USING OSMOMETRY

For Water-Electrolyte Balance Experiments in the Instructional Laboratory

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INTRODUCTION

Selection of suitable physiology experiments for the college or university instructional laboratory is a challenge. On the one hand, we encounter pressures to reduce the numbers of animals used in laboratory experiments. On the other, concerns about HIV and HBV infection and other blood-borne pathogens restricts drawing of human blood samples for instructional use.

At the University of Southern California, we have developed exercises using measurements of the osmotic concentration of urine samples for quantitative study of fluid and electrolyte balance, using nominal values for the osmolarity of body fluids. In many institutions, the 'urine lab' has tended to be primarily phenomenological, rather than analytical. Dip stick tests or microscopic examination of urinary sediments, while available, are rather uninteresting procedures for the population of healthy young adult subjects on college campuses. The instructor can discuss the physiological basis for abnormalities detected by such clinical tests, but in most classes all the students are healthy and the tests are negative. Measurement of specific gravity with hydrometers gives some indication of solutes in the urine, but the resolution of hydrometers is low, and the range of specific gravity between dilute and concentrated urine is narrow.

Osmometry - direct measurement of the molar concentration of the total solutes in an aqueous solution - offers a tool for conducting quantitative experiments in the instructional laboratory. In osmosis the movement of water from compartments of high concentration of water (low concentration of solutes) to compartments of low concentration of water (high concentration of solutes) is driven by the difference in osmotic concentration between compartments. Students frequently have difficulty understanding this phenomenon. Although it is the solute that is responsible for the osmotic pressure, it is a negative pressure resulting in movement of water. Nonetheless this process is fundamental in the control of volume and concentration of the intracellular, interstitial and plasma water compartments.

These laboratory exercises explore water and electrolyte shifts in the body water compartments as reflected in the volume and osmolarity of the excreted urine. Calculations of the body water compartments over periods of a few hours solidify understanding of the underlying physiological events.

The osmotic movement of water across a permeable membrane from a compartment of dilute solution to a compartment of concentrated solution is central to the understanding of biological systems. The driving force for osmotic water movement is the difference in concentration of solutes between two compartments. Osmotic pressure can be calculated from a form of *van't Hoff's Law*:

$$\pi = \phi I R T c$$

in which,

π = osmotic pressure (in atmospheres in this example)

I = number of particles dissociated

R = ideal gas constant (22.4 L-atmos/mol)

T = Kelvin temperature

c = the molar concentration of the solute, and

ϕ is a coefficient accounting for incomplete dissociation of the solute.

The measurement of the osmolar concentration of a solution is based on physical properties of solvents and solutions, particularly the colligative properties. When a solute is added to a solvent, the solution differs from the pure solvent in that:

the osmotic pressure increases,

the boiling point increases,

the vapor pressure decreases, and

the freezing point decreases.

The osmometers used in the instructional laboratories at the University of Southern California employ the last phenomenon. The normal freezing point of water, 0°C, is depressed by 1.86°C for each mole of a non-ionized solute in an aqueous solution. For ionic solvents, each dissociated ion contributes to the osmotic concentration and, consequently, the lowering of the freezing point.

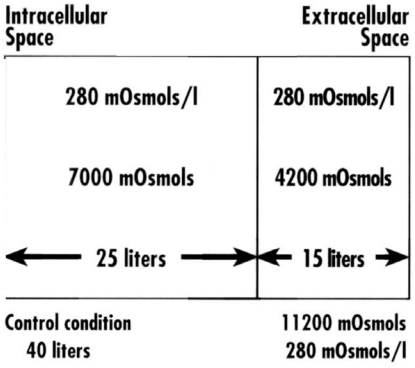
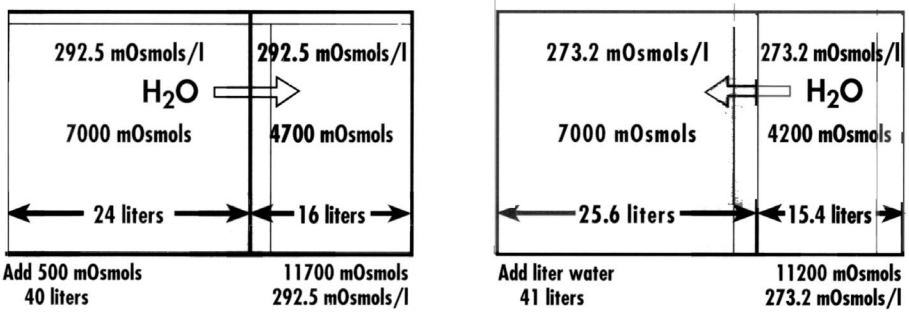


FIGURE 1 - Normal composition of intra- and extracellular water compartments.

In a healthy adult male, water accounts for about 60% of the weight of the body.[1] Since the density of the body is about 1g/ml, the total body volume may be estimated as a percentage of the body weight (1 l ≈ 1 kg). There are methods for direct measurement of total body water (for example, using D_2O as a tracer); however, an estimation from body weight is satisfactory for the laboratory exercises described below. The total body water is distributed between the intracellular space (about 60%) and the extracellular space (about 40%) as shown in Figure 1. The two water spaces[2] are separated by the plasma membrane in which the protein Na^+-K^+-ATP-ase concentrates most of the K^+ inside the cells and most of the Na^+ outside.



Changes in intracellular and extracellular volume following addition of 500 mOsmols of NaCl (lt) and 1 liter of water (rt).

FIGURE 2 - Body compartment volumes in response to water and osmolar loads (heavy lines) compared with control conditions (light lines).

Although the distribution of ion species differs across the plasma membrane, the osmotic concentration (that is, the molar concentration of all dissolved particles) of the body fluids is always equal in intra- and extracellular spaces.[3] As a consequence, any alterations of the osmotic concentration in the extracellular spaces also alters the concentration in the intracellular spaces. Since changes across the cellular membrane involve the movement of water rather than solvent, they necessarily effect changes in cellular volume. Figure 2 shows the effects of adding solvent or solute to the extracellular space. In both cases, the osmolarity is equalized across the cellular membrane by movement of water, which changes the volume of intracellular as well as extracellular space.

As illustrated in the right side of Figure 2, ingestion of solute initially increases the osmolarity of the extracellular compartment. Water then flows into the extracellular space, decreasing the volume of the intracellular space, as the osmolarity is equalized across the plasma membrane.

[1] In females, the average is about 55%. However, with both males and females there is considerable variability related to body composition. The percentage of total body water increases with the lean body weight.

[2] A third water space, the *transcellular*, is not important in the present considerations.

[3] The plasma proteins in the vascular spaces result in a slightly higher osmotic pressure in the plasma than in the interstial spaces. However there is not osmotic gradient across the cell membranes in either the vascular or extravascular spaces.

Conversely, drinking water dilutes the osmolarity and increases the intracellular volume, as shown on the left side of Figure 2. Subsequently water flows into the intracellular compartment, increasing its volume as the osmolarity is equalized across the cellular membrane. Since an organ such as the brain, within the rigid compartment of the skull, cannot tolerate changes in volume, intracellular volume must be maintained. The control of intracellular volume is effected by control of the osmolarity of the body fluids.

In humans, the osmolarity of the plasma is between 275 and 290 mOsmols/liter,[4] and within any individual it is controlled in a very narrow range. For convenience in calculations (such as those in Figure 2) and for experiments on subjects in which plasma osmolarity is not measured, you can use the nominal value of 280 mOsmols/liter.

The composition and volume of body fluids is controlled through the blood plasma. *Osmolarity* is controlled by sensors in the hypothalamus. Increases in plasma osmolarity stimulate release of antidiuretic hormone (ADH) from the posterior pituitary gland to retain fluid by reabsorption of water in the collecting tubules of the kidney. Increases in plasma osmolarity also stimulate thirst. *Plasma volume* is regulated by several mechanisms which control excretion or retention of sodium, including the renin-angiotensin-aldosterone system, the sympathetic nervous system, and the atrial natriuretic hormone.

The effects of several of these mechanisms can be followed non-invasively in the volume and composition of the urine. Since sodium is the major extracellular cation, control mechanisms involving retention or secretion of Na^+ can be followed without direct measurement of $[Na^+]$.

[4] In a strict sense, there is a distinction between osmolarity in units of osmols/liter of solution and osmolality in units of osmols/ kilogram of solution. Since biological fluids have concentrations of only a few hundred milliosmoles per liter, the solute contributes little to the weight of the solution. In these applications, the terms may be used interchangeably, and the measured values for either are within the accuracy of the measurement. Although the instrumentation reports values in mOsmols/kg, we refer to the values incorrectly, but conveniently, as osmolarity.

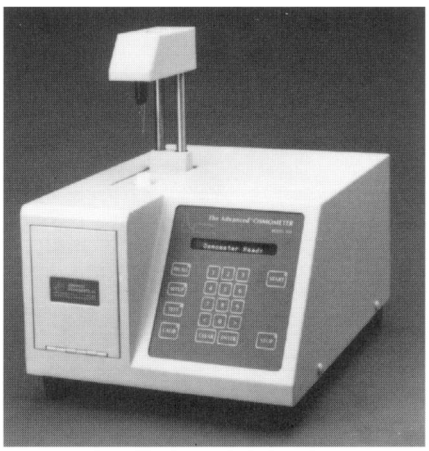


FIGURE 3 - Freezing point osmometer with direct reading (mOsmols/kg)

The use of osmometry for determination of electrolyte and water balance can be incorporated into physiology courses taught at a variety of levels in community colleges, universities, and professional schools. Measurement of urine osmolarity can supplement the procedures in an existing urine lab exercise, providing quantitative observations for calculation of osmotic clearance or free water loss or gain. Or entirely new laboratory exercises on fluid and electrolyte balance can be developed, using the students as subjects by measuring the course of the volume and osmolarity of urine excreted following ingestion of a water or electrolyte load. Such exercises can be enriched by addition of other measurements such as $[Na^+]$, $[K^+]$, $[Cl^-]$ or BUN, depending on the level of the class and the equipment available. The clinical dip-stick tests (glucose, protein, keytones, blood, or leukocytes), although convenient and readily available, typically give negative readings on healthy young adults and consequently are of limited value for teaching physiological principles.

EXPERIMENTAL DESIGN

For the *simplest protocol*, the students should empty their bladders at the beginning of the laboratory period and save that sample for other tests to be done during the lab period. One and a half or two hours later, a second sample can be taken for measurement of osmolar and free water clearance. In the simplest experiment, the time between the two samples should be noted and no food or fluids should be taken during that time.

For a *standard protocol*, the students should drink water equal to 1% of their body weight upon entering the lab - for example a 70 kg subject would drink 700 ml of water. [Supply a scale and a calculator to convert to kilograms, if necessary.] After drinking the water, subjects should empty their bladders, saving that sample for other tests if appropriate; there is very little absorption or redistribution of the ingested water over a period of a few minutes. All of the urine excreted over the next two to three hours (use the same time interval for all subjects, as appropriate to the length of the lab period) must be saved for measurement of volume and osmolarity. For observation of the time course of urine flow, take measurements at 30 minute intervals; otherwise, subjects may urinate as needed, but the last sample must be taken at the end of the experimental period (see Figure 4).

For a *controlled protocol,* there should be a control group and one or more experimental groups. There is considerable latitude in choice of the control and experimental conditions. The control group could be water, as in the standard protocol, with the experimental group ingesting an osmotic load of NaCl. Another possible experiment is for the control group to ingest nothing, with one or more experimental groups taking a water and/or osmotic load. An elegant design is for the control group to take 1% of their body weight of isotonic saline, a hypertonic group take the same amount of NaCl tablets in a minimum of water, and a hypotonic group take 1% of their body weight in water. The time course for each group should be the same as for the standard experiment (see Figure 4).

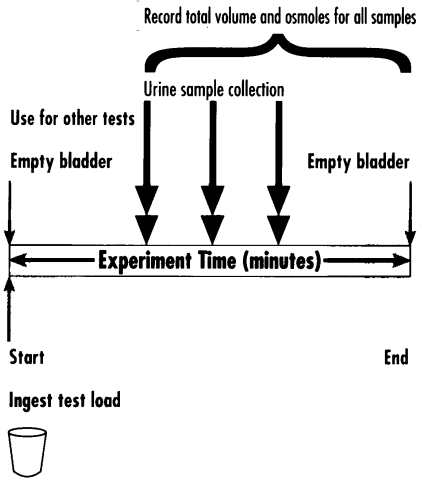


FIGURE 4 - Time line for standard or control procedure.

When schedule allows, it can be instructive to guide a class in consideration and selection of the conditions for control and experimental groups. In this laboratory, students have selected experiments such as decaffeinated vs. regular coffee, or diet vs. regular soft drinks. Whether or not the chosen experimental variable is effective in altering fluid or electrolyte balance, an ingested volume equal to 1% of each subject's body weight is suitable to induce some diuresis in excess of obligatory urine flow during a two- to three-hour collection period. When it is feasible for the class to plan an experiment before the day of the lab session, students can be guided in procedures for eating and drinking schedules prior to the experimental period to insure that all members begin the test period at reasonably similar conditions of hydration.

Advise students not to empty their bladders before coming to the laboratory to insure an adequate volume of sample for initial testing. A few fastidious students may object to collecting and analyzing their own urine samples. If necessary, explain that urine is usually a sterile fluid containing only substances which have been in the blood plasma. Some cajoling may be needed to obtain cooperation; typically humor and camaraderie develop during these exercises. Occasionally female students may wish to wrap a sheet of paper around their specimen cup when the lab occurs during menstruation.

SAMPLE COLLECTION AND ANALYSIS

It is feasible to use ordinary laboratory beakers or even beverage cups for sample collection; however, a small investment in clinical urine sample cups and lids (available in cases of 500 from medical suppliers) maintains a professional image for these exercises. These cups are marked to indicate volume in ounces and milliliters.

Although the conditions necessary for proper collection of urine samples may seem obvious to the instructor, explain them carefully to students:

For the initial urine sample: *At the beginning of the experimental period, each subject must empty his/her bladder as completely as possible.*

The initial urine sample may be used for other urine analysis procedures, but the values from the initial sample may not be used for calculations of water or electrolyte balance in the standard or controlled experiments.

For subsequent urine samples collected during the experimental period: *The entire volume and osmolarity of each sample must be measured and recorded. To avoid any possible loss of sample volume, subjects should take two or three cups each time they go to collect a sample.*

The last urine sample *must be taken at the end of the experimental period and must empty the bladder as completely as possible.*

No food or drink may be taken during the experimental period.

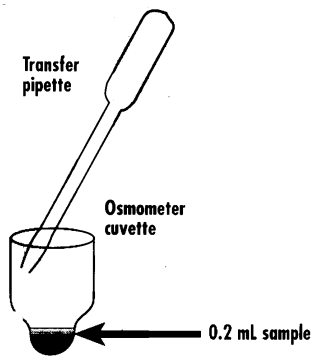


FIGURE 5 - Fill cuvette with 0.2 ml sample.

With standard clinical urine sample cups the volume of the urine can be read directly on the cup. When multiple samples are collected during the experimental period, be sure students record the volume and corresponding osmolarity of each sample. (The exception is using pooled samples; see below.)

In this laboratory, osmolarity is measured with an Advanced Instruments, Inc freezing point osmometer, Figure 3. This instrument measures the molar concentration of all solutes in the sample by its effect in lowering the freezing point. The operator transfers a 0.2 to 0.3 ml aliquot of each specimen into a cuvette (see Figure 5) with a pipette and places the cuvette into the well of the osmometer. Pressing the operate button initiates a sequence in which the cuvette is lowered into a cooling chamber and the temperature sensor inserted. The sample undergoes supercooling; as it warms, the freezing point is detected and converted to a direct reading of milliosmoles / kg (see footnote 1 on terminology).

PRINCIPLES OF WATER BALANCE CALCULATIONS

Most of the fluid and electrolyte calculations are applications of simple mixing equations. The basic format is:

AMOUNT = VOLUME × CONCENTRATION

In these applications, amount is in milliosmoles of solute,
volume is in liters, and
concentration is in milliosmoles/liter.

This basic equation can be solved for:

VOLUME = AMOUNT / CONCENTRATION or
CONCENTRATION = AMOUNT / VOLUME

The next step is merely a statement of the conservation of matter. An amount equals that same amount, merely:

AMOUNT = AMOUNT

When that amount is present in different volumes and different concentrations, the equation becomes:

$VOLUME_1 \times CONCENTRATION_1 = VOLUME_2 \times CONCENTRATION_2$

This is a powerful and versatile equation for use in physiological calculations. The equation for clearance is merely a form of this equation in which,

Vol is the volume of urine per unit time,
U_X is the concentration of X in the urine and
P_X is the concentration of X in the plasma.

Substituting into the equation above,

$U_X \times Vol = P_X \times C_X$ in which

C_X is the clearance - the volume of plasma which contained the amount of the substance appearing in the urine.

In calculation form:

$C_X = Vol \times (U_X / P_X$

OSMOLAR CLEARANCE

Applying these principles to a urine sample collected over a period of time, we can calculate the volume of plasma from which all osmoles of solute have been removed during that time.

For these exercises take plasma osmolarity (P_{OSMOL}) as 280 mOsmol/l.

First calculate urine flow: Urine volume (ml) / time (min) = Flow (ml/min).

Divide by 1000 to convert units to l/min

Use urine measured osmolarity (U_{OSMOL}) in mOsmol/l

C_{OSMOL} = Flow (ml/min) × U_{OSMOL}/ P_{OSMOL}

Example: Find the osmolar clearance for 600 ml of urine with an osmolarity of 140 mOsmol/l over a one hour period.

Urine flow: 600 ml / 60 min = 10 ml / min

C_{OSMOL} = 10 ml/ min × (140 mosmol/l / 280 mosmol/l) = 5 ml/min

(plasma cleared of particles)

FREE WATER CLEARANCE AND RETENTION

Hypo-osmotic urine (osmolarity < 280 mosmol/l) indicates removal of water from the body. A portion of the hypo-osmotic sample was filtered at a concentration of 280 mOsmol/l in the glomerulus; the additional volume indicates free water removal. The same basic equation is used first to calculate the AMOUNT; that is, the number of osmoles in the collected sample. Next, calculate the VOLUME that amount would occupy at a concentration of 280 mOsmol/l.

In the example above,

AMOUNT = VOLUME × CONCENTRATION

= 600 ml × 140 mOsmol/1000 ml = 84 milliosmoles (in sample).

Isosmotic VOLUME = AMOUNT / CONCENTRATION

= 84 mOsmol/280 mOsmols/1000 ml = 300 ml (isosmotic urine)

Free water = Total volume - Isosmotic volume

= 600 ml - 300 ml = 300 ml.

Urine is frequently hyperosmotic (osmolarity > 280 mOsmol/l). In this case, the kidney is reabsorbing water; the glomerulus filters more free water than it excretes.

In the following example, 120 ml of urine is collected over a two hour period with an osmolarity of 840 mOsmol/l. The osmolar clearance is:

$$C_{OSMOL} = Flow \times (U_{OSMOL}/P_{OSMOL})$$
$$= (120 \text{ ml} / 120 \text{ min}) \times (840 \text{ mOsmol/l} / 280 \text{ mOsmol/l})$$
$$= 3 \text{ ml/min}$$

The amount of solute in this sample is 120 ml × 840 mOsmol/1000ml = 100.8 mOsmols

The volume of plasma filtered through the glomerulus to deliver 100.8 mOsmols to the tubule was:

VOLUME = AMOUNT / CONCENTRATION
= 100.8 mOsmols / 280 mOsmols/1000 ml = 360 ml

The free water retained in this example is:

Free water = Isosmotic volume - Excreted volume
= 360 - 120 = 240 ml free water retained.

CALCULATION OF BODY WATER COMPARTMENTS

Estimates total body water (liters):

For males, body weight in kg × 0.60,

For females, body weight in kg × 0.55,

For older or obese subjects, decrease these percentages.

Estimate for total osmoles[5] (milliosmoles):

Total body water (liters) × 280 mOsmols/l.

Estimates for extracellular volume (liters) and osmoles:

Extracellular volume (liters) = Total body volume × 0.40,

Extracellular osmoles = Extracellular volume (liters) × 280 mOsmol/l.

Estimates for intracellular volume (liters) and osmoles:

Intracellular volume (liters) = Total body volume × 0.60,

Intracellular osmoles = Intracellular volume (liters) × 280 mOsmols/l.

[5] The unit for **amount** is **osmoles;** the unit for **concentration** is **milliosmoles per liter.** These terms are not interchangeable; be sure students understand them and use them correctly.

CALCULATIONS FOR MULTIPLE URINE SAMPLES

When subjects collect multiple urine samples during the measurement period, they may combine all samples to obtain the total volume and measure the osmolarity of that pooled sample, or they may use the following as a volume weighted average when osmolarity is measured separately for each sample:

Total urine sample volume = $vol_1 + vol_2 + ... + vol_n$

Average osmolarity excreted =

$[(osmolarity_1 \times vol_1) + (osmolarity_2 \times vol_2) + ... + (osmolarity_n \times vol_n)]$ / Total volume

Osmoles excreted = Average osmolarity excreted × Total urine sample

CALCULATION OF CHANGES FOLLOWING INGESTION OF WATER OR SOLUTE LOAD

Initial changes in extracellular compartments before osmotic equilibrium:

New extracellular volume = Original extracellular volume + Added volume.

New extracellular osmoles = Original extracellular osmoles + Added osmoles

New extracellular osmolarity = New extracellular osmoles / New extracellular volume

Changes in body water compartments after osmotic equilibrium:

New total body volume = Original total body volume + Added volume

New total body osmoles = Original total body osmoles + Added osmoles

New total body osmolarity = New total body osmoles / New total body volume

New extracellular osmolarity = New intracellular osmolarity = New total body osmolarity

Changes in intracellular and extracellular volume after osmotic equilibrium

New intracellular volume = Intracellular osmoles / New total body osmolarity

New extracellular volume = (Orig extracellular osmoles + Added osmoles)/New osmolarity

CALCULATION OF CHANGES FOLLOWING URINE EXCRETION

Final body volume = Original volume + Volume ingested - Volume excreted

Final body osmoles = Original osmoles + Osmoles ingested - Osmoles excreted

Final body osmolarity = Final body osmoles / Final volume

Final intracellular volume = Intracellular osmoles / Final body osmolarity

Final extracellular volume = (Orig extracellular osmoles + osmoles added - osmoles excreted)/Final body osmolarity

CALCULATION OF EXPERIMENTAL CHANGES AND CORRECTION

For each determination of the change in volume, osmoles, or osmolarity for the total body water compartment, the intracellular compartment, or the extracellular compartment (there are nine combinations of values), calculate as follows (multiply by 100 for percentage change):

Experimental change induced = (New value - Original value) / Original value

Physiological response = (New value - Final value) / New value

Overall correction = (Original value - Final value) / Original value

The calculations presented above lead the student through the logical development of the volume and osmolar changes following ingestion of a osmotic and/or water load, and the subsequent physiological adjustments. By normalizing water or osmotic load on the basis of total body water as estimated from body weight, responses between different groups can be compared. The estimates of body water volume and osmolarity introduce little error in overall effects. There is a tacit assumption that ingested osmoles remain in the extracellular space and are not metabolized. An osmolar load of NaCl meets these assumptions most closely. Nonetheless, interesting and informative experiments can be conducted with substances which do not meet such assumptions, especially if the instructor guides the discussion of the assumptions and outcome on the basis of sound physiological principles. In comparing groups receiving different treatments, it is feasible to pool the data for all the subjects in the same group, treating each as having a single large extracellular space and excreting a large urine sample.

The calculations of volumes, concentration, and amounts for the three body water compartments, for initial, new, and final conditions, give students ample opportunity for practicing calculations of quantitative physiological functions. Frequently, the instructor may wish to summarize the data for the whole class and compare groups which received different treatments. A spreadsheet program (such as Lotus 1-2-3® or Microsoft Excel®) is suitable for entering data from individual students (rows) and calculating values (columns). By summing columns and calculating across in rows, both individual and group data may be presented. Students can be encouraged to develop the formulas for spreadsheets of their own; however, they may miss some of the instructional value of these exercises if allowed to use a ready made spreadsheet in lieu of learning the underlying principles of the calculations.

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Dr. Norton received a bachelor's degree from the University of California Santa Barbara (1957), and a doctorate from the University of Buffalo (now SUNY, Buffalo) in 1961. From 1961 to 1963 he was a National Institutes of Health postdoctoral fellow at Tohoku University School of Medicine, Sendai, Japan. He has had appointments in physiological research at Children's Hospital, Los Angeles, Huntington Memorial Hospital, Pasadena, CA and Wadsworth Veteran's Administration Hospital, Los Angeles, CA.

Between 1969 and 1989 he worked for Beckman Instruments, SmithKline Beckman, SensorMedics and Del Mar Avionics in development of physiological instrumentation and applications as research physiologist, applications manager and senior scientist. He has been laboratory manager in the Department of Physiology at UCLA School of Medicine and is currently Laboratory Director in the Department of Biological Sciences at the University of Southern California.

He has authored and coauthored books on the dorsal columns of the spinal cord, use of commercial instrumentation in the space station, exercise physiology and cardiopulmonary rehabilitation.