMETERS, INPUT DATA AND RESULTING PERMEABILITY'
    WRITE(*,*)'CAN BE FOUND IN "O_SMRY.DAT".'
    WRITE(*,*)'THE FILES THAT CAN BE CREATED FOR PLOTIT ARE'
    WRITE(*,*)'FOR THE FOLLOWING DATA SETS.'
    WRITE(*,*)
    WRITE(*,*)'1) CONCENTRATION V.S. DIMENSIONLESS TIME'
    WRITE(*,*)'2) SENSITIVITY COEFFICIENTS V.S. TIME'
    WRITE(*,*)'3) NORMALIZED VOLUME V.S. TIME'
    WRITE(*,*)'4) SUM OF THE SQUARE OF THE ERRORS V.S. PERMEABILITY'
    WRITE(*,*)
    WRITE(*,*)'THE PROGRAM WILL PROMPT THE USER TO ENTER A FILE'
    WRITE(*,*)'NAME FOR EACH OF THE DESIRED DATA SETS TO BE PLOTTED.'
    WRITE(*,*)
    WRITE(*,*)'YOU ARE NOW READY TO START THE PROGRAM.'
 1200 CONTINUE
    WRITE(*,*)
    WRITE(*,*)'DO YOU WISH TO ENTER THE DATA USING (1) THE TERMINAL'
    WRITE(*,*)'OR (2) A PRE-EXISTING INPUT FILE, (ENTER 1 OR 2)?'
```

```
    333       WRITE(*,*)
    334       READ(*,*,ERR=1200) ITERINP
^H                                                                    Page
7 .
                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    335       CALL IONETWO(ITERINP)
    336       IF(ITERINP.EQ.2) THEN
    337          WRITE(*,*)
    338          WRITE(*,*)'PLEASE ENTER THE NAME OF THE INPUT FILE TO BE USED,'
    339          WRITE(*,*)'(ENTER NO MORE THAN 10 CHARACTERS).'
    340          READ(*,'(A10)') ISENS
    341          CALL CHANNAM(ISENS)
    342          WRITE(*,*)
    343          WRITE(*,*)'OKAY, THE PROGRAM IS CRUNCHING.'
    344          GO TO 2999
    345       ENDIF
    346 C
    347       IPRINT = 10
    348 C-----
    349 C     PROMPTING THE USER TO ENTER THE REQUIRED DATA AND PARAMETERS
    350 C     NEEDED TO RUN THE PROGRAM.
    351 C-----
    352 2005  CONTINUE
    353       WRITE(*,*)
    354       WRITE(*,*)'DO YOU WISH TO (1) ENTER DATA FROM A REAL EXPERIMENT'
    355       WRITE(*,*)'OR (2) USE THE PROGRAM FOR A SIMULATION (PSEUDO-'
    356       WRITE(*,*)'EXPERMINT), (ENTER 1 OR 2)?'
    357       WRITE(*,*)
    358       READ(*,*,ERR=2005) IRELPSE
    359       CALL IONETWO(IRELPSE)
    360 2100  CONTINUE
    361       WRITE(*,*)'ENTERING THE PHYSICAL PARAMETERS OF THE SYSTEM:'
    362       WRITE(*,*)
    363       WRITE(*,*)'PLEASE ENTER THICKNESS OF THE DIALYSIS MEMBRANE, '
    364       WRITE(*,*)'(MICRONS).'
    365       WRITE(*,*)
    366       READ(*,*,ERR=2100) RL1
    367       WRITE(*,*)
    368 2105  CONTINUE
    369       WRITE(*,*)'PLEASE ENTER THE THICKNESS OF THE CELL CHAMBER,'
    370       WRITE(*,*)'(MICRONS).'
    371       WRITE(*,*)
    372       READ(*,*,ERR=2105) RL2
    373       WRITE(*,*)
    374 2106  CONTINUE
    375       WRITE(*,*)'PLEASE ENTER THE CELL POSITION, (5-11).'
    376       WRITE(*,*)'(SEE THE DIAGRAM BELOW FOR BETTER UNDERSTANDING.)'
    377       WRITE(*,*)
    378       WRITE(*,*)'    DIALYSIS MEMBRANE             CELL CHAMBER'
    379       WRITE(*,*)'  |                    |                          |'
```

```
    380       WRITE(*,*)'B |                    |                              |'
    381       WRITE(*,*)'U |                    |                              |'
    382       WRITE(*,*)'L |                    |                              |'
    383       WRITE(*,*)'K |                    |                              |'
    384       WRITE(*,*)'  1    2    3    4    5    6    7    8    9    10   11'
    385       WRITE(*,*)'F |                    |                              |'
    386       WRITE(*,*)'L |                    |                              |'
    387       WRITE(*,*)'O |                    |                              |'
    388       WRITE(*,*)'W |                    |                              |'
    389       WRITE(*,*)'  |                    |                              |'
    390       WRITE(*,*)
^H                                                                        Page
8
                                                                       07-20-87
                                                                       19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    391       READ(*,*,ERR=2106) LIP
    392       WRITE(*,*)
    393 2110  CONTINUE
    394       WRITE(*,*)'PLEASE ENTER THE DIFFUSIVITY OF THE SOLUTE INSIDE'
    395       WRITE(*,*)'THE CELL CHAMBER, (METERS*METERS/SEC.), D2.'
    396       WRITE(*,*)
    397       READ(*,*,ERR=2110) D2
    398       WRITE(*,*)
    399 2120  CONTINUE
    400       WRITE(*,*)'DO YOU WISH TO ENTER (1) SEPARATE VALUES FOR THE'
    401       WRITE(*,*)'DIFFUSIVITY OF THE SOLUTE INSIDE THE DIALYSIS'
    402       WRITE(*,*)'MEMBRANE (METERS*METERS/SEC.), D1, AND THE MASS'
    403       WRITE(*,*)'TRANSFER COEFFICIENT, H, OR (2) USE  PRESET'
    404       WRITE(*,*)'VALUES OF D1=D2/10 AND H=10000*D2?'
    405       WRITE(*,*)
    406       READ(*,*,ERR=2120) ISEPPRE
    407       CALL IONETWO(ISEPPRE)
    408       IF(ISEPPRE.EQ.1) THEN
    409 2130  CONTINUE
    410          WRITE(*,*)'PLEASE ENTER D1 (METERS*METERS/SEC.).'
    411          WRITE(*,*)
    412          READ(*,*,ERR=2130) D1
    413          WRITE(*,*)
    414 2140     CONTINUE
    415          WRITE(*,*)'PLEASE ENTER H, (METERS/SEC.)'
    416          WRITE(*,*)
    417          READ(*,*,ERR=2140) H
    418       ELSE IF (ISEPPRE.EQ.2) THEN
    419          D1 = D2/10.0
    420          H = 10000.*D2
    421       ENDIF
    422 2150  CONTINUE
    423       WRITE(*,*)
    424       WRITE(*,*)'PLEASE ENTER THE INITIAL AND FINAL CONCENTRATION, '
    425       WRITE(*,*)'CINIT CINF, (OSMOLALITY).  (ENTER BOTH VALUES AND'
    426       WRITE(*,*)'SEPARATE WITH A SPACE.)'
```

```
    427       WRITE(*,*)
    428       READ(*,*,ERR=2150) CINIT, CINF
    429 2160  CONTINUE
    430       WRITE(*,*)
    431       WRITE(*,*)'PLEASE ENTER THE INACTIVE VOLUME (%).'
    432       WRITE(*,*)
    433       READ(*,*,ERR=2160) VINA
    434       WRITE(*,*)
    435 2170  CONTINUE
    436       WRITE(*,*)
    437       WRITE(*,*)'PLEASE ENTER THE MAGNIFICATION FACTOR.  (USE 5080.0 '
    438       WRITE(*,*)'FOR BTP MEASUREMENTS.  IF YOU ARE ENTERING THE'
    439       WRITE(*,*)'EXACT CELL SIZE ENTER 1.0.)'
    440       WRITE(*,*)
    441       READ(*,*,ERR=2170) RMAG
    442       WRITE(*,*)
    443 2180  CONTINUE
    444       WRITE(*,*)
    445       WRITE(*,2190) RL1,RL2,LIP,D2,D1,H,CINIT,CINF,VINA,RMAG
    446 2190  FORMAT(1X,'THE VALUES ENTERED SO FAR ARE:',/,
^H                                                                    Page
9
                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    447      . 1X,'1) DIALYSIS MEMBRANE THICKNESS = ',E11.3,' MICRONS',/,
    448      . 1X,'2) CELL CHAMBER THICKNESS = ',E11.3,' MICRONS',/,
    449      . 1X,'3) LIPOSOME POSITION (5-11) = ',I2,/,
    450      . 1X,'4) DIFFUSIVITY IN CELL CHAMBER = ',E11.3,' M*M/SEC.',/,
    451      . 1X,'5) DIFFUSIVITY IN DIALYSIS MEMBRANE = ',E11.3,' M*M/SEC.',/,
    452      . 1X,'6) MASS TRANSFER COEFFICIENT = ',E11.3,' M/SEC  ',/,
    453      . 1X,'7) INITIAL CONCENTRATION = ',F7.3,' OSMOLALITY',/,
    454      . 1X,'8) FINAL CONCENTRATION = ',F7.3,' OSMOLALITY',/,
    455      . 1X,'9) INACTIVE VOLUME % = ',F5.2,/,
    456      . 1X,'10) MAGNIFICATION FACTOR = ',F7.1,/)
    457       WRITE(*,*)'DO YOU WISH TO CHANGE ANY OF THEM, (Y/N)?'
    458       READ(*,'(A1)') ICHANG
    459       CALL IYESNO(ICHANG)
    460       IF(ICHANG.EQ.'Y') THEN
    461 2210     CONTINUE
    462          WRITE(*,*)
    463          WRITE(*,*)'PLEASE ENTER THE NUMBER BESIDE THE PARAMETER'
    464          WRITE(*,*)'YOU WISH TO CHANGE, (1-10). '
    465          READ(*,*,ERR=2210) NCHANG
    466 2215     CONTINUE
    467          WRITE(*,*)
    468          WRITE(*,*)'PLEASE ENTER THE NEW VALUE.'
    469          IF(NCHANG.EQ.1) THEN
    470              READ(*,*,ERR=2215) RL1
    471          ELSE IF(NCHANG.EQ.2) THEN
    472              READ(*,*,ERR=2215) RL2
    473          ELSE IF(NCHANG.EQ.3) THEN
```

```
    474            READ(*,*,ERR=2215) LIP
    475          ELSE IF(NCHANG.EQ.4) THEN
    476            READ(*,*,ERR=2215) D2
    477          ELSE IF(NCHANG.EQ.5) THEN
    478            READ(*,*,ERR=2215) D1
    479          ELSE IF(NCHANG.EQ.6) THEN
    480            READ(*,*,ERR=2215) H
    481          ELSE IF(NCHANG.EQ.7) THEN
    482            READ(*,*,ERR=2215) CINIT
    483          ELSE IF(NCHANG.EQ.8) THEN
    484            READ(*,*,ERR=2215) CINF
    485          ELSE IF(NCHANG.EQ.9) THEN
    486            READ(*,*,ERR=2215) VINA
    487          ELSE IF(NCHANG.EQ.10) THEN
    488            READ(*,*,ERR=2215) RMAG
    489          ELSE IF(NCHANG.LE.0.OR.NCHANG.GE.11) THEN
    490             CALL INCORRES
    491            GO TO 2210
    492          ENDIF
    493          GO TO 2180
    494        ENDIF
    495        IF(IRUNAG.GE.1.OR.IMISTAK.EQ.'Y') GO TO 2235
    496        WRITE(*,*)
    497        WRITE(*,*)'IN ORDER FOR THE PROGRAM TO RUN THE USER'
    498        WRITE(*,*)'MUST ALSO ENTER THE FOLLOWING DATA:'
    499 2220  CONTINUE
    500        WRITE(*,*)
    501        WRITE(*,*)'THE TIME RANGE OF THE EXPERIMENT, TM0  TM1, (SEC.),'
    502        WRITE(*,*)'(NOTE:  PLEASE MAKE SURE TM1 IS GREATER THAN THE '
^H                                                                      Page
10
                                                                    07-20-87
                                                                    19:28:31
D Line# 1      7                          Microsoft FORTRAN77 V3.31 August 1985
    503        WRITE(*,*)'TIME OF THE LAST DATA POINT TO BE ENTERED AND ENTER '
    504        WRITE(*,*)'BOTH VALUES WITH A SPACE BETWEEN THEM.)'
    505        READ(*,*,ERR=2220) TM0, TM1
    506 2223  CONTINUE
    507        WRITE(*,*)
    508        WRITE(*,*)'THE TIME STEP, DT, (SEC.).  (NOTE:  THIS TIME STEP IS'
    509        WRITE(*,*)'USED FOR NUMERICAL INTEGRATION - CHOOSE DT SUCH THAT'
    510        WRITE(*,*)'DT .GE. (TM1-TM0)/301  TO PREVENT ARRAY OVERFLOW.)'
    511        READ(*,*,ERR=2223) DT
    512 2225  CONTINUE
    513        WRITE(*,*)
    514        WRITE(*,*)'THE PERMEABILITY RANGE WHICH YOU ARE INVESTIGATING, '
    515        WRITE(*,*)'P0   P1, (MICRONS/SEC.).  (ENTER BOTH VALUES WITH '
    516        WRITE(*,*)'A SPACE BETWEEN THEM.)'
    517        READ(*,*,ERR=2225) P0, P1
    518 2226  CONTINUE
    519        WRITE(*,*)
    520        WRITE(*,*)'THE PERMEABILITY STEP, DP, (MICRONS/SEC.) (NOTE: '
```

```
      WRITE(*,*)'CHOOSE DP SUCH THAT DP .GE. (P1-P0)/301  TO '
      WRITE(*,*)'PREVENT ARRAY OVERFLOW.)'
      READ(*,*,ERR=2226) DP
 2230 CONTINUE
      WRITE(*,*)
      WRITE(*,*)'THE PERMEABILITY VALUE AT WHICH THE SENSITIVITY'
      WRITE(*,*)'COEFFICIENT WILL BE EVALUATED, PSEN, (MICRONS/SEC.) '
      READ(*,*,ERR=2230) PSEN
 2235 CONTINUE
      WRITE(*,*)
      WRITE(*,2240) DT,TM0,TM1,DP,P0,P1,PSEN
 2240 FORMAT(1X,'THE VALUES ENTERED ARE:',/,
     .  1X,'1) TIME STEP = ',F7.2,' SEC.',/,
     .  1X,'2) TIME RANGE = ',F8.1,' SEC.',' TO ',F8.1,' SEC.',/,
     .  1X,'3) PERMEABILITY STEP = ',F7.2,' MICRONS/SEC.',/,
     .  1X,'4) PERMEABILITY RANGE = ',F7.2,' MICRONS/SEC. TO',/,
     .  1X,'                        ',F7.2,' MICRONS/SEC.',/,
     .  1X,'5) INVESTIGATING PERMEABILITY = ',F7.2,' MICRONS/SEC.',/)
      WRITE(*,*)'DO YOU WISH TO CHANGE ANY OF THEM, (Y/N)?'
      READ(*,'(A1)') ICHANG
      CALL IYESNO(ICHANG)
      IF(ICHANG.EQ.'Y')THEN
 2245   CONTINUE
        WRITE(*,*)
        WRITE(*,*)'PLEASE ENTER THE NUMBER BESIDE THE PARAMETER'
        WRITE(*,*)'YOU WISH TO CHANGE, (1-5). '
        READ(*,*,ERR=2245) NCHANG
 2246   CONTINUE
        WRITE(*,*)
        WRITE(*,*)'PLEASE ENTER THE NEW VALUE(S). '
        IF(NCHANG.EQ.1) THEN
          READ(*,*,ERR=2246) DT
        ELSE IF(NCHANG.EQ.2) THEN
          WRITE(*,*)'(BOTH TM0 AND TM1 - SEPARATE WITH A SPACE)'
          READ (*,*,ERR=2246) TM0, TM1
        ELSE IF(NCHANG.EQ.3) THEN
          READ(*,*,ERR=2246) DP
        ELSE IF(NCHANG.EQ.4) THEN
^H                                                                  Page
11

                                                                  07-20-87
                                                                  19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
          WRITE(*,*)'(BOTH P0 AND P1 - SEPARATE WITH A SPACE)'
          READ(*,*,ERR=2246) P0,P1
        ELSE IF(NCHANG.EQ.5) THEN
          READ(*,*,ERR=2246) PSEN
        ELSE IF(NCHANG.LE.0.OR.NCHANG.GE.6) THEN
           CALL INCORRES
           GO TO 2246
        ENDIF
      GO TO 2235
```

```
      ENDIF
C
      IF(IRELPSE.EQ.1) THEN
         IF(IRUNAG.GE.1.OR.IMISTAK.EQ.'Y') THEN
2248        CONTINUE
            WRITE(*,*)
            WRITE(*,*)'DO YOU WISH TO (1) ENTER ALL NEW DATA POINTS'
            WRITE(*,*)'OR (2) REVIEW THE PREVIOUS DATA POINTS, (Y/N)?'
            READ(*,*,ERR=2248) INR
            CALL IONETWO(INR)
            IF(INR.EQ.2)GO TO 2261
            IF(INR.EQ.1) THEN
               I=0
               WRITE(*,*)
               GO TO 2250
            ENDIF
         ENDIF
         I = 0
         WRITE(*,*)
         WRITE(*,*)'SINCE YOU HAVE CHOSEN THE REAL-EXPERIMENTAL'
         WRITE(*,*)'OPTION THE DATA POINTS MUST BE ENTERED.  BUT'
2250     CONTINUE
         WRITE(*,*)'BEFORE ENTERING THE DATA, IS THERE A TIME'
         WRITE(*,*)'DELAY THAT YOU WOULD LIKE TO HAVE SUBTRACTED'
         WRITE(*,*)'FROM THE TIME ARRAY YOU WILL BE ENTERING,'
         WRITE(*,*)'(Y/N)?'
         READ(*,'(A1)')IDEL
         CALL IYESNO(IDEL)
         IF(IDEL.EQ.'N') THEN
            DELAY = 0.0
         ELSE IF(IDEL.EQ.'Y') THEN
2251        CONTINUE
            WRITE(*,*)
            WRITE(*,*)'PLEASE ENTER THE TIME DELAY TO BE SUBTRACTED,'
            WRITE(*,*)'(SEC.).'
            READ(*,*,ERR=2251) DELAY
            WRITE(*,2252) DELAY
2252        FORMAT(/,' THE TIME DELAY THAT WILL BE SUBTRACTED IS ',
     .              F7.4,' SEC.',/,/,' DO YOU WISH TO CHANGE IT, (Y/N)?')
            READ(*,'(A1)') ICHANG
            CALL IYESNO(ICHANG)
            IF(ICHANG.EQ.'Y') GO TO 2251
         ENDIF
         WRITE(*,*)
         WRITE(*,*)'DO YOU WISH TO ENTER THE RADIUS USING UNITS'
         WRITE(*,*)'OF (1) MICRONS OR (2) CENTIMETERS?'
2253     READ(*,*,ERR=2253) IMICCEN
```

```
      CALL IONETWO(IMICCEN)
      WRITE(*,*)
      WRITE(*,*)'PLEASE ENTER THE DATA POINTS,'
      IF(IMICCEN.EQ.1)THEN
         WRITE(*,*)'TIME(I) (SEC.)  RADIUS(I) (MICRONS)'
      ELSE IF(IMICCEN.EQ.2) THEN
         WRITE(*,*)'TIME(I) (SEC.)  RADIUS(I) (CENTIMETERS)'
      ENDIF
      WRITE(*,*)'(ENTER BOTH VALUES AND SEPARATE WITH A SPACE.)'
      WRITE(*,*)'(NOTE:  ENTER 0.0 0.0 FOR THE LAST DATA POINT.) '
      WRITE(*,*)
 2255 I = I+1
      WRITE(*,2256) I
 2256 FORMAT(1X,'ENTER POINT ',I3)
 2257 READ(*,*,ERR=2257) TMA(I), RA(I)
       IF((TMA(I).GT.0.).AND.(RA(I).GT.0.)) TMA(I) = TMA(I) - DELAY
      IF(I.EQ.1) RAMAX = RA(I)
      IF(I.GE.2.AND.RA(I).GT.RAMAX) RAMAX = RA(I)
      IF((TMA(I).GT.0.).OR.(RA(I).GT.0.)) GO TO 2255
      ICOUNT = I-1
      IF(TM1.LE.TMA(ICOUNT)) THEN
          WRITE(*,*)
          WRITE(*,*)'PLEASE MAKE SURE THE TIME LIMIT, TM1, IS'
          WRITE(*,*)'LARGER THAN THE TIME OF THE LAST DATA POINT.'
      WRITE(*,*)'IF YOU DO NOT CHANGE THIS THE PROGRAM WILL STOP.'
          WRITE(*,*)'(RELAX YOU WILL HAVE A CHANCE TO CHANGE IT.)'
          WRITE(*,*)
      ENDIF
 2260 CONTINUE
      WRITE(*,*)'THE NEXT TABLE WILL SHOW YOU THE POINTS YOU HAVE'
      WRITE(*,*)'JUST ENTERED.  IF YOU HAVE ENTERED MORE THAN 20'
      WRITE(*,*)'POINTS THE TABLE WILL STOP SPOOLING EVERY 20 POINTS'
      WRITE(*,*)'TO ALLOW YOU TO REVIEW THE POINTS ENTERED.  MAKE'
      WRITE(*,*)'A NOTE OF WHICH POINT YOU WISH TO CHANGE OR'
      WRITE(*,*)'INSERT AND PRESS [RETURN] TO CONTINUE NOTE, YOU'
      WRITE(*,*)'YOU WILL ONLY BE ABLE TO CHANGE OR INSERT ONE '
      WRITE(*,*)'POINT AT A TIME.'
      WRITE(*,*)'(NOW PRESS [RETURN] TO CONTINUE.)'
      READ(*,'(A1)') ICHANG
 2261 CONTINUE
      WRITE(*,*)
      WRITE(*,*)'THE DATA POINTS YOU HAVE ENTERED ARE: '
      WRITE(*,2262) DELAY
 2262 FORMAT(' (INCLUDING THE TIME DELAY OF ',F8.4,' SEC.)')
      WRITE(*,*)
      WRITE(*,*)'  J       TIME(J)      RADIUS(J)'
      IF(IMICCEN.EQ.1) THEN
      WRITE(*,*)'          (SEC.)       (MICRONS)'
      ELSE IF(IMICCEN.EQ.2) THEN
      WRITE(*,*)'          (SEC.)       (CENTIMETERS)'
      ENDIF
      DO 2265 J=1,ICOUNT+1
```

```
1   667              WRITE(*,2263) J, TMA(J), RA(J)
1   668 2263         FORMAT(1X,I3,5X,F8.2,5X,F8.2)
1   669              IF((J/20)*20.EQ.J) THEN
1   670                 WRITE(*,*)
^H                                                                    Page
13
                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
1   671                 WRITE(*,*)'PRESS [RETURN] TO CONTINUE.'
1   672                 READ(*,'(A1)') IMORE
1   673              ENDIF
1   674 2265      CONTINUE
    675           IF(IBACK.EQ.1) GO TO 2281
    676           WRITE(*,*)
    677           WRITE(*,*)'DO YOU WISH TO CHANGE OR INSERT ANY OF THE POINTS,'
    678           WRITE(*,*)'(Y/N)?'
    679           READ(*,'(A1)') ICHANG
    680           CALL IYESNO(ICHANG)
    681           IF(ICHANG.EQ.'Y') THEN
    682 2266            CONTINUE
    683                 WRITE(*,*)
    684                WRITE(*,*)'ENTER (1) TO CHANGE AND (2) TO INSERT.'
    685                READ(*,*,ERR=2266) ICHAINS
    686                CALL IONETWO(ICHAINS)
    687             IF(ICHAINS.EQ.1) THEN
    688 2267            CONTINUE
    689                WRITE(*,*)
    690                WRITE(*,*)'PLEASE ENTER THE INDEX NUMBER  J'
    691                READ(*,*,ERR=2267) JI
    692                IF(JI.LE.0.OR.JI.GE.I+1) THEN
    693                    CALL INCORRES
    694                    GO TO 2267
    695                 ENDIF
    696                WRITE(*,*)
    697                WRITE(*,*)'PLEASE ENTER THE NEW VALUES FOR '
    698                WRITE(*,*)'TIME(J) AND RADIUS(J). '
    699 2270           READ(*,*,ERR=2270) TMA(JI), RA(JI)
    700                GO TO 2260
    701             ELSE IF(ICHAINS.EQ.2) THEN
    702 2272            CONTINUE
    703                WRITE(*,*)
    704                WRITE(*,*)'ENTER THE INDEX NUMBER J YOU WISH TO '
    705                WRITE(*,*)'CHANGE, (OR PUSH DOWN).'
    706                READ(*,*,ERR=2272) JI
    707                IF(JI.LE.0.OR.JI.GE.I+1) THEN
    708                    CALL INCORRES
    709                    GO TO 2272
    710                ENDIF
    711                I = I+1
    712                DO 2278 J=JI,I-1
1   713                   TMA( I+JI-J ) = TMA( (I-1)+JI-J )
```

```
1   714                   RA( I+JI-J ) = RA( (I-1)+JI-J )
1   715 2278            CONTINUE
    716                 WRITE(*,*)
    717                 WRITE(*,*)'PLEASE ENTER THE NEW VALUES FOR'
    718                 WRITE(*,*)'TIME(J) AND RADIUS(J).'
    719 2280            READ(*,*,ERR=2280) TMA(JI), RA(JI)
    720                 GO TO 2260
    721               ENDIF
    722             ENDIF
    723 2281        CONTINUE
    724             IF((IRUNAG.GT.0).OR.(IMISTAK.EQ.'Y')) THEN
    725                 WRITE(*,*)
    726                 WRITE(*,*)'DO YOU WISH TO HAVE A TIME DELAY SUBTRACTED'
^H                                                                        Page
14

                                                                      07-20-87
                                                                      19:28:31
D Line# 1     7                                Microsoft FORTRAN77 V3.31 August 1985
    727                 WRITE(*,*)'FROM THE DATA POINTS, (Y/N)?'
    728                 READ(*,'(A1)') IDEL
    729                 CALL IYESNO(IDEL)
    730                 IF(IDEL.EQ.'Y') THEN
    731 2282                CONTINUE
    732                     WRITE(*,*)
    733                     WRITE(*,*)'PLEASE ENTER THE DELAY TO BE SUBTRACTED.'
    734                     READ(*,*,ERR=2282) DELAY
    735                     WRITE(*,*)
    736                     WRITE(*,2283) DELAY
    737 2283                FORMAT(' THE DELAY ENTERED IS ',F10.4,' (SEC.)',/,
    738       .                 ' DO YOU WISH TO CHANGE IT, (Y/N)?')
    739                     READ(*,'(A1)') ICHANG
    740                     IF(ICHANG.EQ.'Y') GO TO 2282
    741                 ELSE IF(IDEL.EQ.'N') THEN
    742                     DELAY = 0.0
    743                     GO TO 2341
    744                 ENDIF
    745                 DO 2284 J = 1,ICOUNT
1   746                     TMA(J) = TMA(J) - DELAY
1   747 2284            CONTINUE
    748                 IBACK = 1
    749                 GO TO 2261
    750               ENDIF
    751               IBACK = 0
    752               RINIT = RA(1)
    753             ELSE IF(IRELPSE.EQ.2) THEN
    754               IF(IRUNAG.GE.1.OR.IMISTAK.EQ.'Y') GO TO 2319
    755               WRITE(*,*)
    756               WRITE(*,*)'SINCE YOU HAVE CHOSEN THE PSEUDO-EXPERIMENTAL'
    757               WRITE(*,*)'OPTION, THE FOLLOWING PARAMETERS MUST ALSO'
    758               WRITE(*,*)'BE ENTERED.'
    759               WRITE(*,*)
    760 2300          CONTINUE
```

```
    761           WRITE(*,*)'THE PERMEABILITY VALUE WITH WHICH THE PROGRAM'
    762           WRITE(*,*)'GENERATES PSEUDO-EXPERIMENTAL DATA, PTRU, '
    763           WRITE(*,*)'(MICRONS/SEC.).'
    764           READ(*,*,ERR=2300) PTRU
    765 2303      CONTINUE
    766           WRITE(*,*)
    767           WRITE(*,*)'DO YOU WISH TO ENTER THE INITIAL RADIUS USING UNITS'
    768           WRITE(*,*)'OF (1) MICRONS OR (2) CENTIMETERS, (ENTER 1 OR 2)?'
    769           READ(*,*,ERR=2303) IMICCEN
    770           CALL IONETWO(IMICCEN)
    771 2305      CONTINUE
    772           WRITE(*,*)
    773           WRITE(*,*)'PLEASE ENTER THE INITIAL RADIUS OF THE CELL, RINIT,'
    774           IF(IMICCEN.EQ.1) WRITE(*,*)'(MICRONS).'
    775           IF(IMICCEN.EQ.2) WRITE(*,*)'(CENTIMETERS).'
    776           READ(*,*,ERR=2305) RINIT
    777 2310      CONTINUE
    778           WRITE(*,*)
    779           WRITE(*,*)'SINCE THIS IS A SIMULATION THE DATA POINTS '
    780           WRITE(*,*)'GENERATED WILL HAVE A RADIUS HISTORY THAT WILL'
    781           WRITE(*,*)'LOOK EXACTLY LIKE THAT OF THEORY, THEREFORE'
    782           WRITE(*,*)'THE PROGRAM ALLOWS THE USER TO IMPOSE A PSEUDO-'
^H                                                                      Page
15

                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    783           WRITE(*,*)'RANDOMNESS FACTOR, DR, ON THE DATA.  DR CAN BE'
    784           WRITE(*,*)'THOUGHT OF AS THE MAGNITUDE OF THE VARIATION'
    785           WRITE(*,*)'IN MEASURING THE RADIUS OF THE CELL.'
    786           WRITE(*,*)'PLEASE ENTER DR NOW.'
    787           IF(IMICCEN.EQ.1) WRITE(*,*)'(MICRONS).'
    788           IF(IMICCEN.EQ.2) WRITE(*,*)'(CENTIMETERS).'
    789           READ(*,*,ERR=2310) DR
    790           WRITE(*,*)
    791           WRITE(*,*)'WHEN USING THIS OPTION THE USER CAN CHANGE'
    792           WRITE(*,*)'THE TIME STEP IN TWO REGIONS TO STUDY THE '
    793           WRITE(*,*)'EFFECT OF DATA SPACING OF PARAMETER ESTIMATION.'
    794           WRITE(*,*)'DO YOU WISH TO DO THIS, (Y/N)? '
    795           READ(*,'(A1)') IMORE
    796           CALL IYESNO(IMORE)
    797           IF(IMORE.EQ.'N') THEN
    798              TM01 = TM1
    799              DT1 = DT
    800              DT2 = DT
    801           ELSE IF(IMORE.EQ.'Y') THEN
    802 2311         CONTINUE
    803              WRITE(*,*)
    804              WRITE(*,*)'PLEASE ENTER THE FIRST TIME STEP, DT1, (SEC.). '
    805              READ(*,*,ERR=2311) DT1
    806 2312         CONTINUE
    807              WRITE(*,*)
```

```
    808             WRITE(*,*)'PLEASE ENTER THE SECOND TIME STEP, DT2, (SEC.) '
    809             READ(*,*,ERR=2312) DT2
    810 2313        CONTINUE
    811             WRITE(*,*)
    812            WRITE(*,*)'PLEASE ENTER THE INTERMEDIATE TIME LIMIT, TM01,'
    813             WRITE(*,*)'(SEC.) FOR THE FIRST TIME INTERVAL.  (TM01  '
    814             WRITE(*,*)'TO TM1 IS ASSUMED TO BE THE SECOND TIME  '
    815             WRITE(*,*)'INTERVAL.)'
    816             READ(*,*,ERR=2313) TM01
    817          ENDIF
    818 C
    819 2319     CONTINUE
    820          IF(IMICCEN.EQ.1) XMICCEN ='MICRONS'
    821          IF(IMICCEN.EQ.2) XMICCEN ='CENTIMETERS'
    822          WRITE(*,*)
    823          WRITE(*,2320) PTRU,RINIT,XMICCEN,DR,XMICCEN,DT1,DT2,TM01
    824 2320     FORMAT(1X,'THE VALUES ENTERED FOR THE PSEUDO-EXPERIMENTAL'
    825       .   ,' OPTION ARE:',/,
    826       . 1X,'1) PSEUDO-PERMEABILITY = ',F8.1,' MICRONS/SEC.',/,
    827       . 1X,'2) INITIAL CELL RADIUS = ',E11.3,1X,A11,/,
    828       . 1X,'3) RADIUS RANDOMNESS = ',E11.4,1X,A11,/,
    829       . 1X,'4) FIRST TIME STEP = ',F7.2,' SEC.',/,
    830       . 1X,'5) SECOND TIME STEP = ',F7.2,' SEC.',/,
    831       . 1X,'6) INTERMEDIATE TIME LIMIT = ',F8.1,' SEC.',/)
    832          WRITE(*,*)'DO YOU WISH TO CHANGE ANY OF THEM, (Y/N)?'
    833          READ(*,'(A1)') ICHANG
    834          CALL IYESNO(ICHANG)
    835          IF(ICHANG.EQ.'Y') THEN
    836 2330        CONTINUE
    837             WRITE(*,*)
    838             WRITE(*,*)'PLEASE ENTER THE NUMBER BESIDE THE PARAMETER'
^H                                                                      Page
16

                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    839             WRITE(*,*)'YOU WISH TO CHANGE, (1-5). '
    840             READ(*,*,ERR=2330) NCHANG
    841 2340        CONTINUE
    842             WRITE(*,*)
    843             WRITE(*,*)'PLEASE ENTER THE NEW VALUE. '
    844             IF(NCHANG.EQ.1) THEN
    845                 READ(*,*,ERR=2340) PTRU
    846             ELSE IF(NCHANG.EQ.2) THEN
    847                 READ(*,*,ERR=2340) RINIT
    848             ELSE IF(NCHANG.EQ.3) THEN
    849                 READ(*,*,ERR=2340) DR
    850             ELSE IF(NCHANG.EQ.4) THEN
    851                 READ(*,*,ERR=2340) DT1
    852             ELSE IF(NCHANG.EQ.5) THEN
    853                 READ(*,*,ERR=2340) DT2
    854             ELSE IF(NCHANG.EQ.6) THEN
```

```
    855                 READ(*,*,ERR=2340) TM01
    856               ELSE IF(NCHANG.LE.0.OR.NCHANG.GE.7) THEN
    857                 CALL INCORRES
    858                 GO TO 2330
    859               ENDIF
    860             GO TO 2319
    861           ENDIF
    862         ENDIF
    863 C
    864 2341    CONTINUE
    865         IF1 = 0
    866         IF2 = 0
    867         IF3 = 0
    868         IF4 = 0
    869         CON1 ='O_CON1.DAT'
    870         CON2 ='O_CON2.DAT'
    871         SENC ='O_SENC.DAT'
    872         VOL1 ='O_VOL1.DAT'
    873         VOL2 ='O_VOL2.DAT'
    874         SUMR ='O_SUMR.DAT'
    875         WRITE(*,*)'DO YOU WISH TO HAVE FILES CREATED SO THAT YOU CAN'
    876         WRITE(*,*)'USE PLOTIT TO GENERATE GRAPHICAL OUTPUT, (Y/N)?'
    877         READ(*,'(A1)') IGRAPH
    878         CALL IYESNO(IGRAPH)
    879         IF(IGRAPH.EQ.'Y') THEN
    880 2342      CONTINUE
    881           WRITE(*,*)
    882           WRITE(*,*)'THE OUTPUT CAN BE VIEWED IN THE FOLLOWING WAYS:'
    883           WRITE(*,*)
    884           WRITE(*,*)'1) CONCENTRATION V.S. DIMENSIONLESS TIME'
    885           WRITE(*,*)'2) SENSITIVITY COEFFICIENT V.S. TIME'
    886           WRITE(*,*)'3) NORMALIZED VOLUME V.S. TIME'
    887           WRITE(*,*)'4) SUM OF SQUARE OF ERRORS V.S. PERMEABILITY'
    888           WRITE(*,*)
    889           WRITE(*,*)'WHICH GRAPHICAL OPTION WOULD YOU LIKE (1-4), -'
    890           WRITE(*,*)'(PLEASE CHOOSE ONE GRAPH AT A TIME).'
    891 2343      CONTINUE
    892           READ(*,*,ERR=2343) IOPTGR
    893           IF(IOPTGR.LE.0.OR.IOPTGR.GE.5) THEN
    894             CALL INCORRES
^H                                                                    Page
17
                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    895             GO TO 2342
    896           ENDIF
    897           WRITE(*,*)
    898           WRITE(*,*)'(NOTE: WHEN ENTERING THE FILE NAME USE 10'
    899           WRITE(*,*)' CHARACTERS OR LESS.)'
    900           IF(IOPTGR.EQ.1) THEN
    901             IF1 = 1
```

```
 902 2344          CONTINUE
 903               WRITE(*,*)'THE DATA GENERATED FOR THE GRAPH CONCENTRATION'
 904               WRITE(*,*)'V.S. DIMENSIONLESS TIME REQUIRES THE USER TO '
 905               WRITE(*,*)'CHOOSE TWO FILE NAMES.'
 906               WRITE(*,*)
 907               WRITE(*,*)'PLEASE ENTER THE NAME FOR THE FIRST DATA SET,'
 908               WRITE(*,*)'(CA(I) V.S. TIME(I)/TMAX).'
 909               READ(*,'(A10)',ERR=2344) CON1
 910               CALL CHANNAM(CON1)
 911 2345          CONTINUE
 912               WRITE(*,*)
 913               WRITE(*,*)'PLEASE ENTER THE NAME FOR THE SECOND DATA SET,'
 914               WRITE(*,*)'(CB(I) V.S. TIME(I)/TMAX).'
 915               READ(*,'(A10)',ERR=2345) CON2
 916               CALL CHANNAM(CON2)
 917            ELSE IF(IOPTGR.EQ.2) THEN
 918               IF2 = 1
 919 2346          CONTINUE
 920               WRITE(*,*)'PLEASE ENTER THE FILE NAME YOU DESIRE FOR THE'
 921               WRITE(*,*)'GRAPH - SENSITIVITY COEFFICIENT V.S. TIME.'
 922               WRITE(*,*)'(SEN(I) V.S. TIME(I)).'
 923               READ(*,'(A10)',ERR=2346) SENC
 924               CALL CHANNAM(SENC)
 925            ELSE IF(IOPTGR.EQ.3) THEN
 926               IF3 = 1
 927 2347          CONTINUE
 928               WRITE(*,*)'THE DATA GENERATED FOR THE GRAPH OF NORMALIZED'
 929               WRITE(*,*)'VOLUME V.S. TIME REQUIRES THE USER TO CHOOSE'
 930               WRITE(*,*)'TWO FILE NAMES.  THE FIRST FILE WILL CONTAIN'
 931               IF(IRELPSE.EQ.1) THEN
 932                 WRITE(*,*)'THE ACTUAL DATA POINTS ENTERED (WHICH ARE'
 933                 WRITE(*,*)'CONVERTED TO NORMALIZED VOLUME).  THE '
 934                 WRITE(*,*)'SECOND FILE WILL CONTAIN THE DATA FOR THE'
 935                 WRITE(*,*)'BEST FIT CURVE BASED OF THE PERMEABILITY'
 936                 WRITE(*,*)'ESTIMATED FROM THE DATA ENTERED.'
 937               ELSE IF(IRELPSE.EQ.2) THEN
 938                 WRITE(*,*)'THE SIMULATED DATA POINTS, WITH AN IMPOSED'
 939                 WRITE(*,*)'RANDOMNESS TO THE DATA, BASED ON THE TRUE'
 940                 WRITE(*,*)'PERMEABILITY ENTERED.  THE SECOND FILE WILL'
 941                 WRITE(*,*)'CONTAIN THE DATA FOR THE BEST FIT CURVE'
 942                 WRITE(*,*)'BASED ON THE TRUE PERMEABILITY ENTERED ALSO.'
 943               ENDIF
 944               WRITE(*,*)
 945               WRITE(*,*)'PLEASE ENTER THE NAME FOR THE FIRST DATA SET,'
 946               WRITE(*,*)'(VOLA(I) V.S. TIME(I)).'
 947               READ(*,'(A10)',ERR=2347) VOL1
 948               CALL CHANNAM(VOL1)
 949 2348          CONTINUE
 950               WRITE(*,*)
```

```
 951              WRITE(*,*)'PLEASE ENTER THE NAME FOR THE SECOND DATA SET,'
 952              WRITE(*,*)'(VOLC(I) V.S. TIME(I)).'
 953              READ(*,'(A10)',ERR=2348) VOL2
 954              CALL CHANNAM(VOL2)
 955            ELSE IF(IOPTGR.EQ.4) THEN
 956              IF4 = 1
 957 2349         CONTINUE
 958              WRITE(*,*)'PLEASE ENTER THE FILE NAME YOU DESIRE FOR THE'
 959           WRITE(*,*)'GRAPH - SUM OF SQUARE OF ERRORS V.S. PERMEABILITY,'
 960              WRITE(*,*)'(SUM(I) V.S. P(I)).'
 961              READ(*,'(A10)',ERR=2349) SUMR
 962              CALL CHANNAM(SUMR)
 963            ENDIF
 964            WRITE(*,*)
 965            WRITE(*,*)'DO YOU WISH TO CHOOSE ANOTHER FILE NAME FOR'
 966            WRITE(*,*)'ANOTHER DATA SET, (Y/N)?'
 967            READ(*,'(A1)') IANOTH
 968            CALL IYESNO(IANOTH)
 969            IF(IANOTH.EQ.'Y') GO TO 2342
 970         ENDIF
 971         WRITE(*,*)
 972         WRITE(*,*)'HAVE YOU MADE ANY MISTAKES THAT YOU WOULD LIKE '
 973         WRITE(*,*)'ANOTHER CRACK AT ENTERING/CHANGING THE PARAMETERS '
 974         WRITE(*,*)'OR DATA AGAIN, (Y/N)?'
 975         READ(*,'(A1)') IMISTAK
 976         CALL IYESNO(IMISTAK)
 977         IF(IMISTAK.EQ.'Y') GO TO 2180
 978         WRITE(*,*)
 979         WRITE(*,*)'OKAY, THE PROGRAM IS NOW CRUNCHING.'
 980         GO TO 3999
 981 C-----
 982 C       OPTION ITERINP = 2:  ENTERING THE INPUT FILE
 983 C-----
 984 2999    CONTINUE
 985         OPEN(11,FILE=ISENS,STATUS='OLD')
 986         READ(11,'(A11)') PROBLEM
 987         READ(11,3100) RL1,RL2,LIP
 988 3100    FORMAT(///,1X,F11.6,F11.6,I2)
 989         READ(11,3110) D1,D2,H
 990 3110    FORMAT(//,1X,E9.3,2X,E9.3,2X,E9.3)
 991         READ(11,3120) CINIT, CINF
 992 3120    FORMAT(//,1X,F11.6,F11.6)
 993         READ(11,3125) VINA,RMAG
 994 3125    FORMAT(//,1X,F11.6,F11.6)
 995         READ(11,3130) DT,TM0,TM1,DELAY
 996 3130    FORMAT(//,1X,F11.6,F11.6,F11.6,F11.6)
 997         READ(11,3140) DP,P0,P1,PSEN
 998 3140    FORMAT(//,1X,F11.6,F11.6,F11.6,F11.6)
 999         READ(11,3145) RINIT
1000 3145    FORMAT(//,1X,F11.6)
```

```
   1001        READ(11,3150) PTRU,DR,TM01,DT1,DT2
   1002 3150   FORMAT(///,1X,F11.6,F11.6,F11.6,F11.6,F10.6)
   1003        READ(11,3160) IRELPSE,IMICCEN
   1004 3160   FORMAT(///,1X,I1,10X,I1)
   1005        READ(11,3170) IF1,IF2,IF3,IF4,IPRINT
   1006 3170   FORMAT(///,1X,I1,10X,I1,10X,I1,10X,I1,10X,I2,/)
^H                                                                    Page
19

                                                                   07-20-87
                                                                   19:28:31
D Line# 1      7                          Microsoft FORTRAN77 V3.31 August 1985
   1007        READ(11,3175) CON1,CON2,SENC,VOL1,VOL2,SUMR
   1008 3175   FORMAT(//,1X,A10,1X,A10,/,1X,A10,/1X,A10,1X,A10,/,1X,A10,///)
   1009 C
   1010        IF(IMICCEN.EQ.1) XMICCEN='MICRONS'
   1011        IF(IMICCEN.EQ.2) XMICCEN='CENTIMETERS'
   1012 C
   1013 C-----
   1014 C      WRITING THE INPUT DATA TO THE OUTPUT FILE O_SENS.DAT
   1015 C-----
   1016        WRITE(*,3180) ISENS
   1017 3180   FORMAT(/,' THE INPUT FILE ',A10,' HAS BEEN ENTERED.')
   1018 3999   CONTINUE
   1019        IF(IOPNAG.EQ.0) THEN
   1020           OPEN(10,FILE='O_SENS.DAT',STATUS='NEW')
   1021           OPEN(20,FILE='O_SMRY.DAT',STATUS='NEW')
   1022        ENDIF
   1023        WRITE(10,4000) ISENS
   1024 4000   FORMAT(1X,'**********************  THE INPUT FILE ',A10,
   1025       .       '  **********************',/)
   1026        WRITE(10,4005)
   1027 4005   FORMAT(/,'RL1,RL2,LIP ARE',/)
   1028        WRITE(10,*)RL1,RL2,LIP
   1029        WRITE(10,4010)
   1030 4010   FORMAT(/,'D1,D2,H ARE:',/)
   1031        WRITE(10,*) D1,D2,H
   1032        WRITE(10,4020)
   1033 4020   FORMAT(/,'CINIT,CINF ARE:',/)
   1034        WRITE(10,*) CINIT,CINF
   1035        WRITE(10,4030)
   1036 4030   FORMAT(/,'VINA,RMAG ARE:',/)
   1037        WRITE(10,*) VINA,RMAG
   1038        WRITE(10,4040)
   1039 4040   FORMAT(/,'DT,TM0,TM1,DELAY ARE:',/)
   1040        WRITE(10,*) DT,TM0,TM1,DELAY
   1041        WRITE(10,4050)
   1042 4050   FORMAT(/,'DP,P0,P1,PSEN ARE:',/)
   1043        WRITE(10,*) DP,P0,P1,PSEN
   1044        WRITE(10,4060)
   1045 4060   FORMAT(/,'RINIT IS:',/)
   1046        WRITE(10,*) RINIT
   1047        WRITE(10,4070)
```

```
   1048 4070  FORMAT(/,'PTRU,DR,TM01,DT1,DT2 ARE:',/)
   1049       WRITE(10,*) PTRU,DR,TM01,DT1,DT2
   1050       WRITE(10,4080)
   1051 4080  FORMAT(/,'IRELPSE,IMICCEN ARE:',/)
   1052       WRITE(10,*) IRELPSE, IMICCEN
   1053       WRITE(10,4090)
   1054 4090  FORMAT(/,'IF1,IF2,IF3,IF4,IPRINT ARE:',/)
   1055       WRITE(10,*) IF1,IF2,IF3,IF4,IPRINT
   1056       WRITE(10,4100)
   1057 4100  FORMAT(/,'CON1,CON2,SENC,VOL1,VOL2,SUMR ARE:'
   1058      .         ,/)
   1059       WRITE(10,4110) CON1,CON2,SENC,VOL1,VOL2,SUMR
   1060 4110  FORMAT(A10,5X,A10,/,A10,/,A10,5X,A10,/,A10)
   1061 C
   1062 C-----
^H                                                                    Page
20

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
   1063 C     USING SUBROUTINE MBCON TO PREDICT THE CONCENTRATION
   1064 C     CHANGE INSIDE THE CELL CHAMBER
   1065 C-----
   1066       WRITE(*,*)
   1067       WRITE(*,*)'ENTERING SUBROUTINE MBCON.'
   1068       CALL MBCON(TMS,CA,CB,IP)
   1069       WRITE(*,*)
   1070       WRITE(*,*)'LEAVING MBCON.'
   1071 C-----
   1072 C     IF IF1=1 (YES), WRITE DATA TO CON1 AND CON2
   1073 C-----
   1074       IF(IF1.EQ.0) GO TO 4150
   1075          OPEN(14,FILE=CON1,STATUS='NEW')
   1076          OPEN(15,FILE=CON2,STATUS='NEW')
   1077          DO 4140 I=1,IP
1  1078             WRITE(14,4135) TMS(I),CA(I)
1  1079             WRITE(15,4135) TMS(I),CB(I)
1  1080 4135        FORMAT(1X,F6.4,1X,',',1X,E9.3)
1  1081 4140     CONTINUE
   1082          WRITE(*,*)
   1083          WRITE(*,*)'THE DATA FOR THE GRAPH CONCETRATION V.S. '
   1084          WRITE(*,*)'DIMENSIONLESS TIME HAS BEEN SENT TO FILES -'
   1085          WRITE(*,4142) CON1, CON2
   1086 4142     FORMAT(1X,A10,' AND ',A10)
   1087          CLOSE(14,STATUS='KEEP')
   1088          CLOSE(15,STATUS='KEEP')
   1089 4150  CONTINUE
   1090 C-----
   1091 C     INITIALIZATION OF THE TIME VARIABLES AND RINIT
   1092 C-----
   1093       IT=INT((TM1-TM0)/DT)+1
   1094       DO 4170 I=1,IT
```

```
1  1095          TMS(I)=TM0+(I-1)*DT
1  1096          TMB(I)=TMS(I)
1  1097 4170  CONTINUE
   1098        IF(IMICCEN.EQ.1) THEN
   1099           RINIT = RINIT/RMAG
   1100        ELSE IF(IMICCEN.EQ.2) THEN
   1101           RINIT = RINIT*10000./RMAG
   1102        ENDIF
   1103 C-----
   1104 C      CALCULATE R(TM,P) AND R(TM,P+DP)
   1105 C-----
   1106        WRITE(*,*)
   1107        WRITE(*,*)'CALCULATING R(TM,P).'
   1108        CALL RGKT(TMS,RS,IT,DT,RINIT,PSEN)
   1109        WRITE(*,*)
   1110        WRITE(*,*)'CALCULATING R(TM,P+DP).'
   1111        CALL RGKT(TMB,RB,IT,DT,RINIT,PSEN+EP1*PSEN)
   1112 C-----
   1113 C      CALCULATE SENSITIVITY COEFF. (SEN)
   1114 C-----
   1115        WRITE(10,4175)
   1116 4175  FORMAT(//,1X,'**************  DIMENSIONLESS SENSIVITITY ',
   1117       .           'COEFFICIENT VERSUS TIME  ************')
   1118        WRITE(*,*)
^H                                                                    Page
21

                                                                   07-20-87
                                                                   19:28:31
D Line# 1      7                          Microsoft FORTRAN77 V3.31 August 1985
   1119        WRITE(*,*)'CALCULATING SENSITIVITY COEFFICIENT, SEN.'
   1120        WRITE(10,4180)
   1121 4180  FORMAT(/,6X,'TIME(I)',7X,'SEN(I)',/)
   1122        DO 4190 J=1,IT
1  1123           SEN(J)=(RB(J)-RS(J))*PSEN/RINIT/(EP1*PSEN)
1  1124           WRITE(10,4185)TMS(J),SEN(J)
1  1125 4185      FORMAT(5X,F7.2,5X,E10.3)
1  1126 4190  CONTINUE
   1127 C-----
   1128 C      IF IF2 = 1 (YES), WRITE DATA TO SENC
   1129 C-----
   1130        IF(IF2.EQ.0)GO TO 4230
   1131 C-----
   1132           OPEN(16,FILE=SENC,STATUS='NEW')
   1133           DO 4220 I=1,IT
1  1134             WRITE(16,4210) TMS(I),SEN(I)
1  1135 4210        FORMAT(1X,F8.2,1X,',',1X,E9.3)
1  1136 4220      CONTINUE
   1137           WRITE(*,*)
   1138           WRITE(*,*)'THE DATA FOR THE GRAPH OF THE SENSITIVITY '
   1139           WRITE(*,*)'COEFFICIENTS V.S. TIME HAS BEEN SENT TO FILE -'
   1140           WRITE(*,'(1X,A10)') SENC
   1141           CLOSE(16,STATUS='KEEP')
```

```
   1142 4230  CONTINUE
   1143       WRITE(10,*)
   1144 4235  FORMAT(1X,'*****************************  RADIUS VERSUS TIME',
   1145      .        '  **************************')
   1146 C-----
   1147 C     READING THE EXPERIMENTAL DATA AND CONVERTING TO MICRONS
   1148 C-----
   1149       IF (IRELPSE.EQ.1.AND.ITERINP.EQ.2) THEN
   1150          WRITE(10,4235)
   1151          I=0
   1152          WRITE(10,4240)
   1153 4240    FORMAT(/,6X,'TM(I)',10X,'R(I)',/)
   1154 4250    I=I+1
   1155          READ(11,*)TMA(I),RA(I)
   1156          RSAVE(I) = RA(I)
   1157          IF(RA(I).GT.0.) TMA(I) =TMA(I)-DELAY
   1158          WRITE(10,4253) TMA(I), RA(I)
   1159          IF(IMICCEN.EQ.1) THEN
   1160             RA(I) = RA(I)/RMAG
   1161          ELSE IF(IMICCEN.EQ.2) THEN
   1162             RA(I) = RA(I)*10000./RMAG
   1163          ENDIF
   1164          IF(I.EQ.1) RAMAX = RA(I)
   1165          IF(I.GE.2) THEN
   1166               IF(RA(I).GT.RAMAX) RAMAX = RA(I)
   1167          ENDIF
   1168          IF ((TMA(I).GT.0.).OR.(RA(I).GT.0.)) GO TO 4250
   1169          ICOUNT=I-1
   1170          IF (TM1.LE.TMA(ICOUNT)) THEN
   1171             WRITE(*,*)
   1172             WRITE(*,*)'PLEASE MAKE SURE TM1 IS LARGER THAN THE TIME'
   1173             WRITE(*,*)'OF THE LAST DATA POINT, AND RUN IT AGAIN.'
   1174             GO TO 6000
^H                                                                      Page
22

                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
   1175          END IF
   1176 C-----
   1177 C     CONVERTING INPUT DATA, ENTERED BY THE TERMINAL, TO MICRONS
   1178 C-----
   1179       ELSE IF(IRELPSE.EQ.1.AND.ITERINP.EQ.1) THEN
   1180          WRITE(10,4235)
   1181          WRITE(10,4240)
   1182          DO 4255 K=1,ICOUNT+1
1  1183             RSAVE(K) = RA(K)
1  1184             WRITE(10,4253) TMA(K),RA(K)
1  1185 4253        FORMAT(5X,F7.2,5X,E11.5)
1  1186 4255    CONTINUE
   1187          IF(IMICCEN.EQ.1)THEN
   1188              DO 4260 K=1,ICOUNT
```

```
1  1189                  RA(K) = RA(K)/RMAG
1  1190 4260          CONTINUE
   1191              RAMAX = RAMAX/RMAG
   1192           ELSE IF(IMICCEN.EQ.2) THEN
   1193               DO 4270 K=1,ICOUNT
1  1194                  RA(K) = RA(K)*10000./RMAG
1  1195 4270          CONTINUE
   1196               RAMAX = RAMAX*10000./RMAG
   1197           ENDIF
   1198 C-----
   1199 C     GENERATING THE SIMULATED (PSEUDO) EXPERIMENTAL DATA
   1200 C-----
   1201        ELSE IF(IRELPSE.EQ.2) THEN
   1202           ICOUNT = ((TM01-TM0)/DT1+(TM1-TM01)/DT2+1)
   1203           CALL RGKT(TMS,RB,IT,DT,RINIT,PTRU)
   1204           DO 4280 J=1,IT
1  1205             TMA(J) = TMS(J)
1  1206             RC(J) = RB(J)
1  1207 4280      CONTINUE
   1208           TMA(1) = TM0
   1209           DO 4290 J=2,ICOUNT
1  1210              IF (TMA(J-1).LT.TM01) THEN
1  1211                 TMA(J)=TMA(J-1)+DT1
1  1212              ELSE IF (TMA(J-1).GE.TM01) THEN
1  1213                 TMA(J)=TMA(J-1)+DT2
1  1214              END IF
1  1215 4290      CONTINUE
   1216           IF(IMICCEN.EQ.1) THEN
   1217               DR = DR/RMAG
   1218           ELSE IF(IMICCEN.EQ.2) THEN
   1219               DR = DR*10000./RMAG
   1220           ENDIF
   1221           RAMAXC = RC(1)
   1222           DO 4292 J=2,IT
1  1223                 IF(RC(J).GT.RAMAXC) RAMAXC = RC(J)
1  1224 4292      CONTINUE
   1225           RAMAX = RB(1)
   1226           RA(1) = RB(1)
   1227           DO 4300 J=2,ICOUNT
1  1228              ITM=INT(TMA(J)/DT)+1
1  1229              IF (ITM.GE.IT) THEN
1  1230                 RA(J)=RB(ITM)+DR*RANND(XSEED,ISC)
^H                                                                    Page
23

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
1  1231              ELSE
1  1232                 DRA=(RB(ITM+1)-RB(ITM))*(TMA(J)-(ITM-1)*DT)/DT
1  1233                 RA(J)=RB(ITM)+DRA+DR*RANND(XSEED,ISC)
1  1234              END IF
1  1235              IF(RA(J).GT.RAMAX) RAMAX = RA(J)
```

```
1  1236 4300     CONTINUE
   1237          WRITE(10,*)
   1238       WRITE(10,4302)
   1239 4302  FORMAT(1X,'**********  SIMULATED RADIUS VERSUS TIME'
   1240      .        '  **************')
   1241          WRITE(10,*)
   1242          DO 4305 J=1,ICOUNT+1
1  1243             IF(IMICCEN.EQ.1) THEN
1  1244                  RSAVE(J) = RA(J)*RMAG
1  1245             ELSE IF(IMICCEN.EQ.2) THEN
1  1246                  RSAVE(J) = RA(J)*RMAG/10000.
1  1247             ENDIF
1  1248 4305     CONTINUE
   1249          WRITE(10,4240)
   1250          DO 4310 J = 1,ICOUNT
1  1251               WRITE(10,4253) TMA(J), RSAVE(J)
1  1252 4310     CONTINUE
   1253       ENDIF
   1254 C-----
   1255 C     CALCULATING THE NORMALIZED VOLUME
   1256 C-----
   1257       DO 4315 I=1,ICOUNT
1  1258          VOLA(I)=(RA(I)/RAMAX)**3
1  1259          IF(IRELPSE.EQ.2) VOLC(I) =(RC(I)/RAMAXC)**3
1  1260 4315  CONTINUE
   1261 C-----
   1262 C     CALCULATE SUM OF ERROR OF SQUARE FOR P VALUES FROM
   1263 C     P0 TO P1
   1264 C-----
   1265       WRITE(*,*)
   1266       WRITE(*,*)'CALCULATING SUM OF SQUARE OF ERRORS FROM P0 TO P1.'
   1267       IP=(P1-P0)/DP+1
   1268       DO 4330 I=1,IP
1  1269          P(I)=P0+(I-1)*DP
1  1270          CALL RGKT(TMB,RB,IT,DT,RINIT,P(I))
1  1271          SUM(I)=0.
1  1272          DO 4320 J=1,ICOUNT
2  1273             ITM=INT(TMA(J)/DT)+1
2  1274             IF (ITM.GE.IT) THEN
2  1275                RN=RB(ITM)
2  1276             ELSE
2  1277                DRN=(RB(ITM+1)-RB(ITM))*(TMA(J)-(ITM-1)*DT)/DT
2  1278                RN=RB(ITM)+DRN
2  1279             END IF
2  1280             SUM(I)=SUM(I)+(RN-RA(J))**2
2  1281 4320     CONTINUE
1  1282 4330  CONTINUE
   1283 C-----
   1284 C
   1285 C-----
   1286       WRITE(10,4332)
^H                                                                    Page
```

```
24
                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
   1287 4332  FORMAT(///,1X,'**************  SUM OF SQUARES OF ERRORS ',
   1288      .               'VERSUS PERMEABILITY  ***************')
   1289       WRITE(10,4335)
   1290 4335  FORMAT(/,'   P(J)        SUM(J)',/)
   1291       DO 4338 J=1,IP
1  1292          WRITE(10,4460) P(J),SUM(J)
1  1293 4338  CONTINUE
   1294 C
   1295       SUMIN=SUM(1)
   1296       PEST = P(1)
   1297       DO 4340 I=2,IP
1  1298          IF (SUMIN.GT.SUM(I)) THEN
1  1299             SUMIN=SUM(I)
1  1300             PEST=P(I)
1  1301          END IF
1  1302 4340   CONTINUE
   1303       CALL RGKT(TMB,RC,IT,DT,RINIT,PEST)
   1304       CALL RGKT(TMB,RB,IT,DT,RINIT,PEST+EP1*PEST)
   1305       DO 4350 J=1,IT
1  1306          SEN(J) = (RB(J)-RC(J))/(EP1*PEST)
1  1307 4350  CONTINUE
   1308       DO 4360 J=1,ICOUNT
1  1309          ITM=INT(TMA(J)/DT)+1
1  1310          IF (ITM.GE.IT) THEN
1  1311             SEN(J)=SEN(ITM)
1  1312          ELSE
1  1313             DSEN=(SEN(ITM+1)-SEN(ITM))*(TMA(J)-(ITM-1)*DT)/DT
1  1314             SEN(J)=SEN(ITM)+DSEN
1  1315          END IF
1  1316 4360  CONTINUE
   1317       SENSUM=0.
   1318       DO 4365 I=1,ICOUNT
1  1319          SENSUM=SENSUM+SEN(I)**2
1  1320 4365  CONTINUE
   1321 C----
   1322 C     DUMPING SUMMARY INPUT PARAMETERS, INPUT DATA AND RESULTING
   1323 C     PEST, SDP, SUMIN TO 'O_SMRY.DAT'
   1324 C----
   1325       WRITE(20,*)
   1326       WRITE(20,4366)
   1327 4366  FORMAT(//,'************************  THIS IS FILE',
   1328      .          ' O_SMRY.DAT  ************************',//)
   1329       WRITE(20,4367)
   1330 4367  FORMAT(/,'THE INPUT PARAMETERS AND DATA WERE:',//)
   1331       WRITE(20,2190) RL1,RL2,LIP,D2,D1,H,CINIT,CINF,VINA,RMAG
   1332       WRITE(20,*)
   1333       WRITE(20,2240) DT,TM0,TM1,DP,P0,P1,PSEN
   1334       IF(IRELPSE.EQ.1) THEN
```

```
   1335          WRITE(20,*)
   1336          WRITE(20,*)'THE DATA POINTS ENTERED WERE:'
   1337          WRITE(20,2262) DELAY
   1338          WRITE(20,*)
   1339          WRITE(20,*)'  J       TIME(J)      RADIUS(J)'
   1340          IF(IMICCEN.EQ.1) THEN
   1341          WRITE(20,*)'           (SEC.)      (MICRONS)'
   1342          ELSEIF(IMICCEN.EQ.2) THEN
^H                                                                    Page
25
                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
   1343          WRITE(20,*)'           (SEC.)      (CENTIMETERS)'
   1344          ENDIF
   1345          DO 4368 J=1,ICOUNT+1
1  1346             WRITE(20,2263) J,TMA(J),RA(J)
1  1347 4368     CONTINUE
   1348         ELSEIF(IRELSPE.EQ.2) THEN
   1349          WRITE(20,*)
   1350          WRITE(20,2320) PTRU,RINIT,XMICCEN,DR,XMICCEN,DT1,DT2,TM01
   1351         ENDIF
   1352 C----
   1353 C     THE ESITMATED PERMEABILITY
   1354 C----
   1355       WRITE(10,4375)
   1356       WRITE(20,4375)
   1357 4375  FORMAT(///,'*************************  THE RESULTING '
   1358      .           'PERMEABILITY  ************************',/)
   1359       SDP=SQRT(SUMIN/(ICOUNT-1)/SENSUM)
   1360       WRITE(10,4380)PEST
   1361       WRITE(20,4380)PEST
   1362 4380  FORMAT(/,'THE LOCAL MINIMUM OCCURS AT P =',F8.3,
   1363      .         ' MICRONS/SEC.')
   1364       WRITE(10,4385)SDP
   1365       WRITE(20,4385)SDP
   1366 4385  FORMAT(/,'THE STANDARD DEVIATION OF ESTIMATED P IS  ',E8.3)
   1367       WRITE(10,4390)SUMIN
   1368       WRITE(20,4390)SUMIN
   1369 4390  FORMAT(/,'THE MINIMUM VALUE OF SUM IS',F8.3)
   1370 C
   1371 C
   1372       IF(IRELPSE.EQ.1) THEN
   1373           DO 4400 J=1,IT
1  1374              IF(J.EQ.1) RAMAXC = RC(1)
1  1375              IF(J.GE.2.AND.RC(J).GT.RAMAXC) RAMAXC = RC(J)
1  1376 4400      CONTINUE
   1377           DO 4410 J=1,IT
1  1378              VOLC(J) = (RC(J)/RAMAXC)**3
1  1379 4410      CONTINUE
   1380       ENDIF
   1381 C-----
```

```
   1382 C     IF IF3 = 1 (YES), WRITE THE DATA TO VOL1 AND VOL2
   1383 C-----
   1384       IF(IF3.EQ.0) GO TO 4450
   1385          OPEN(17,FILE=VOL1,STATUS='NEW')
   1386          OPEN(18,FILE=VOL2,STATUS='NEW')
   1387          DO 4430 J=1,ICOUNT
1  1388             WRITE(17,4420) TMA(J),VOLA(J)
1  1389 4420        FORMAT(1X,F7.2,1X,',',1X,F7.5)
1  1390 4430     CONTINUE
   1391          DO 4431 J=1,IT
1  1392             WRITE(18,4420) TMS(J), VOLC(J)
1  1393 4431     CONTINUE
   1394          WRITE(*,*)
   1395          WRITE(*,*)'THE DATA FOR THE GRAPH NORMALIZED VOLUME V.S.'
   1396          WRITE(*,*)'TIME HAS BEEN SENT TO FILES -'
   1397          WRITE(*,4432) VOL1, VOL2
   1398 4432     FORMAT(1X,A10,' AND ',A10)
^H                                                                    Page
26

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
   1399          CLOSE(17,STATUS='KEEP')
   1400          CLOSE(18,STATUS='KEEP')
   1401 C
   1402 4450  CONTINUE
   1403 C-----
   1404 C     IF IF4 = 1 (YES), WRITE THE DATA TO SUMR
   1405 C-----
   1406       IF(IF4.EQ.0) GO TO 6000
   1407          OPEN(19,FILE=SUMR,STATUS='NEW')
   1408          DO 4470 J=1,IP
1  1409             WRITE(19,4460) P(J),SUM(J)
1  1410 4460        FORMAT(1X,F8.2,1X,',',1X,E9.2)
1  1411 4470     CONTINUE
   1412          WRITE(*,*)
   1413          WRITE(*,*)'THE DATA FOR THE GRAPH OF THE SUM OF SQUARE OF'
   1414          WRITE(*,*)'ERRORS V.S. PERMEABILITY HAS BEEN SENT TO FILE - '
   1415          WRITE(*,'(1X,A10)') SUMR
   1416          CLOSE(19,STATUS='KEEP')
   1417 6000  CONTINUE
   1418       WRITE(*,*)
   1419       WRITE(*,6100) PEST,SDP,SUMIN
   1420 6100  FORMAT(' THE ESTIMATED PERMEABILITY IS ',F8.3,' MICRONS/SEC.',//,
   1421      .       ' THE STANDARD DEVIATION IS ',E9.3,' MICRONS/SEC.',//,
   1422      .       ' THE MINIMUM SUM OF THE SQUARE OF THE ERRORS IS ',E9.3)
   1423 C-----
   1424 C      RESETTING THE RA(I) = RSAVE(I) AND RINIT, RAMAX TO ORIGNAL VALUES
   1425 C-----
   1426       IF(IMICCEN.EQ.1) THEN
   1427           RINIT = RINIT * RMAG
   1428           RAMAX = RAMAX * RMAG
```

```
   1429           IF(IRELPSE.EQ.2) DR = DR * RMAG
   1430           DO 280 K = 1, ICOUNT+1
1  1431               RA(K) = RSAVE(K)
1  1432 280       CONTINUE
   1433       ELSE IF(IMICCEN.EQ.2) THEN
   1434           RINIT = RINIT *RMAG/10000.
   1435           RAMAX = RAMAX *RMAG/10000.
   1436           IF(IRELPSE.EQ.2) DR = DR *RMAG/10000.
   1437           DO 290 K = 1,ICOUNT+1
1  1438               RA(K) = RSAVE(K)
1  1439 290       CONTINUE
   1440       ENDIF
   1441 C-----
   1442 C     OPTION TO SAVE THE INPUT FILE
   1443 C-----
   1444       WRITE(*,*)
   1445       WRITE(*,*)'DO YOU WISH TO SAVE THE INPUT DATA IN A FILE,'
   1446       WRITE(*,*)'(Y/N)?'
   1447       READ(*,'(A1)') ISAVE
   1448       CALL IYESNO(ISAVE)
   1449 319   CONTINUE
   1450       IF(ISAVE.EQ.'Y') THEN
   1451          IF(IEXP.EQ.1) GO TO 331
   1452 320      CONTINUE
   1453          WRITE(*,*)
   1454          WRITE(*,*)'WHAT WOULD YOU LIKE TO NAME THIS FILE,'
^H                                                                    Page
27

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
   1455          WRITE(*,*)'(ENTER NO MORE THAN 10 CHARATERS)?'
   1456          READ(*,'(A10)',ERR=320) NAMFIL
   1457          OPEN(12,FILE=NAMFIL,STATUS='NEW')
   1458          WRITE(12,325)
   1459 325      FORMAT(1X,'THIS IS THE FILE YOU HAD SAVED.  YOU CAN '
   1460     .    ,'USE THIS FILE AS',/,' AN INPUT FILE IF YOU DESIRE BY'
   1461     .    ,' ACCESSING THIS FILE ',/,' WHEN YOU ARE PROMPTED FOR'
   1462     .    ,' THE NAME OF AN INPUT FILE.')
   1463 331      CONTINUE
   1464          WRITE(12,*)'RL1        RL2        LIP'
   1465          WRITE(12,332)RL1,RL2,LIP
   1466 332      FORMAT(1X,F5.2,6X,F7.2,4X,I2,/)
   1467          WRITE(12,*)'D1         D2         H'
   1468          WRITE(12,333) D1,D2,H
   1469 333      FORMAT(1X,E9.3,2X,E9.3,2X,E9.3,/)
   1470          WRITE(12,*)'CINIT      CINF'
   1471          WRITE(12,334) CINIT,CINF
   1472 334      FORMAT(1X,F8.3,3X,F8.3,/)
   1473          WRITE(12,*)'VINA       RMAG'
   1474          WRITE(12,335) VINA,RMAG
   1475 335      FORMAT(1X,F5.2,6X,F7.2,/)
```

```
   1476          WRITE(12,*)'DT          TM0         TM1         DELAY'
   1477          WRITE(12,336) DT,TM0,TM1,DELAY
   1478 336      FORMAT(1X,F6.3,5X,F7.2,4X,F7.2,4X,F7.2,/)
   1479          WRITE(12,*)'DP          P0          P1          PSEN'
   1480          WRITE(12,337) DP,P0,P1,PSEN
   1481 337      FORMAT(1X,F5.2,5X,F6.2,6X,F6.2,5X,F6.2,5X,/)
   1482          WRITE(12,*)'RINIT'
   1483          WRITE(12,338) RINIT
   1484 338      FORMAT(1X,E9.3,/)
   1485          WRITE(12,339)
   1486 339      FORMAT(1X,'THE PARAMETERS FOR THE LINE BELOW ARE FOR ',
   1487       .  'SIMULATION OPTION:')
   1488          WRITE(12,*)'PTRU        DR          TM01        DT1         DT2'
   1489          WRITE(12,340) PTRU,DR,TM01,DT1,DT2
   1490 340      FORMAT(1X,F6.2,5X,F7.3,4X,F7.2,4X,F6.3,5X,F6.3,/)
   1491          WRITE(12,341)
   1492 341      FORMAT(1X,'THESE PARAMETERS BELOW ARE FOR DATA INPUT '
   1493       .  ,'CONTROL:')
   1494          WRITE(12,*)'IRELPSE  IMICCEN'
   1495          WRITE(12,342) IRELPSE, IMICCEN
   1496 342      FORMAT(1X,I1,10X,I1,/)
   1497          WRITE(12,*)'THESE PARAMETERS ARE FOR DATA OUTPUT CONTROL:'
   1498          WRITE(12,*)'IF1         IF2         IF3         IF4         IPRINT'
   1499          WRITE(12,345) IF1,IF2,IF3,IF4,IPRINT
   1500 345      FORMAT(1X,I1,10X,I1,10X,I1,10X,I1,10X,I2,/)
   1501          WRITE(12,346)
   1502 346      FORMAT(1X,'THESE ARE THE FILE NAMES FOR THE GRAPHICAL ',
   1503       .  'OUTPUT:')
   1504          WRITE(12,347) CON1,CON2,SENC,VOL1,VOL2,SUMR
   1505 347      FORMAT(/,1X,A10,1X,A10,/1X,A10,/1X,A10,1X,A10,/1X,A10,/)
   1506          WRITE(12,349)
   1507 349      FORMAT(1X,'THE LAST GROUP BELOW ARE THE DATA POINTS:')
   1508          WRITE(12,*)'   TMA(I)       RA(I)'
   1509          DO 360 I =1,ICOUNT
1  1510             WRITE(12,350) TMA(I), RA(I)
^H                                                                    Page
28

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
1  1511 350          FORMAT(1X,F9.4,5X,F9.4)
1  1512 360       CONTINUE
   1513           WRITE(12,*)' 0.0           0.0'
   1514           CLOSE(12,STATUS='KEEP')
   1515           IF(IQUIT.EQ.'Y') GO TO 600
   1516           WRITE(*,*)
   1517           WRITE(*,362) NAMFIL
   1518 362       FORMAT(' OKAY, THE FILE ',A10,' HAS BEEN SAVED.')
   1519        ENDIF
   1520        CLOSE(11,STATUS='KEEP')
   1521 C-----
   1522 C     OPTION TO RUN THE PROGRAM AGAIN
```

```
1523 C-----
1524       WRITE(*,*)
1525       WRITE(*,*)'DO YOU WISH TO RUN THE PROGRAM AGAIN, (Y/N)?'
1526       READ(*,'(A1)') IAGAIN
1527       CALL IYESNO(IAGAIN)
1528       IOPNAG = IOPNAG + 1
1529       IF(IAGAIN.EQ.'Y') THEN
1530 500       CONTINUE
1531 C-----
1532 C     OPTION TO ENTER NEW DATA OR REVIEW OLD DATA
1533 C-----
1534           WRITE(*,*)
1535           WRITE(*,*)'DO YOU WISH TO (1) ENTER ALL NEW DATA OR '
1536           WRITE(*,*)'(2) USE AND REVIEW THE DATA ALREADY ENTERED,'
1537           WRITE(*,*)'(ENTER 1 OR 2)?'
1538           READ(*,*,ERR=500) INEWREV
1539           CALL IONETWO(INEWREV)
1540           DELAY = 0.0
1541           IBACK = 0
1542           IF(INEWREV.EQ.1) THEN
1543               IRUNAG = 0
1544               GO TO 2000
1545           ELSE IF(INEWREV.EQ.2) THEN
1546               ITERINP = 1
1547               IRUNAG = IRUNAG + 1
1548               GO TO 2180
1549           ENDIF
1550       ENDIF
1551 C-----
1552 C      STOPPING THE PROGRAM.
1553 C-----
1554 600   WRITE(*,*)
1555       WRITE(*,*)'OKAY, PROGRAM DONE.'
1556       CLOSE(10,STATUS='KEEP')
1557       CLOSE(20,STATUS='KEEP')
1558       END

Name    Type        Offset P Class

A1      REAL             8   /C1    /
A2      REAL            16   /C1    /
CA      REAL         15668
CB      REAL         15768
CINF    REAL            24   /C3    /
^H                                                                  Page
29
                                                                 07-20-87
                                                                 19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
CINIT   REAL            20   /C3    /
CON1    CHAR*10      15964
CON2    CHAR*10      15974
```

COUT	REAL	16	/C2 /
D1	REAL	12	/C3 /
D2	REAL	16	/C3 /
DELAY	REAL	15904	
DMY327	REAL	0	LARGE
DP	REAL	15908	
DR	REAL	15928	
DRA	REAL	19444	
DRN	REAL	19560	
DSEN	REAL	19732	
DT	REAL	8	/C2 /
DT1	REAL	15936	
DT2	REAL	15940	
EP1	REAL		PARAMETER
F			EXTERNAL
H	REAL	28	/C3 /
I	INTEGER*4	16028	
IAGAIN	CHAR*1	21420	
IANOTH	CHAR*1	18080	
IBACK	INTEGER*4	15876	
ICHAIN	INTEGER*4	17444	
ICHANG	CHAR*1	16748	
ICOUNT	INTEGER*4	16024	
IDEL	CHAR*1	17212	
IEXP	INTEGER*4	15892	
IF1	INTEGER*4	4	/C1 /
IF2	INTEGER*4	15952	
IF3	INTEGER*4	15956	
IF4	INTEGER*4	15960	
IFIRST	CHAR*1	15889	
IGRAPH	CHAR*1	18074	
II	INTEGER*4		PARAMETER
IMICCE	INTEGER*4	15948	
IMISTA	CHAR*1	15888	
IMORE	CHAR*1	17442	
INEWRE	INTEGER*4	21422	
INR	INTEGER*4	17208	
INT			INTRINSIC
IOPNAG	INTEGER*4	15884	
IOPTGR	INTEGER*4	18076	
IP	INTEGER*4	18972	
IPRINT	INTEGER*4	0	/C1 /
IQUIT	CHAR*1	15890	
IRELPS	INTEGER*4	15944	
IRELSP	INTEGER*4	19904	
IRUNAG	INTEGER*4	15880	
ISAVE	CHAR*1	16032	
ISC	INTEGER*4	15872	
ISENS	CHAR*10	16038	
ISEPPR	INTEGER*4	16048	
IT	INTEGER*4	19024	
ITERIN	INTEGER*4	16034	

```
ITM     INTEGER*4      19440
^H                                                                    Page
30
                                                                    07-20-87
                                                                    19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
J       INTEGER*4      17410
JI      INTEGER*4      17448
K       INTEGER*4      19384
LIP     INTEGER*4          8   /C3    /
N1      INTEGER*4              PARAMETER
N3      INTEGER*4              PARAMETER
N4      INTEGER*4              PARAMETER
N5      INTEGER*4              PARAMETER
NAMFIL  CHAR*10        20516
NCHANG  INTEGER*4      16750
O       REAL           18472
P       REAL           14464
PO      REAL           15912
P1      REAL           15916
PEST    REAL           19716
PROBLE  CHAR*11        18081
PSEN    REAL           15920
PTRU    REAL           15924
RA      REAL            6036
RAMAX   REAL           17358
RAMAXC  REAL           19428
RANND   REAL                  FUNCTION
RB      REAL            7240
RC      REAL           13260
RINIT   REAL               0   /C2    /
RL1     REAL               0   /C3    /
RL2     REAL               4   /C3    /
RMAG    REAL           15896
RN      REAL           19556
RS      REAL           10852
RSAVE   REAL           12056
SDP     REAL           20024
SEN     REAL            8444
SENC    CHAR*10        15984
SENSUM  REAL           19736
SQRT                          INTRINSIC
SUM     REAL            9648
SUMIN   REAL           19712
SUMR    CHAR*10        16014
TM0     REAL           15900
TM01    REAL           15932
TM1     REAL              12   /C2    /
TMA     REAL              16
TMB     REAL            1220
TMS     REAL            4832
VINA    REAL               4   /C2    /
```

```
VOL1    CHAR*10        15994
VOL2    CHAR*10        16004
VOLA    REAL            3628
VOLC    REAL            2424
XMICCE  CHAR*11        17558
XSEED   REAL           15868


   1559 C-----
   1560 C     SUBROUTINE RGKT(X,Y,N,DX,Y0,P)
^H                                                                      Page
31
                                                                     07-20-87
                                                                     19:28:31
D Line# 1      7                              Microsoft FORTRAN77 V3.31 August 1985
   1561 C
   1562 C     USE RUNGE-KUTTA METHOD TO SOLVE ORDINARY DIFFERENTIAL
   1563 C     EQUATION
   1564 C
   1565 C     X: INDEPENDENT VARIABE
   1566 C
   1567 C     Y: DEPENDENT VARIABLE
   1568 C
   1569 C     N: DIMENSION OF X(N) AND Y(N)
   1570 C
   1571 C     DX: INCREMENT OF X
   1572 C
   1573 C     Y0: INITIAL CONDITION OF Y
   1574 C
   1575 C     P: PARAMETER
   1576 C
   1577 C     F: THE SUPLLIED FUNCTION.  (DY/DX=F(X,Y))
   1578 C-----
   1579       SUBROUTINE RGKT(X,Y,N,DX,Y0,P)
   1580       DIMENSION X(N),Y(N)
   1581       Y(1)=Y0
   1582       DO 1 I=1,N-1
1  1583          RK1=DX*F(X(I),Y(I),P)
1  1584          RK2=DX*F(X(I)+DX/2.,Y(I)+RK1/2.,P)
1  1585          RK3=DX*F(X(I)+DX/2.,Y(I)+RK2/2.,P)
1  1586          RK4=DX*F(X(I)+DX,Y(I)+RK3,P)
1  1587          Y(I+1)=Y(I)+(RK1+2*RK2+2*RK3+RK4)/6.
1  1588 1     CONTINUE
   1589       RETURN
   1590       END

Name    Type         Offset P Class

DX      REAL             12 *
F       REAL                    FUNCTION
I       INTEGER*4     21426
N       INTEGER*4         8 *
```

```
P       REAL              20 *
RK1     REAL           21434
RK2     REAL           21438
RK3     REAL           21442
RK4     REAL           21446
X       REAL               0 *
Y       REAL               4 *
Y0      REAL              16 *


   1591 C-----
   1592 C     FUNCTION F(X,Y,Z)
   1593 C
   1594 C     X: INDEPENDENT VARIABLE
   1595 C
   1596 C     Y: DEPENDENT VARIABLE
   1597 C
   1598 C     Z: PARAMETER
   1599 C-----
^H                                                                      Page
32
                                                                     07-20-87
                                                                     19:28:31
D Line# 1      7                                Microsoft FORTRAN77 V3.31 August 1985
   1600        FUNCTION F(X,Y,Z)
   1601        PARAMETER (N4=301,PI=3.14159)
   1602        COMMON /C2/RINIT,VINA,DT,TM1,COUT(N4)
   1603        COMMON /C3/RL1,RL2,LIP,D1,D2,CINIT,CINF,H
   1604        V=0.018
   1605        I=INT(X/DT)+1
   1606        C0=COUT(I)+(COUT(I+1)-COUT(I))*(X-(I-1)*DT)/DT
   1607        V0=(4.*PI*(RINIT**3))/3.
   1608        VIN=VINA*V0/100.
   1609        F=-Z*V*(C0-CINIT*(V0-VIN)/(4.*PI*Y**3/3.-VIN))
   1610        RETURN
   1611        END

Name    Type         Offset P Class

C0      REAL          21458
CINF    REAL             24   /C3    /
CINIT   REAL             20   /C3    /
COUT    REAL             16   /C2    /
D1      REAL             12   /C3    /
D2      REAL             16   /C3    /
DT      REAL              8   /C2    /
H       REAL             28   /C3    /
I       INTEGER*4     21454
INT                           INTRINSIC
LIP     INTEGER*4         8   /C3    /
N4      INTEGER*4             PARAMETER
PI      REAL                  PARAMETER
```

```
RINIT   REAL              0   /C2    /
RL1     REAL              0   /C3    /
RL2     REAL              4   /C3    /
TM1     REAL             12   /C2    /
V       REAL          21450
V0      REAL          21462
VIN     REAL          21466
VINA    REAL              4   /C2    /
X       REAL              0 *
Y       REAL              4 *
Z       REAL              8 *


   1612 C-----
   1613 C     FUNCTION RANND(XSEED,ISC)
   1614 C
   1615 C     RANDOM VARIABLE GENERATOR
   1616 C     NORMAL DISTRIBUTION WITH STANDARD DEVIATION EQUAL TO 1.
   1617 C-----
   1618       FUNCTION RANND(XSEED,ISC)
   1619 C     DOUBLE PRECISION RANDOM
   1620 C
   1621 C     GENERATING A UNIFORM RANDOM NUMBER
   1622 C
   1623       INTEGER A,X
   1624       IF(ISC.EQ.0) X = XSEED
   1625 C
   1626       A = 2**10 + 3
^H                                                                    Page
33

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
   1627       M = 2**20
   1628       FM = M
   1629       X = MOD(A*X, M)
   1630       FX = X
   1631       XSEED = X
   1632       Z = FX/FM
   1633 C
   1634 C     CONVERTING THE RANDOM NUMBER WITH A UNIFORM DISTRIBUTION TO A
   1635 C     RANDOM NUMBER HAVING A NORMAL DISTIBUTION
   1636 C
   1637 C     R = RANDOM()
   1638       R = Z
   1639       A0=2.30753
   1640       A1=0.27061
   1641       B1=0.99299
   1642       B2=0.04481
   1643       IF (R-0.5) 10,10,20
   1644 10    AK=1.
   1645       GO TO 30
```

```
   1646 20     AK=-1.
   1647        R=R-0.5
   1648 30     T=SQRT(ALOG(1./(R*R)))
   1649        E=T-(A0+A1*T)/(1.+B1*T+B2*T*T)
   1650        RANND=AK*E
   1651        ISC = ISC + 1
   1652        RETURN
   1653        END

Name     Type         Offset P Class

A        INTEGER*4     21474
A0       REAL          21498
A1       REAL          21502
AK       REAL          21514
ALOG                           INTRINSIC
B1       REAL          21506
B2       REAL          21510
E        REAL          21522
FM       REAL          21482
FX       REAL          21486
ISC      INTEGER*4         4 *
M        INTEGER*4     21478
MOD                            INTRINSIC
R        REAL          21494
SQRT                           INTRINSIC
T        REAL          21518
X        INTEGER*4     21470
XSEED    REAL              0 *
Z        REAL          21490


   1654 C
   1655        SUBROUTINE IONETWO(ITEST)
   1656 31     CONTINUE
   1657        IF(ITEST.LE.0.OR.ITEST.GE.3) THEN
   1658           WRITE(*,*)
^H                                                                    Page
34
                                                                   07-20-87
                                                                   19:28:31
D Line# 1      7                          Microsoft FORTRAN77 V3.31 August 1985
   1659           WRITE(*,*)'**  INCORRECT RESPONSE **'
   1660           WRITE(*,*)'PLEASE ENTER  1  OR  2'
   1661           WRITE(*,*)
   1662           READ(*,*) ITEST
   1663           GO TO 31
   1664        ENDIF
   1665        RETURN
   1666        END

Name     Type         Offset P Class
```

```
ITEST  INTEGER*4         0 *

   .

  1667 C
  1668       SUBROUTINE IYESNO(ITEST)
  1669       CHARACTER *1 ITEST
  1670 32    CONTINUE
  1671       IF(ITEST.NE.'Y'.AND.ITEST.NE.'N') THEN
  1672          WRITE(*,*)
  1673          WRITE(*,*)'**  INCORRECT RESPONSE **'
  1674          WRITE(*,*)'PLEASE ENTER  "Y"  OR  "N"'
  1675          WRITE(*,*)
  1676          READ(*,'(A1)') ITEST
  1677          GO TO 32
  1678       ENDIF
  1679       RETURN
  1680       END

Name    Type         Offset P Class

ITEST  CHAR*1             0 *


  1681 C
  1682       SUBROUTINE CHANNAM(FILNAM)
  1683       CHARACTER *1 ICHANG
  1684       CHARACTER *10 FILNAM
  1685 33    CONTINUE
  1686       WRITE(*,*)
  1687       WRITE(*,35) FILNAM
  1688 35    FORMAT(1X,'THE FILE NAME ENTERED IS ',A10)
  1689       WRITE(*,*)
  1690       WRITE(*,*)' DO YOU WISH TO CHANGE IT,(Y/N)? '
  1691       READ(*,'(A1)') ICHANG
  1692       CALL IYESNO(ICHANG)
  1693       IF(ICHANG.EQ.'Y') THEN
  1694          WRITE(*,*)
  1695 36       CONTINUE
  1696          WRITE(*,*)'PLEASE ENTER THE NEW NAME. '
  1697          WRITE(*,*)
  1698          READ(*,'(A10)',ERR=36) FILNAM
  1699          GO TO 33
  1700       ENDIF
  1701       RETURN
  1702       END
^H                                                                    Page
35

                                                                   07-20-87
                                                                   19:28:31
D Line# 1     7                        Microsoft FORTRAN77 V3.31 August 1985
```

```
Name    Type         Offset P Class

FILNAM CHAR*10            0 *
ICHANG CHAR*1         21562


   1703        SUBROUTINE INCORRES
   1704        WRITE(*,*)
   1705        WRITE(*,*)'**  INCORRECT RESPONSE  **'
   1706        RETURN
   1707        END

Name    Type         Offset P Class



Name    Type           Size   Class

C1                       24   COMMON
C2                     1220   COMMON
C3                       32   COMMON
CHANNA                        SUBROUTINE
F       REAL                  FUNCTION
INCORR                        SUBROUTINE
IONETW                        SUBROUTINE
IYESNO                        SUBROUTINE
MBCON                         SUBROUTINE
RANND   REAL                  FUNCTION
RGKT                          SUBROUTINE
SENS                          PROGRAM


 Pass One    No Errors Detected
           1707 Source Lines
^Z
```

```
^H                                                                    Page
1

                                                                   06-28-87
                                                                   20:18:55
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
       1      SUBROUTINE MBCON(TM,CA,CB,IP)
       2 C$DEBUG
       3 $INCLUDE: 'IMSL'
       1 $LARGE: DMY327
       2      DIMENSION DMY327(1)
       4 C-----
       5 C    JOB: 1. PREDICT THE CONCENTRATION CHANGE INSIDE THE CELL
       6 C             CHAMBER
       7 C          2. PLOTTING CONCENTRATION VERSUS TIME CHART
       8 C-----
       9      PARAMETER (II=2,III=3,N4=301)
      10      REAL L1,L2,M
      11      DIMENSION CN(11),CO(11),CE(11,11),
      12     &          WK1(154),CONC(11),CA(25),CB(25),
      13     &          DC(25),TM(25),X(II,II),Y1(II),Y2(II),WK2(10)
      14      COMMON /C1/IPRINT,IF1,A1(II),A2(II)
      15      COMMON /C2/RINIT,VINA,DT,TM1,COUT(N4)
      16      COMMON /C3/RL1,RL2,LIP,D1,D2,CINIT,CINF,H
      17 C-----
      18 C    INITIALIZATION
      19 C-----
      20      N1 = 4
      21      N2 =6
      22      L1=RL1*1.0E-6
      23      L2=RL2*1.0E-6
      24      TMAX=TM1
      25      DX1=L1/N1
      26      DX2=L2/N2
      27      RX=DX2/DX1
      28      P1=DT*D1/DX1/DX1
      29      P2=DT*D2/DX2/DX2
      30      B1=H*DX1/D1
      31      M=2./(1+RX)
      32      DO 1 I=1,N1+N2+1
1     33         CN(I)=0.
1     34         CO(I)=0.
1     35         DO 1 J=1,N1+N2+1
2     36            CE(I,J)=0.
2     37 1    CONTINUE
      38      ICOUNT=0
      39 C-----
      40 C    USING THE BACKWARD DIFFERENCE METHOD TO CALCULATE THE
      41 C    CONCENTRATION INSIDE THE CELL CHAMBER
      42 C-----
      43      WRITE(10,151)
      44 151  FORMAT(///,1X,'*************************  THE CONCENTRATION',
      45     ,           ' HISTORY  *************************')
```

```
     46 C-----
     47 C        INPUT VALUES TO THE COEFFICIENT MATRIX
     48 C-----
     49        CE(1,1)=1+2*P1+2*P1*B1
     50        CE(1,2)=-2*P1
     51        DO 2 I=2,N1
1    52           CE(I,I-1)=-P1
1    53           CE(I,I)=1+2*P1
1    54           CE(I,I+1)=-P1
^H                                                                    Page
2
                                                                     06-28-87
                                                                     20:18:55
D Line# 1     7                                Microsoft FORTRAN77 V3.31 August 1985
1    55 2     CONTINUE
     56        CE(N1+1,N1)=-P1*M
     57        CE(N1+1,N1+1)=1+P1*M+P1*M*(D2/D1)/RX
     58        CE(N1+1,N1+2)=-P1*M*(D2/D1)/RX
     59        DO 3 I=N1+2,N1+N2
1    60           CE(I,I-1)=-P2
1    61           CE(I,I)=1+2*P2
1    62           CE(I,I+1)=-P2
1    63 3     CONTINUE
     64        CE(N1+N2+1,N1+N2)=-2*P2
     65        CE(N1+N2+1,N1+N2+1)=1+2*P2
     66        WRITE(*,*)
     67        WRITE(*,*)'CALCULATING THE COEFFICIENT MATRIX -'
     68        WRITE(*,*)'ENTERING LEQT2F (1)'
     69 6     CO(1)=CO(1)+2*B1*P1
     70 C-----
     71 C     CALCULATE THE COEFFICIENT MATRIX
     72 C-----
     73        NX1 = N1+N2+1
     74        MX = 1
     75        IDGT1 = 3
     76        CALL LEQT2F(CE,MX,NX1,NX1,CO,IDGT1,WK1,IER1)
     77        IX =1
     78        IF(IER1.NE.0) THEN
     79           WRITE(*,*)
     80           WRITE(*,110) IX,IER1,ICOUNT
     81 110       FORMAT(' IER',I1,' = ',I5,5X,'ICOUNT='I4)
     82        ENDIF
     83 C-----
     84 C     PUT CO INTO CN FOR NEXT CALCULATION
     85 C-----
     86        ICOUNT=ICOUNT+1
     87        DO 4 I=1,N1+N2+1
1    88           CN(I)=CO(I)
1    89 4     CONTINUE
     90        COUT(ICOUNT)=CN(LIP)*(CINF-CINIT)+CINIT
     91 C     COUT(ICOUNT)=CINF+(CINIT-CINF)*EXP(-(ICOUNT-1)*DT/19.6)
     92 C-----
```

```
     93 C     CHECK TO SEE WHETHER IT IS TIME TO OUTPUT THE DATA
     94 C-----
     95       IF (ICOUNT/IPRINT*IPRINT.EQ.ICOUNT) THEN
     96          WRITE(10,101)ICOUNT*DT
     97 101      FORMAT(/,'CONCENTRATION DISTRIBUTION AT TIME=',
     98     .             F8.2,'SEC. IS',)
     99          DO 5 I=1,N1+N2+1
1    100            CONC(I)=CN(I)*(CINF-CINIT)+CINIT
1    101 5        CONTINUE
    102       WRITE(10,*)
    103       WRITE(10,*)'    DIALYSIS MEMBRANE          CELL CHAMBER'
    104       WRITE(10,*)'  |                    |                          |'
    105       WRITE(10,*)'B |                    |                          |'
    106       WRITE(10,*)'U |                    |                          |'
    107       WRITE(10,*)'L |                    |                          |'
    108       WRITE(10,*)'K |                    |                          |'
    109       WRITE(10,*)'  |                    |                          |'
    110       WRITE(10,*)'  1         3          5         7          9        11'
^H                                                                        Page
3

                                                                      06-28-87
                                                                      20:18:55
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    111       WRITE(10,201) CONC(1),CONC(3),CONC(5),CONC(7),CONC(9),CONC(11)
    112 201   FORMAT(1X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3)
    113       WRITE(10,*)'  |                    |                          |'
    114       WRITE(10,*)'  |    2          4    |     6          8       10    |'
    115       WRITE(10,202) CONC(2),CONC(4),CONC(6),CONC(8),CONC(10)
    116 202   FORMAT(3X,E9.3,2X,E9.3,1X,E9.3,1X,E9.3,1X,E9.3)
    117       WRITE(10,*)'  |                    |                          |'
    118       WRITE(10,*)'F |                    |                          |'
    119       WRITE(10,*)'L |                    |                          |'
    120       WRITE(10,*)'O |                    |                          |'
    121       WRITE(10,*)'W |                    |                          |'
    122       WRITE(10,*)'  |                    |                          |'
    123       WRITE(10,*)
    124       WRITE(10,*)
    125          IP=ICOUNT/IPRINT
    126          IF (CN(N1+1).GE.1.) CN(N1+1)=1.-1.E-6
    127          IF (CN(N1+N2+1).GE.1.) CN(N1+N2+1)=1.-1.E-6
    128          CA(IP)=-LOG(1-CN(N1+1))
    129          CB(IP)=-LOG(1-CN(N1+N2+1))
    130          DC(IP)=CONC(N1+1)-CONC(N1+N2+1)
    131          TM(IP)=ICOUNT*DT/TMAX
    132       END IF
    133 C-----
    134 C     CHECK TO SEE WHETHER IT IS TIME TO STOP THE EXECUTION
    135 C-----
    136       IF (ICOUNT*DT.LT.TMAX+DT) GO TO 6
    137       WRITE(*,*)
    138       WRITE(*,*)'LEAVING LEQT2F (1)'
    139 C-----
```

```
    140 C     FINDING THE BEST LINEAR FIT FOR THE CONCENTRATION VERSUS
    141 C     TIME POINTS
    142 C-----
    143       DO 10 I=1,2
1   144          X(I,2)=0.
1   145          Y1(I)=0.
1   146          Y2(I)=0.
1   147 10    CONTINUE
    148       DO 11 I=1,IP
1   149          X(1,2)=X(1,2)+TM(I)
1   150          X(2,2)=X(2,2)+TM(I)**2
1   151          Y1(1)=Y1(1)+CA(I)
1   152          Y1(2)=Y1(2)+CA(I)*TM(I)
1   153          Y2(1)=Y2(1)+CB(I)
1   154          Y2(2)=Y2(2)+CB(I)*TM(I)
1   155 11    CONTINUE
    156       X(2,1)=X(1,2)
    157       X(1,1)=IP
    158       NX2 = 2
    159       IDGT2 = 3
    160       IDGT3 = 3
    161       WRITE(*,*)
    162       WRITE(*,*)'FINDING BEST LINEAR FIT FOR CONCENTRATION V.S. TIME -'
    163       WRITE(*,*)
    164       WRITE(*,*)'ENTERING LEQT2F (2)'
    165       CALL LEQT2F(X,MX,NX2,NX2,Y1,IDGT2,WK2,IER2)
    166       WRITE(*,*)
^H                                                                    Page
4

                                                                   06-28-87
                                                                   20:18:55
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
    167       WRITE(*,*)'LEAVING LEQT2F (2)'
    168       IX = 2
    169       IF(IER2.NE.0) THEN
    170          WRITE(*,*)
    171          WRITE(*,120) IX, IER2
    172 120      FORMAT(' IER',I1,' = ',I3)
    173       ENDIF
    174       DO 200 K = 1,2
1   175           A1(K) = Y1(K)
1   176 200   CONTINUE
    177       WRITE(*,*)
    178       WRITE(*,*)'ENTERING LEQT2F (3)'
    179       CALL LEQT2F(X,MX,NX2,NX2,Y2,IDGT3,WK2,IER3)
    180       WRITE(*,*)
    181       WRITE(*,*)'LEAVING LEQT2F (3)'
    182       IX = 3
    183       IF(IER3.NE.0) THEN
    184          WRITE(*,*)
    185          WRITE(*,120) IX, IER2
    186       ENDIF
```

```
    187       DO 210 K =1,2
1   188          A2(K) = Y2(K)
1   189 210   CONTINUE
    190       WRITE(10,103)A1(1),A1(2)
    191 103   FORMAT(/,' THE EQUATION FOR THE LINEAR BEST FIT FOR THE',/,
    192      .         ' CONCENTRATION VERSUS TIME IS,',/,
    193      .         ' Y = ',F6.3,' + ',F6.3,' *X',/)
    194       WRITE(10,103)A2(1),A2(2)
    195       RETURN
    196       END

Name    Type         Offset P Class

A1      REAL              8   /C1    /
A2      REAL             16   /C1    /
B1      REAL           1460
CA      REAL              4 *
CB      REAL              8 *
CE      REAL            936
CINF    REAL             24   /C3    /
CINIT   REAL             20   /C3    /
CN      REAL            704
CO      REAL            748
CONC    REAL            792
COUT    REAL             16   /C2    /
D1      REAL             12   /C3    /
D2      REAL             16   /C3    /
DC      REAL            836
DMY327  REAL              0   LARGE
DT      REAL              8   /C2    /
DX1     REAL           1440
DX2     REAL           1444
H       REAL             28   /C3    /
I       INTEGER*4      1468
ICOUNT  INTEGER*4      1484
IDGT1   INTEGER*4      1632
^H                                                                  Page
5

                                                                 06-28-87
                                                                 20:18:55
D Line# 1     7                          Microsoft FORTRAN77 V3.31 August 1985
IDGT2   INTEGER*4      1876
IDGT3   INTEGER*4      1880
IER1    INTEGER*4      1636
IER2    INTEGER*4      1884
IER3    INTEGER*4      1912
IF1     INTEGER*4         4   /C1    /
II      INTEGER*4             PARAMETER
III     INTEGER*4             PARAMETER
IP      INTEGER*4        12 *
IPRINT  INTEGER*4         0   /C1    /
IX      INTEGER*4      1640
```

```
J        INTEGER*4        1476
K        INTEGER*4        1908
L1       REAL             1428
L2       REAL             1432
LIP      INTEGER*4           8   /C3    /
LOG                              INTRINSIC
M        REAL             1464
MX       INTEGER*4        1628
N1       INTEGER*4        1420
N2       INTEGER*4        1424
N4       INTEGER*4                PARAMETER
NX1      INTEGER*4        1624
NX2      INTEGER*4        1872
P1       REAL             1452
P2       REAL             1456
RINIT    REAL                0   /C2    /
RL1      REAL                0   /C3    /
RL2      REAL                4   /C3    /
RX       REAL             1448
TM       REAL                0 *
TM1      REAL               12   /C2    /
TMAX     REAL             1436
VINA     REAL                4   /C2    /
WK1      REAL               16
WK2      REAL              664
X        REAL              632
Y1       REAL              648
Y2       REAL              656


    197
    198
    199

Name     Type          Size   Class

C1                       24   COMMON
C2                     1220   COMMON
C3                       32   COMMON
LEQT2F                        SUBROUTINE
MBCON                         SUBROUTINE


 Pass One     No Errors Detected
             199 Source Lines
^Z
```

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Mazur, P., "Cryobiology: The freezing of biological systems," Science 168, 939-949 (1970).

2. Morris, G.J., "Liposomes as a Model System for Investigating Freezing Injury," Effects of Low Temperatures of Biological Membranes, Academic Press Inc. (London) LTD., 241-262 (1981).

3. McGrath, J.J., "A Microscope Diffusion Chamber for the Determination of the Equilibrium and Non-equilibrium Response of Individual Cells," Submitted to the Journal of Microscopy, March 1985.

4. Blok, M.C., vanDeenan, L.L.M. & deGeir, J., "The Effect of Cholesterol Incoporation on the Temperature Dependence of Water Permation Through Liposomal Membranes Prepared From Phosphatidycholines," Biochemica Biophysica ACTA, 464 509-518 (1977).

5. Hempling, H.G., "Permeability of the Ehrlich ascites Tumor Cells to Water," Journal of General Physiology, 44 365-379 (1960).

6. Savitz, D., Sidel, V.M. & Solomon, A.K., "Osmotic Properties of Human Red Cells," Journal of General Physiology, 48 79-94 (1964).

7. McGrath, J.J. & Tu, S.M., "On the Determination of Cell Membrane Water Permeability Using a Microscope Diffusion Chamber - I. Solute Concentration History Within a Diffusion Chamber," Submitted to the Journal of Membrane Biology.

8. Wendt, R.P., Klein, E., Bresler, E.H., Holland, F.F., Serino, R.M. & Villa, H., "Sieving Properties of Hemodialysis Membranes," Journal of Membrane Science 5, 23-49 (1979).

9. Kedem, O. & Katchalsky, A., "Thermodynamic Analysis of the Permeability of Biological Membranes to Non-Electrolytes," Biochimica Et Biophysica ACTA, 27, 229 (1958).

10. Katchalsky, A. & Curran, P.F., Nonequilibrium Thermodynamics in Biophysics, Havrard University Press, Cambridge, Massachusetts, p.87 (1965).

11. Beck, J.V. & Arnold, K.J., Parameter Estimation in Engineering and Science, John Wiley & Sons, New York, pp.1,18 (1977).

12. Boroske, E., Elwenspoek, M. and Helfrich, W., "Osmotic Shrinkage of Giant Egg-Lecithin Vesicles", Journal of Biophysics 34, pp.95-109 (1981).

13. McGrath, J.J., Callow, R.A. and Melkerson, M., The Response of Cell-Size Unilamillar Liposomes to Osmotic Pretrabation and to Freezing-Thawing, Cryobiology 20, p.712 (1983).

14. Hempling, H.G., Thompson, S. & Dupre, A., "Osmotic Properties of Human Lymphocyte", Journal of Cell Physiology 93, pp.293-302 (1977).

15. Porsche, A.M., Körber, Ch., Englich, S., Hartmann, U., & Rau, G., "Determination of the Permeability of Human Lymphocytes with a Microscope Diffusion Chamber", Submitted to Cryobiology, (1985).

16. Blok, M.C., van Deenen, L.L.M. & de Gier, J., "Effect of the Gel to Liquid Crystalline Phase Transition on the Osmotic Behavior of Phosphitidylcholine Liposomes", Biochimica Et Biophysica ACTA 433, pp.1-12 (1976).

17. Reeves, J.P. & Dowben, R.M., Water Permeability of Phospholipid Vesicles, Journal of Membrane Biology 3, pp.123-141 (1970).

18. Callow, R.C., Thesis M.S., Thermodynamic Modelling and Cryomicroscopy of Large Unilameller Liposomes, Mechanical Engineering Department, Michigan State Univerisity, (1983).

19. Terwilliger, T.C. & Solomon, A.K., "Osmotic Water Permeability of Human Red Cells", Journal of General Physiology 77, pp.549-570 (1981).

20. Kays, W.M. & Crawford, M.E., Convective Heat and Mass Transfer, McGraw-Hill Book Company, New York, New York, (1980).

21. Tu, J., personal notes.

22. Shabana, M., Thesis M.S., Cryomicroscope Investigation and Thermodynamic Modeling of the Freezing of Unfertilized Hamster Ova, Mechanical Engineering Department, Michigan State University, (1983).

23. Hempling, H.G., "Heats of Activation for the Exosmotic Flow of Water Across the Membrane of Leucocytes and Leukemic Cells", Journal of Cell Physiology 81, pp.1-10 (1973).