Chapter 6

Water and Solute Movement Through Red Blood Cell Membranes

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Contents

Introduction

The movement of water and solutes through cell membranes is a very important aspect of physiology. In this experiment, you will study some or all of the following processes:

A. *Osmosis* in the presence of various concentrations of *nonpermeable* solutes, i.e., in solutions of different tonicities;
B. *Relative permeability* of solutes of different molecular weights, and, therefore, of different *sizes*;
C. *Relative permeability* of solutes with different *lipid solubilities*;
D. *Relative permeability* of a solute at different temperatures.

The entire experiment requires two laboratory periods to finish completely. Usually, osmosis (A) is studied during the first laboratory, and the remaining processes (B–D) are studied during the second laboratory. Turbidity measurements of blood solutions are made in order to obtain data on the osmotic responses of red blood cells (RBCs) as they interact with various solutions. Many of the original experiments for use in the undergraduate laboratory are described in Grinnell (1976). For the study of osmosis, steady-state turbidity measurements can be made after the RBCs have equilibrated with solutions of various tonicities. Such measurements are indicative of the average RBC volume up to the point where hemolysis occurs, and they can be easily obtained with the standard use of the Spectronic 20. However, if one wants to measure the initial response of a given solution to the addition of RBCs, then one needs to measure transient changes in turbidity. In this laboratory, the Spectronic 20 is interfaced with the MacLab system so that transient changes in turbidity can be measured (Figure 6.1). This is done so that accurate measurements of hemolysis times can be made in order to obtain information on the relative permeabilities of various solutes.

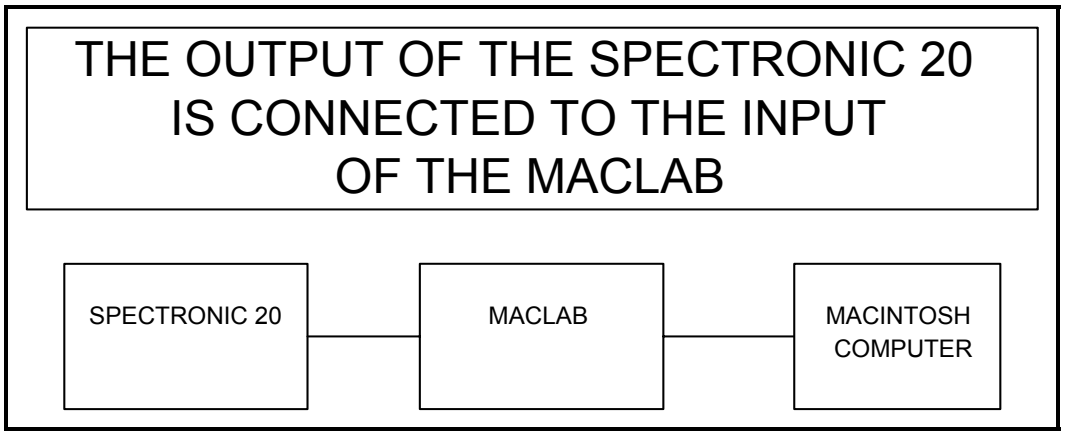

Figure 6.1. Block diagram of the MacLab system used to record transient changes in the turbidity of various blood/solute solutions.

The experiment consists of six exercises. It is not necessary that all six be completed by each student or student group. Each exercise is summarized very briefly as an **action statement** which, hopefully, will challenge a student to apply critical thinking skills in order to create a specific experimental protocol and then to actually perform the experiment. The action statements and pertinent background material should be presented to the student at least one week prior to the laboratory.

Materials

Each *group* should have the following:

Macintosh computer
Maclab
Spectronic 20D
Cuvettes (8)
Test tube rack for cuvettes (1)
Thermometer (1)
Erlenmeyer flask with rubber stopper, 50 ml (1)
Clear plastic, screw cap centrifuge tubes, graduated, 10 ml (16)
Test tube rack for centrifuge tubes (1)
Squirt bottle containing distilled water (1)
Kimwipes (1)
Beaker, 400 ml (1)
Beakers, 50 ml (2)
Beakers, 150 ml (2)
Graduated cylinder, 100 ml (1)
Volumetric flask, 100 ml (1)
Marking pencil (1)
Variable lambda pipette, 1000 µl (1)
Volumetric pipette, 5 ml (1)
Rubber pipetter bulb

The laboratory should have the following:

Water bath, 40°C
Water bath, 60°C
Distilled water (500 ml/group)
Defibrinated sheep blood (5 ml/group)
Balance (1/2 groups)
Sucrose (100 gm/group)
NaCl (30 gm/group)
Solutions
- 0.9 gm% NaCl (500 ml)
- A minimum of 100 ml (or 50 ml/group) of the following solutions should be placed into erlenmeyer flasks:
- 500 mM urea
- 90 mM NaCl

500 mM urea + 90 mM NaCl
500 mM glycerol
500 mM glucose
500 mM ethylene glycol
500 mM 1,3 propanediol
500 mM 2,3 butanediol
3 m 1-propanol
3 m ethanol
3 m methanol
Volumetric pipettes, 5 ml (11)
Rubber pipetter bulb (11)
Kimwipes (2)

Student Outline

Introduction

This laboratory exercise deals with the movement of water and solutes across cell membranes. You should be generally familiar with the fluid mosaic model of the membrane because such an understanding will allow you to think about what portion(s) (lipid and/or protein) a particular substance passes through or "uses" on its way across the membrane. You should also have a general understanding of carrier mediated transport and of the role of channels in membrane transport. The concept of membrane permeability is important. The permeability coefficient, P_S refers to the ease with which solute S can cross a given membrane; the units for this coefficient are cm/second. Thus, P_S is really the velocity of solute movement through a membrane. There are two schools of thought with respect to how solutes cross the RBC membrane. Macy (1984) thinks that facilitated diffusion is the mechanism of solute permeation; on the other hand, Solomon's laboratory provides evidence favoring the existence of a single pore through which all solutes and water pass (Solomon et al., 1983).

Nonpermeable Solutes

In this part of the experiment, you will be concerned with the relationship between cell volume and osmotic gradients across the cell membranes of erythrocytes. The osmotic gradients are to be varied by changing the tonicity of the bathing medium. Tonicity refers to the concentration of nonpermeable solutes in the bathing medium relative to the normal value of intracellular fluid. The key point about tonicity is that it deals with *nonpermeable solutes* in fluids which bathe *living cells*.

Questions: What are the definitions of isotonic, hypertonic, and hypotonic solutions? What is the value (in mOsm/kg H_2O) for the normal isotonic concentration? What are the chemical and osmotic concentrations of a 0.9 g % solution of NaCl? Give an example of a highly *permeable* solute. What would be the tonicity of a solution containing only a highly permeable solute? Explain thoroughly.

Ideally, it would be desirable to measure red blood cell *diameter* in this part of the experiment. What would you expect the relationship to be between RBC diameter and the tonicity of the bathing medium? (For this kind of question, it is usually quite helpful to sketch a graph in which you *plot* what you think is the relationship between the two variables under consideration; be sure to include the correct scientific units on the coordinates.) We will, however, not measure cell diameter directly with a microscope. An *indirect* measure will be used instead: the *turbidity* of cell suspensions will be

measured using a spectrophotometer (Spectronic 20). This is how it works: the light which passes through the cell suspension is set to a wavelength of 510 nm. Light at this wavelength is *not* absorbed by hemoglobin but, rather, by the intact cells whose membranes reflect light. This particular procedure is an example of a light-scattering technique. In fact, as the cells shrink, they reflect greater amounts of light. That light which is reflected and adsorbed by the cells does not pass through to the light sensor in the spectrophotometer; therefore, the per cent transmittance (%T) decreases as the turbidity of the solution increases. On the other hand, as the cells swell to diameters greater than normal the turbidity of the cell suspension decreases. The %T increases because the cell membranes reflect less light. Finally, the %T increases still further when the cells begin to *hemolyze*. For the first part of this experiment, you will be making steady-state measurements of turbidity with the Spectronic 20. (However, an optional part of this experiment would involve transient measurements of turbidity in order to learn about the initial rate of osmosis as a function of tonicity.)

With regard to the experiments in which the nonpermeable solutes are to be studied, you will be interested only in *steady-state* values of %T. It is helpful to realize that solutions which are only slightly hypotonic will not cause hemolysis but only swelling of the cells. Hemolysis begins when the tonicity of the solution is about 0.54 of normal, i.e., of isotonicity. (Question: if isotonicity equals 300 mOsm/Kg H_2O, what is the tonicity (in mOsm/Kg H_2O) of a solution in which hemolysis just begins to occur?) Therefore, your %T readings will represent a quantitative measure of the osmotic flow of water into and out of red blood cells.

This part of the experiment will also illustrate that a single molecule of NaCl behaves differently than does a single molecule of sucrose with respect to osmosis. What do you think is this difference? Explain.

Permeable Solutes

In the second part of this experiment, you will focus on indirect permeability measurements. In other words how fast will a given solute (or water) cross the cell membrane? The experimental design will involve the creation of a diffusion gradient for the solute that is being examined; the concentration gradient will promote the influx of the solute into the cell. But how will turbidity measurements help us here? The turbidity of the solution will actually increase because of the osmotic flow of water into the cell. Eventually the RBCs will hemolyze, and the hemolysis time is dependent on how fast the solute entered the cells in the first place, i.e., it is a function of P_S. An influx of water occurs because once the solute diffuses into the cell, the intracellular fluid becomes hyper-osmotic relative to the extracellular fluid, and, thus, water enters the cells by osmosis. It should now be clear that such indirect permeability measurements require transient changes in turbidity to be obtained.

Reading the meter on the Spectronic 20 will not allow one to make accurate measurements of transient changes in the turbidity of a solution. One must take the signal from the Spectronic 20 and connect it to a scientific recorder (such as an oscilloscope, a physiograph, or a computer used as a recording device) which permits the recording of a signal, which is a voltage, as a function of time. The newer Spectronic 20s have an output connector which permits an analogue signal to be readily connected to a recorder. We will use MacLab to record transient changes in turbidity or per cent Transmittance (%T) in our experiments (Figure 6.1). You will use the *units* conversion feature of *chart*, so that the vertical scale (y-axis) will indicate %T rather than voltage. The Spectronic 20 has been designed so that when it displays 100 %T, the analogue signal at its output connector is 1 V; when the machine displays 0 %T, the output voltage is 0 V.

As explained below, a transient turbidity measurement from a given blood/test solution, prepared by adding blood to that test solution, can give a quantitative index of the permeability coefficient of the substance being tested. The substance can be water, urea, glycerol, glucose, or ethanol, for example. Thus, from such measurements, one can deduce *relative permeabilities* for each of the test substances. This part of the experiment will focus on permeable solutes which will be allowed to diffuse into the RBCs because of concentration gradients that you will create. Examples of transient turbidity measurements are illustrated in Figure 6.2.

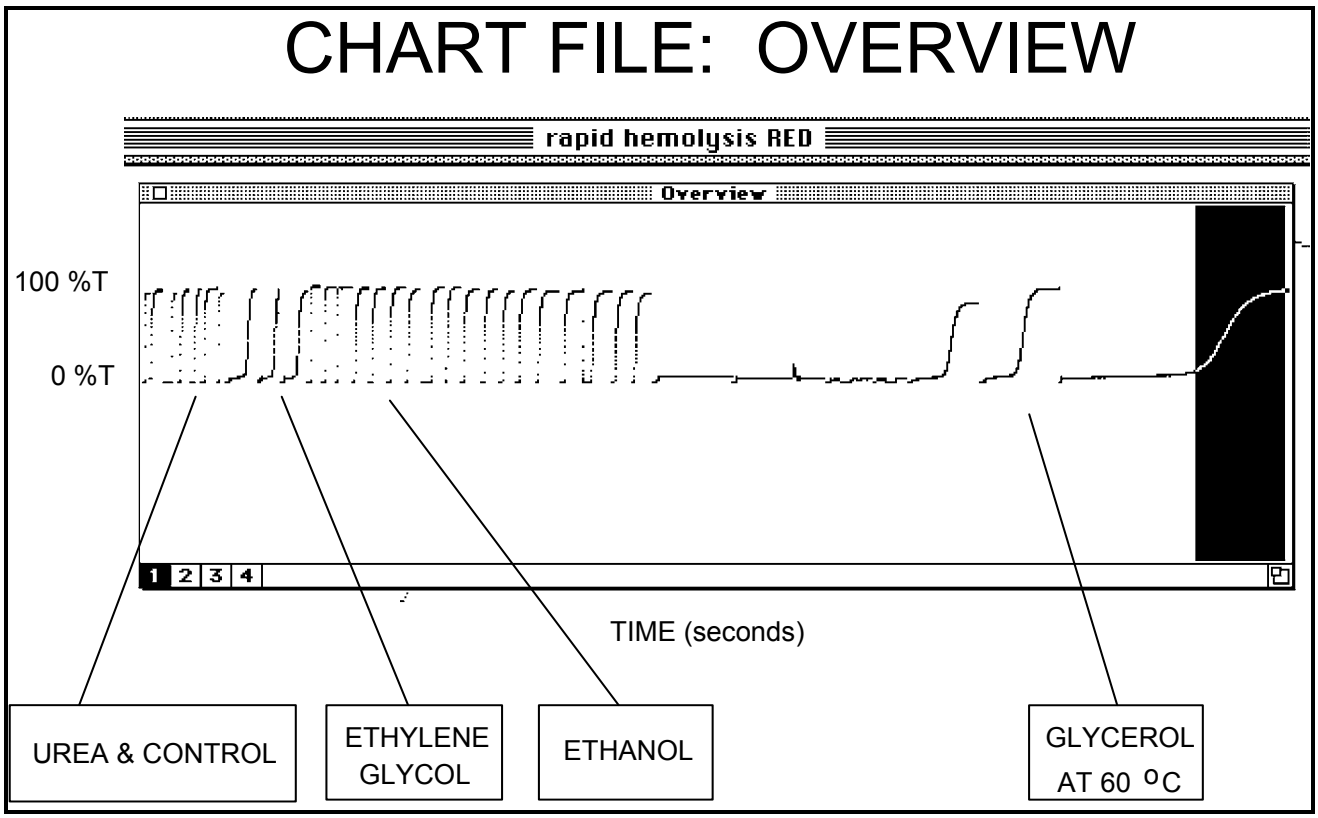

Figure 6.2. Individual records of percent transmittance (%T) versus time (seconds) for a variety of solutes are shown on a compressed time base. 100 %T corresponds to hemolysis, and low values of %T, which are close to 0 %T, correspond to blood solutions in which hemolysis has not occurred. This is an example of a CHART file for an experiment in which relative permeabilities for several solutes were measured. Most of the measurements were performed at room temperature. Glycerol, however, was studied at three different temperatures, one of which was 60°C. Note also that glycerol causes hemolysis considerably more slowly than does, for example, ethanol. Figures 6.3–6 show %T as a function of time more accurately than does this figure.

The remainder of the experiment deals with the relative permeability of various solutes. The membrane *permeability* of a substance refers to the ease with which it can move through a given membrane. The permeability coefficient has the units of cm/second (i.e., of velocity). In the present experiments, the *hemolysis time* will be measured for each of the test substances; it will be assumed that the hemolysis time will be a reliable index of the permeability coefficient. Do you agree with this assumption? Both the rate at which the cell diameter changes and the hemolysis time are dependent on the permeability of a given solute. In these experiments, the initial solute concentration will be 500 mM in the bathing (extracellular) solution while the intracellular concentration will be,

essentially, zero. Therefore, a concentration gradient will be created in order to promote the diffusion of the solute into the cell. *This solute movement into the cell will be accompanied by the osmotic flow of water into to the cell as well.*

In the permeability studies, you will also be concerned about which region of the cell membrane a given solute can pass through: protein channels and/or the lipid bilayer.

Protocol

Blood

You will be provided with 5 ml of sheep blood which has been defribinated so that it cannot clot. Dilute this blood sample to a total volume of 50 ml by adding 45 ml of 0.9 gm % NaCl. Store in a stoppered Erlenmeyer flask. You will use a 0.5 ml automatic pipette to obtain blood samples from this flask. In order to insure that you are working with almost identical blood samples each time, be sure to invert the flask 2–3 times before you take a blood sample. Such a procedure will prevent the blood cells from settling to the bottom of the flask.

Exercise 1: Design an experiment in which blood is to be added to equimolar solutions of sucrose and urea in order to compare and contrast the tonicities and turbidities of the two solute/blood solutions.

This is a quick experiment that can be done by simply observing what happens when blood is added to each of these solutions. Do you need any control solutions? Consider distilled water; what is its tonicity? This exercise can be regarded as a demonstration for your group. What solute concentration should you use? Why? After you have added the blood and made your observations, discuss the physiological significance of this experiment before proceeding to the next exercise.

What conclusions can be made with respect to the tonicities of the two solute solutions? Is it possible to make a hypertonic solution when the only solute used is a highly permeable one? Explain.

Exercise 2: Find the effect of tonicity on the turbidity of various solute/blood solutions.

This experiment should be designed so that you will be able to work with a wide range of tonicities and with sufficient intermediate tonicities so that you will be able to observe the transition between hypertonic and hypotonic solutions. What solutes should you use, permeable or nonpermeable? Should you use electrolytes and/or nonelectrolytes? Explain. The data you obtain should be plotted. What do you think such a plot will look like? In other words, predict the relationship between tonicity and turbidity.

Consult Appendix A for instructions on how to make turbidity measurements with the Spectronic 20.

Consult Appendix B for a detailed explanation as to how you might perform Exercise 2.

Exercise 3: In order to become introduced to transient turbidity measurements, design an experiment which will allow you to compare and contrast the hemolysis times of distilled water and 500 mM urea.

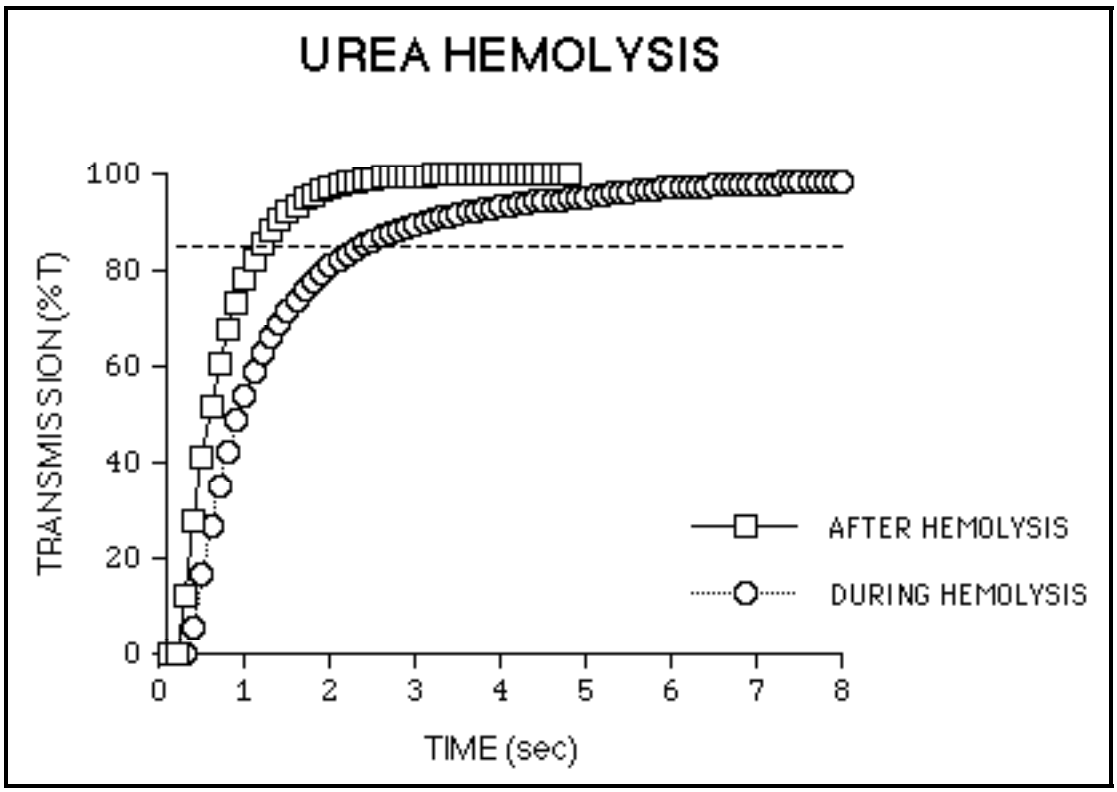

Figure 6.3. This graph, made with Cricket Graph, shows that the turbidity of a urea/blood solution increased within seconds to near 100 %T (DURING HEMOLYSIS). The *hemolysis time* = 2.3 seconds. Although TIME 0 corresponds to the instant the cuvette containing the urea/blood solution was inserted into the Spectronic 20, the actual mixing of the blood with the urea solution occurred a few seconds beforehand. In order to check the speed of the Spectronic 20, the same cuvette containing the hemolyzed blood was re-inserted into the machine (AFTER HEMOLYSIS). The RESPONSE TIME = 1.1 seconds. Data from CHART were saved as two text files (see Appendix E) which were then imported to Cricket Graph. Horizontal dashed line indicates 85% of plateau values; for these experiments, the hemolysis and response times were taken as the time necessary for %T to equal 85% (dashed, horizontal line) of the plateau value.

Measure the *hemolysis time* and the *response time* (Figure 6.3) for distilled water and 500 mM urea. For rapidly permeating solutes, such as urea and alcohols, hemolysis will occur quickly, usually within the range of 2–30 sec. Therefore, you will use MacLab (Appendix C) to record these rather rapid changes in turbidity. Since these measurements will be obtained *quickly*, you are encouraged to make repeated measurements. Two to three repeated measurements are desirable. This is a good practice because it allows one to obtain *reproducible results*. Consider making at least three measurements with distilled water and at least three measurements with 500 mM urea. The procedure is as follows:

Prepare a BLANK consisting of 4.5 ml of distilled water plus 0.5 ml of the diluted blood solution, and make sure the Spectronic 20 is properly adjusted to read 0 and 100 %T (Appendix A).

Hemolysis Time

Transfer 4.5 ml of a given test solution into a cuvette. Trigger CHART. Add 0.5 ml of diluted blood; mix the solution by placing your finger over the top of the cuvette and inverting it two or three times. Then place the cuvette in the Spectronic 20 and *measure the %T as a function of time.* This aspect of the procedure should be done as quickly and as accurately as possible; it is a technique that requires some practice. Since some test solutions will result in very rapid hemolysis (e.g., hemolysis times of 3–6 seconds), we will use the CHART/MacLab system to permit us to make accurate measurements of %T as a function of time. In summary, you will first trigger CHART, then you will add the blood to the test solution (as described above), mix quickly, and insert the cuvette into the machine. CHART will continuously record %T as a function of time. Before the cuvette is inserted, the %T will be zero, but once the cuvette is inserted, the %T will increase, in some cases, very rapidly, to 100 %T. After a plateau, usually at a %T which is close to 100 %T, has been reached, then STOP the CHART recording. Up-date (i.e., SAVE) your CHART file after each measurement.

For the present experiment, we define *hemolysis time* as the time required for the %T of a given blood solution to reach 85% of its plateau value.

Response Time

Immediately after recording the *hemolysis time* for a given test solution, remove the cuvette containing the hemolyzed blood solution (test solution). As a check on how fast the Spectronic 20 can respond to the mere insertion of a cuvette containing *previously* hemolyzed blood, trigger CHART again, and re-insert the hemolyzed cuvette containing the hemolyzed blood. STOP the CHART recording when a plateau has been reached. The *response time* is the time it takes for the %T to shift from 0 %T to 85% of the plateau value.

Comment on response time: The response time measurements are useful for two reasons. In addition to checking the speed with which the system can respond to the insertion of a cuvette, it also provides a quantitative measure of the plateau value, which corresponds to complete hemolysis. Because of differences in solutes and because of small changes in blood concentration from one sample to the next, the plateau value for a given test solution may be different from that of the blank, which, as you recall, was made by adding blood to distilled water. Such differences in plateau values are, however, not terribly important because of the way in which we have defined hemolysis time, i.e. as the time to 85% of the plateau value. If you should decide, later on, to plot data from two or more solutes on the same graph, you might consider normalizing the data such that the plateau value always corresponds to 100% for each solute.

For a given test solution to produce meaningful results, the RESPONSE TIME should always be less than the HEMOLYSIS TIME. Explain.

Exercise 4: Find the effect of lipid solubility of various solutes on *hemolysis time*.

What is a good measure of lipid solubility? For suggestions of which solutes to study, you may consult the following: a.) the classic study by Collander (1937); b.) Appendix D.

Exercise 5: Find the effect of molecular weight of various solutes on *hemolysis time*.

The following solutes can be studied:

Solution	Molecular Weight
500 mM urea	60.06
500 mM ethylene glycol	62.05
500 mM 1,3 propanediol	76.10
500 mM 2,3 butanediol	90.12
500 mM glycerol	92.09
500 mM glucose	180.16

Are there any other solutes that you would like to add to those listed above? The di-hydroxy alcohols (1,3 propanediol and 2,3 butanediol) have recently been shown to permeate the RBC membrane slowly (Macy and Karan, 1994).

***Important Note*:** One can begin to monitor the turbidity of the solutions containing the slowly permeating solutes first. These solutes are glycerol and glucose. However, this does not mean that you cannot perform additional measurements with the Spectronic 20 *while these slowly permeating solutes continue to react with their respective blood samples.* That is, after obtaining a turbidity measurement from a blood solution containing a slowly permeating solute, identify it with an appropriate COMMENT in your CHART file and then place the cuvette containing that solution *into a test tube rack* until it is time to obtain a subsequent measurement. (CHART keeps time in three modes: time of day; time from the start of a record (file); time from the start of a block from within a given record.) One would want to take a measurement every 3–5 minutes; however, if the %T begins to increase, then you should take measurements more frequently or, even, continuously. Meanwhile, that is, in between measurements, your Spectronic 20 can be used to make measurements of other solute-blood solutions (like, for example, blood added to a urea solution or blood added to a glucose solution).

Exercise 6: Find the effect of temperature on *hemolysis time* for a given solute.

Select a solute from the list presented under Exercise 5. What type of solute would be a good candidate for a temperature study? Suggested temperatures (in °C) are as follows: 23 (room temperature); 40; 60.

Additional Experiments

Exercise 7: Find the effect of tonicity on the rate of osmosis/hemolysis.

This is an interesting experiment that is a natural sequel to those experiments associated with Exercise 2. Instead of measuring steady-state turbidities, one would measure the initial or transient changes in the turbidity of various nonpermeable solute/blood solutions. CHART allows one to obtain the initial slope of the %T versus time data.

Exercise 8: Design an experiment in which you interact permeable and non-permeable solutes on the osmotic behavior of RBCs.

For example, will the addition of 80 mM NaCl effect the hemolysis time of a 500 mM urea solution?

Exercise 9: Design an experiment in which you examine various pharmacological blockers on the hemolysis time of a given solute.

This is a challenging experiment because you may find it necessary to search the current literature for specific ideas and blocking substances. For example, one could search Medline using the following search terms: *erythrocyte membrane* and water *channels.*

Exercise 10: Find the effect of tonicity on RBC diameter and count.

What do you think would happen to a population of RBCs as they were exposed to a mildly hypotonic solution? Would each cell in that population behave in an identical fashion to all the rest? Or, perhaps, would some cells hemolyze while others expand slightly? This is an exciting experiment that requires only a microscope as the primary analytical tool.

Data Analysis

The critical thinking used to create a given protocol should also indicate the specific way(s) the data are to be analyzed and displayed. Students, and not the instructor, should make decisions with regard to data analysis. Small group discussions can help students decide on the specific graphs, figures, and tables to make. Graphs and figures should have visual impact. After the data have been submitted, the instructor can arrange to project selected graphs, figures and tables for continued discussions in which the data are interpreted with respect to the underlying scientific principles.

The time a student spends on data analysis is dependent on the level of sophistication required by the instructor and/or desired by the student. One of the least time-consuming forms of data analysis involves the production of selected printouts of CHART records; such figures would be produced directly from within CHART. Cricket Graph can be used to make simple or complex graphs. Hemolysis times can be measured directly from a CHART file. Such data should be entered into your laboratory notebook with the intent of eventually incorporating them into a data table and/or into specific graphs. Tabular data can easily be displayed in a spreadsheet, which allows text to displayed along with the numbers. One could then use a *screen capture utility* (e.g., Flash-it) to capture the appropriate portion of the spreadsheet and import that image into a drawing program or a word processor. In order to create a *scientific figure* in which both text and graphics are combined: First, capture one or more CHART recordings and import them to a drawing program; second, use the text tool in that program to label your recordings (e.g., see Figure 6.4). A considerable amount of time would be necessary to construct a graph containing multiple plots (e.g., Figure 6.5). Appendix E describes two techniques used for the transfer of data from CHART to Cricket Graph.

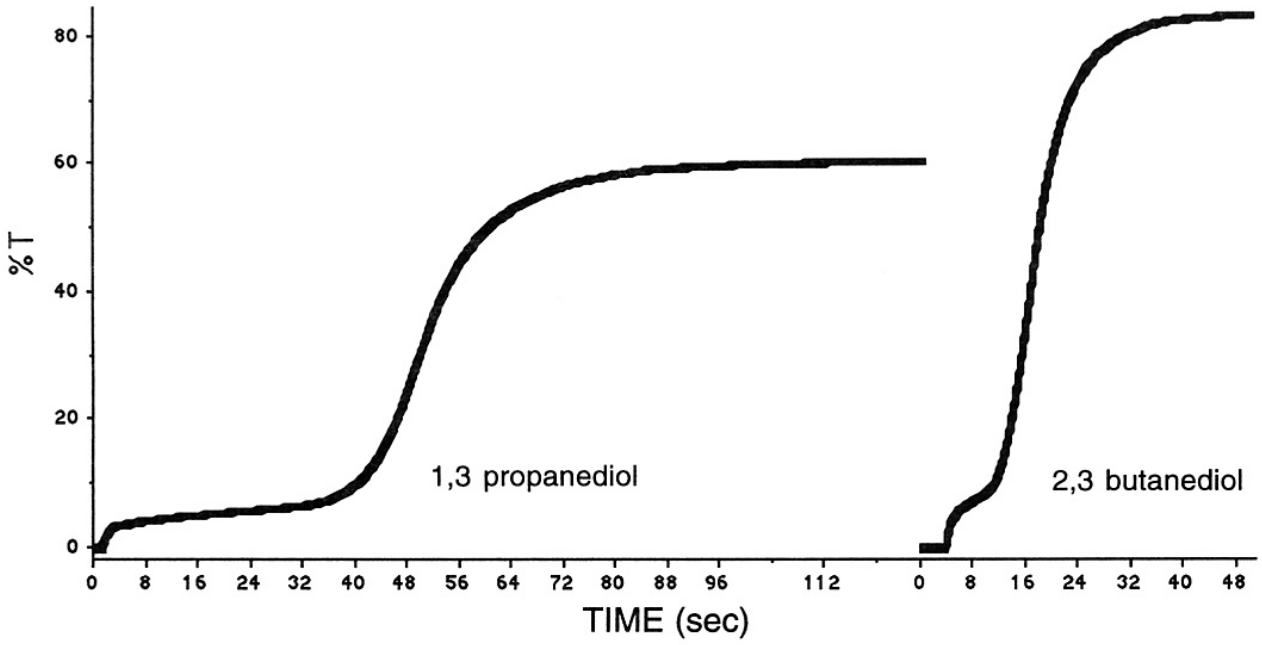

Figure 6.4. Hemolysis of sheep RBCs induced by di-hydroxy alcohols. Note the "latency" at the start of each trace. The zeros on the horizontal axis correspond to TIME ZERO for each of the two solutes tested. Per cent transmittance (%T) is plotted on the vertical axis against time in seconds. This figure was made by capturing a CHART image as it was displayed in the Zoom Window of CHART; Flash-it was used for the screen capture. The image was then displayed in Cricket Draw for labeling. After grouping all of the objects into a single object, the image was copied to the Clipboard and pasted into WordPerfect. From within WordPerfect, one has the option of creating a *frame* and/or a *caption* for any graphic image.

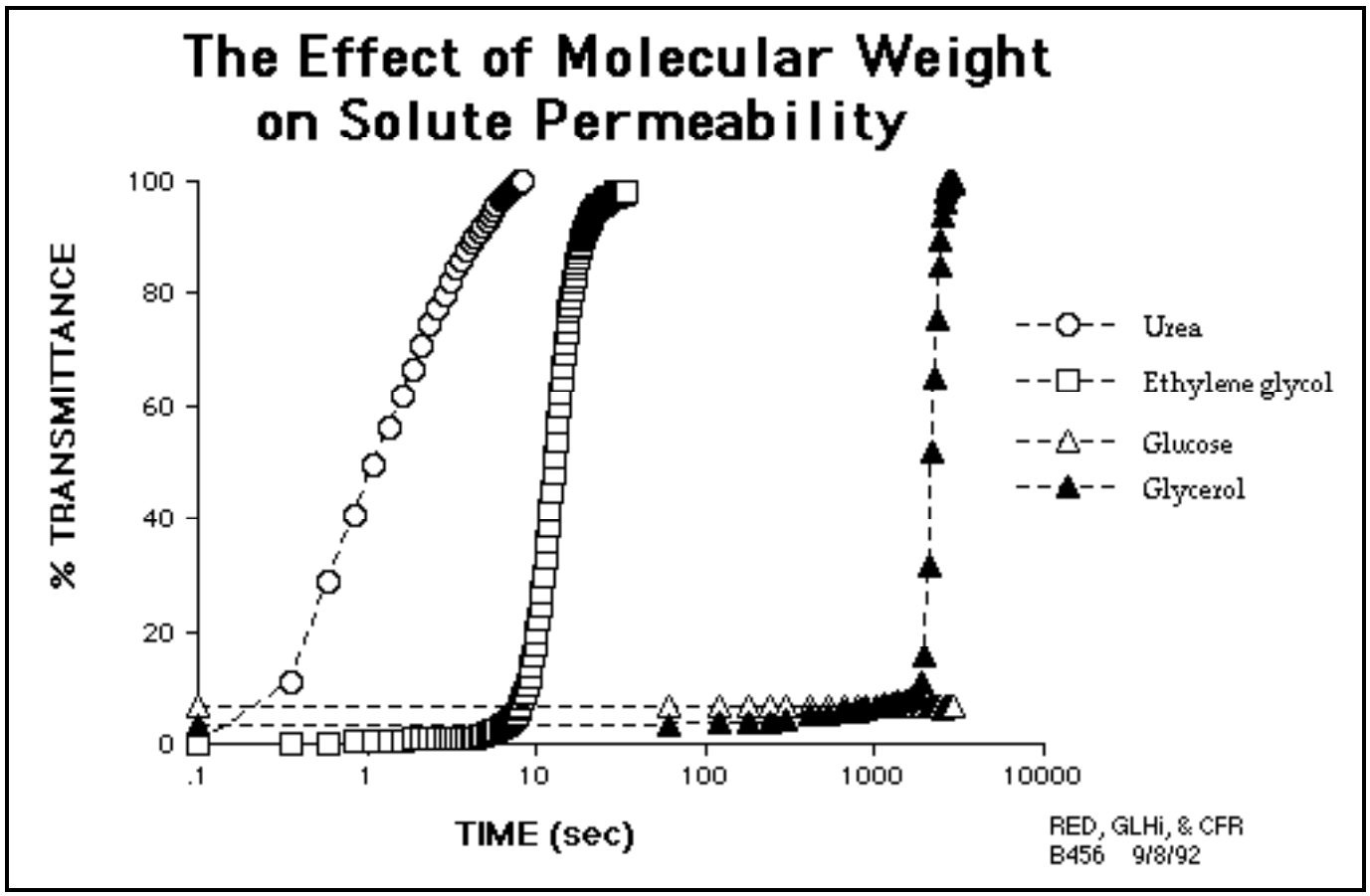

Figure 6.5. Turbidity (%T) measurements of various solute/blood solutions are plotted as a function of time. *Time zero*, which is the time the cuvette containing a particular solute/blood solution was placed into the Spectronic 20, for each of the solutions is approximated by the origin of the horizontal axis (0.1 seconds). The log scale on the horizontal axis allows one to easily visualize the differences in the relative permeabilities of the solutes studied.

Notes for the Instructor

The title of each exercise is an *action statement* which, hopefully, will prompt the student to use critical thinking in the design and execution of that exercise. For me, this represents a major change from the lengthy, cookbook approach to laboratory instruction. Students should receive their action statements and pertinent information, in the form of demonstrations and/or handouts, on scientific techniques one or two weeks prior to the experiment. This coupled with class discussions of specific protocols developed by the students should satisfactorily prepare your students to perform the experiments.

As a minimum, your students should have access to a Spectronic 20 for turbidity measurements. (A Spectronic 20D is a digital instrument which has an analogue output connector located on the bottom; using this connector permits one to easily attach the spectrophotometer to the MacLab input

(Figure 6.1). The older, non-digital Spectronic 20s can also be connected to the MacLab; however, an electrical technician will have to remove the instrument cover and directly attach wires across the meter.) If you wish, you may assign only a few of the exercises to your students. For example, if you do not intend to deal with transient turbidity measurements, then your students can do exercises 1, 2, and 10. If you would like to become involved in transient measurements but do not have a MacLab or a similar scientific recorder, you could still have your students work with the slowly penetrating solutes, which means that the following solutes, which are rapidly penetrating, should not be used: urea, methanol, ethanol, and propanol.

To work exclusively with nonpermeable solutes, both steady-state and transient turbidity measurements can be obtained by doing exercises 2 and 8; the latter exercise provides data on the rate of osmosis, which is especially interesting as one tests solutions of increasing hypotonicity.

Regarding data analysis, the challenge is for you to act as a facilitator so that your students will decide for themselves how best to report their data. I place special emphasis on graphs. For data obtained with the nonpermeable solutes, students should make two graphs. First, chemical concentration (mM) for both NaCl and sucrose versus %T, and, second, osmotic concentration (mOsm/kg H_2O) for these two solutes versus %T. For the transient data, you have a choice depending on how much time you want your students to spend. Having the students create data tables containing hemolysis times is simple, efficient, and effective. Relative permeability can be expressed as a ratio of hemolysis times; for example, one could divide each hemolysis time into the shortest time (which is that for 3M propanol). From the study of tabular data, students may decide, for example, to plot hemolysis time as a function of molecular weight, hemolysis time as a function of temperature for a given solute, and the rate of osmosis as a function of tonicity. In addition to the tabular listing of hemolysis times, the creation of time course plots can produce graphs which very clearly communicate the relative RBC permeability of various test solutions.

Having your students interpret their data based on physiological concepts is most important. Such interpretations can be assigned as an integral part of a laboratory report and/or as an oral presentation in class. It is very important that students gain experience at scientific expression, both written and oral. Students should be encouraged to use critical thinking in order to decide which particular interpretations to include in their reports. Data from experiments in which the nonpermeable solutes were used allow students to discuss osmosis, osmotic gradients, and tonicity. Data from experiments in which the permeable solutes were used can be more challenging to interpret with respect to specific transport mechanisms. Urea, for example, is small enough to easily pass through a membrane channel. Two additional possibilities for urea transport are facilitated diffusion and diffusion directly through the bilipid layer due to momentary breaks in its molecular structure. Quantitative measures of lipid solubility are not readily available except for the oil to water partition coefficients for propanol, methanol, and ethanol. It is interesting to note that predictions from these coefficients indicate that the hemolysis time for ethanol should be less (faster) than is that for methanol, but, in fact, the opposite results are obtained, and critical study of Collander's data (which appear in Figure 4–23 of Eckert (1988)) indicates that methanol permeates giant *Chara* cells more readily than does ethanol.

Literature Cited

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APPENDIX A

Using the Spectronic 20 to Measure the Osmotic Activity of RBCs

Learning to use the Spectronic 20 is relatively easy. You will work with a BLANK which consists of a specific volume (e.g., 4.5 ml) of distilled water to which has been added 0.5 ml of the blood solution prepared as instructed (see page 105). Thus, a hemolyzed blood solution will serve as a BLANK. With the wavelength set to 510 nm, insert the BLANK into the Spectronic 20, and adjust the control, located near the right side of the machine, so that 100 %T is displayed. Then remove the BLANK, and adjust the "zero-adjust" control, located near the left side of the machine, so that 0 %T is displayed. You will have to repeat these two procedures several times before the Spectronic 20 is properly set. *Plan on checking these settings periodically as you progress through the various measurements.*

APPENDIX B

Nonpermeable Solutes

Sodium Chloride

Prepare 100 ml of 1M NaCl. This will be your stock *solution* from which you will make diluted solutions of NaCl. (When working with a stock solution, it is helpful to realize that 1 M/L = 1 mM/ml.)

You are to make-up 10.0 ml each of the following NaCl/blood solutions whose final NaCl concentrations are (in mM): 70, 80, 90, 95, 100, 105, 110, 115, 120, 125, 130, 150 (isotonic), 200, and 300. The most efficient way to make these solutions is to use calibrated test tubes and a pipette. Pipette the correct volume of your stock solution into such a test tube; then fill that test tube up to its 9.0 ml mark with distilled water. Finally, add 1.0 ml of diluted blood in order to bring the total volume to 10.0 ml; slowly invert several times in order to mix the blood with the solute solution. When you have finished this part, you will have 14 test tubes, each containing a particular NaCl/blood solution.

In about fifteen minutes, an osmotic equilibrium will have been reached; at that time, the turbidity of the blood solutions can be measured with the Spectronic 20. Visually inspect your solutions as they appear in the test tube rack. Can you detect a variation in the turbidity? Which are the most turbid and which are the least turbid? BEFORE you begin to make measurements with the Spectronic 20, ask your lab instructor to examine your solutions. It is possible for one to use only a single cuvette to make the turbidity measurements. Simply rinse the cuvette three times with distilled water and shake it "dry" prior to working with the next blood solution. After you have made a given measurement, pour the blood solution back into its test tube, rather than discarding it. Later on, you may wish to repeat the turbidity measurement of that solution. After you have obtained an initial set of measurements, show your data to your instructor.

Sucrose

Prepare 100 ml of a 1 molal solution of sucrose. Prepare 10.0 ml each of the following sucrose/blood solutions whose sucrose concentrations are (in mM): 80, 120, 140, 160, 180, 190, 200, 210, 220, 230, 240, 250, 260, 300, 400, 600. *Follow the same procedure as outlined above for NaCl.*

APPENDIX C
CHART Settings

It is assumed that you have already been introduced to use of MACLAB. The HELP on version 3.3.8 of CHART is excellent.

In order to make transient turbidity measurements with MACLAB, use CHART with the following settings:

i. INPUT AMPLIFIER = Channel 1
   - Range: 2 volts

ii. UNIT CONVERSION
   - 1.0 volt = 100 %T
   - 0 volt = 0 %T

iii. SPEED = 500 milliseconds/division (40 samples/second)

iv. TRIGGER = USER

Use COMMENTS to properly label each measurement. It is very good practice to UP-DATE or SAVE your CHART file after every measurement; if your computer should fail (i.e., crash or "hang-up"), then you will not have lost much data.

APPENDIX D
Alcohols

The following alcohols can be studied:

Solution	***K****
3.0 M 1-propanol	1.09
3.0 M ethanol	0.26
3.0 M methanol	0.14

*K is equal to the oil/water partition coefficient.

APPENDIX E
Plotting Transient Turbidity Data

There are two ways to plot transient turbidity data which have been recorded with CHART. One way involves the transfer of ALL the data points (in this case: *seconds* versus *%T*) contained in a selected portion of a CHART file to a graphing program (e.g., see Figure 6.3). Once the data are properly SELECTED, then you can either transfer them directly to your graphing program via the CLIPBOARD or save them as a TEXT file for subsequent plotting. Figure 6.6 illustrates the important steps in SELECTING data and in making a text file. First place the MARKER at TIME ZERO; to do this accurately, use the Zoom Window (Figure 6.6A). Next, close the Zoom Window and SELECT that portion of your data which you wish to import to your graphing program (left-hand portion of Figure 6.6B); make sure that the left-hand border of your SELECTED data corresponds to 0 seconds, i.e., to the position of the MARKER. Finally, either COPY the data to the CLIPBOARD or SAVE the SELECTED data by properly selecting the correct boxes in the SAVE AS dialogue boxes (right-hand portion of Figure 6.6B; Figure 6.6C).

You should not transfer more than a few hundred points, because you may experience long waits for the data to be plotted. Therefore, if your selected data contains several hundred or more points, use the DATA PAD feature of CHART. This permits one to select specific points, rather than a continuous series of points, for transfer to the CLIPBOARD. Consult your CHART instruction manual and/or HELP, within CHART, for information on the correct use of the DATA PAD. Before copying data to the DATA PAD, be sure to place the MARKER on TIME ZERO, as described above.

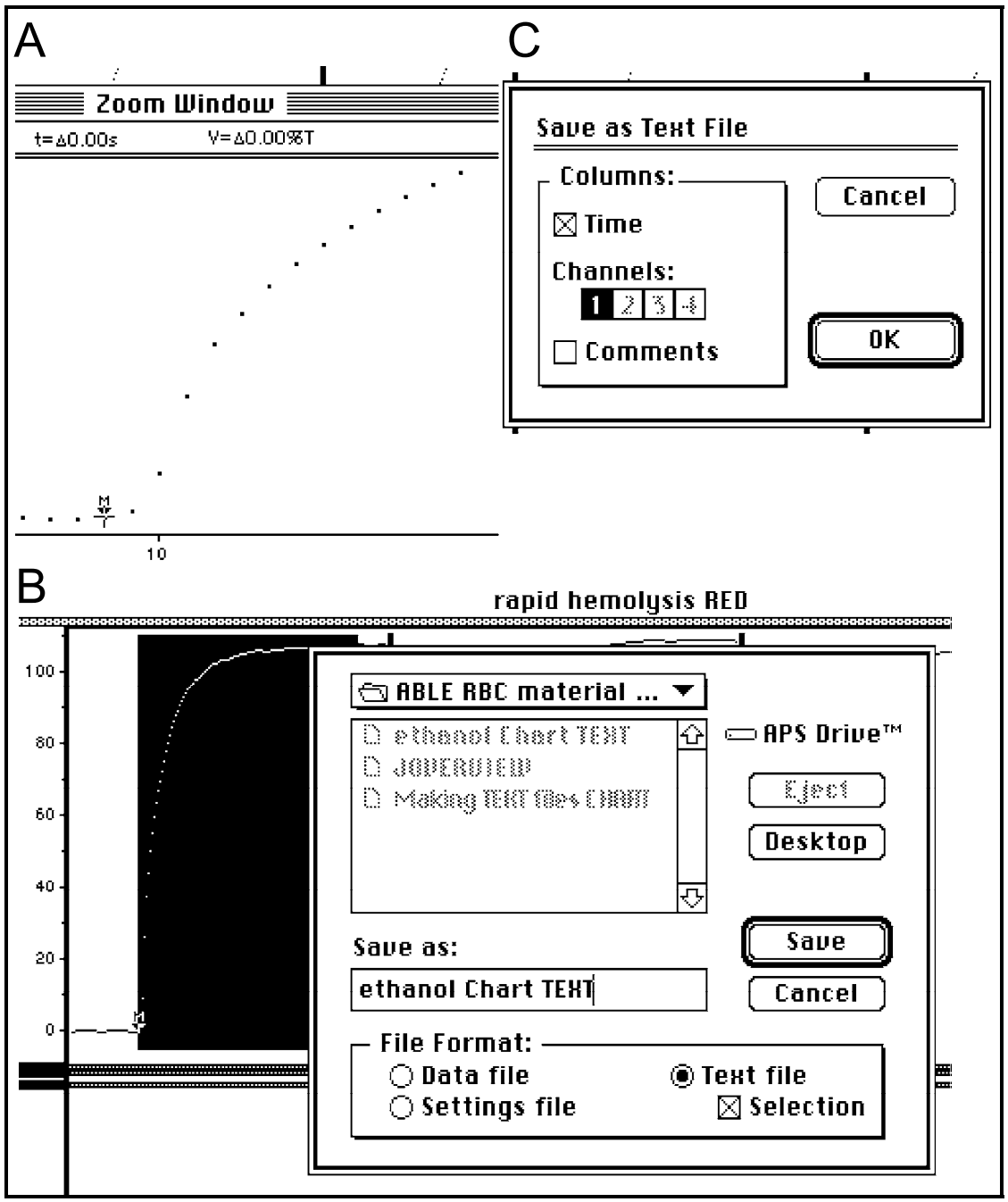

Figure 6.6. SELECTING data and creating a TEXT file from within CHART. Once the data are SELECTED, they can then either be transferred to a graphing program via the CLIPBOARD or saved as a TEXT file for subsequent plotting. The correct positioning of the MARKER (M) at TIME ZERO is very important not only for SELECTING data but also for the correct use of the DATA PAD (see text).