MEMORANDUM
RM-5396-PR
MAY 1970

UNIQUE MATHEMATICAL MODELS OF INDIVIDUAL BLOOD

E. C. DeLand, E. Magnier and J. V. Maloney, Jr.

PREPARED FOR:
UNITED STATES AIR FORCE PROJECT RAND

The RAND Corporation
SANTA MONICA • CALIFORNIA

MEMORANDUM
RM-5396-PR
MAY 1970

UNIQUE MATHEMATICAL MODELS OF INDIVIDUAL BLOOD

E. C. DeLand, E. Magnier and J. V. Maloney, Jr.

This research is supported by the United States Air Force under Project RAND—Contract No. F44620-67-C-0045—monitored by the Directorate of Operational Requirements and Development Plans, Deputy Chief of Staff, Research and Development, Hq USAF. Views or conclusions contained in this study should not be interpreted as representing the official opinion or policy of Rand or of the United States Air Force.

The RAND Corporation
1700 MAIN ST. • SANTA MONICA • CALIFORNIA • 90406

PREFACE

This Memorandum postulates and discusses some methods of mathematically simulating the blood composition of individual patients on a clinical basis. This particular research is based upon fundamental work, previously reported by investigators at The RAND Corporation and at the University of California, Los Angeles, Department of Thoracic Surgery, on the modeling of the biochemistry of the respiratory functions for statistically normal human blood. As one of a series of Rand publications dealing with patient management and delivery of health services, this research is directed toward an understanding of the interrelated biochemical factors important for preventive medical care and monitoring of treatment responses in hospitals. Since military personnel are subject to unusual stresses, and because of the tradition of highly successful medical care in the military services, these studies are particularly relevant to the medical care of DoD personnel. The cooperation of the USAF in sponsoring these fundamental studies is gratefully acknowledged.

The earlier work developed basic methods and procedures for construction of the abstract, computer-based models. Here, we extend these procedures and show that given sufficient data from the laboratory, such a model can be derived for individual patients. Continuing work at the National Institutes of Health, Bethesda, Maryland and at the University of Missouri, School of Medicine, Columbia, Missouri is attempting to derive the minimum necessary conditions to construct an individual model, and to make clinical applications of the resulting automated procedures. We do not address these two questions here.

The models described simulate the steady-state distribution of chemical species between the plasma and red cells of blood, but not the kinetics or time-dependent states of

such systems. This work, therefore, examines the conditional distribution at a moment in the time history of the patient, and considers the proposition that the chemical pattern of the blood can be used to characterize the patient. If this proves valid for certain states, it may follow that such models would be useful for experimental fluid therapy.

SUMMARY

This research investigates the applicability of mathematical and computer procedures for the simulation of theoretically normal human blood biochemistry to the problem of simulating the unique blood of certain clinical patients. This Memorandum reviews only briefly the earlier methods for the steady-state simulation of large biochemical systems, and then adapts the basic concepts to the problem of automatically modeling the unique blood of an individual. The procedures and algorithms are discussed, and, in particular, it is shown that given sufficient laboratory data a unique model can automatically be defined.

In thirty separate laboratory experiments, a model of individual blood was obtained and tested against the real blood *in vitro*, under various chemical stresses. Results indicate satisfactory agreement except in cases where time-dependent or kinetic parameters are involved as in potassium-ion uptake by the cells. These results have encouraged the belief that with subsequent improvement, in particular, development of procedures based upon minimal data, modeling procedures will be applicable and useful in a clinical setting.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. Helen Bugyi, M.S., who developed several original techniques for the chemical analysis of many of the red cell and whole blood species that are a part of the data of this Memorandum. Mrs. Bugyi also supervised the analysis of most of the chemistries that are a part of this Memorandum.

The following U.S. Public Health Service grants have contributed to various portions of this research:

UCLA Health Science Computing Facility USPHS Grant FR 3,

UCLA School of Medicine, Thoracic Surgery-Pediatric Cardiology Interdisciplinary USPHS Grant No. GM 13242,

Dr. Magnier's Career Development Fellowship, USPHS Grant No. 5F3-HE-19, 070-04,

The RAND Corporation's USPHS Research Grants No. HE 08665-02 and GM 15896, as well as the U.S. Air Force Project RAND support for Dr. DeLand.

CONTENTS

FIGURES

TABLES

I. INTRODUCTION

This Memorandum deals with the physiology of blood, in particular those aspects that are related to its respiratory function and its acid-base chemistry. Consequently, particular attention is given to the distribution of proteins (albumin, globulins, and hemoglobin), electrolytes, water and other miscellaneous substances in the blood. The discussion is directed to researchers in clinical biochemistry although acquaintance with certain mathematical and modeling procedures is assumed in order not to divert the argument. These procedures, adequately referenced, are not essential in detail in order to follow the development of the research.

The work described concerns the creation of a computer-based mathematical model† of the blood of an *individual* patient that interrelates many of the known characteristics for that blood, such as its oxygen saturation curves, Gibbs-Donnan effect, cationic pumps, carbamino reactions, and the titration curves of albumin and hemoglobin. Essentially, a mathematical model of the biochemistry of blood will consist of a set of equations similar, for example, to the Henderson-Hasselbach equation [2,3,4] and derived from the theoretical biochemistry of blood, plus a set of constraints that are consequences both of the properties of semi-permeable membranes and of the conservation of mass and charge. This concept of a model is discussed by McLean [5]. The set of such equations is solved simultaneously to simulate the

†We define a model as a set of chemical equations and mathematical constraints that describe many of the steady-state interrelationships of the numerous chemical species in the blood. A "solution" of the model is the result of simultaneously solving a set of equivalent mathematical equations and constraints to give the concentrations of each product species at equilibrium or at the simulated biological "steady-state," and to give the distribution of species between plasma and red cells. For a background discussion of modeling, see Ref. 1.

distribution of species across the red cell membrane at biological steady-state.

Note that the Henderson-Hasselbach equation of the bicarbonate system (and the larger model we describe) is a deterministic or analytical model, not a statistical model. Such a model attempts to predict empirical data by simulating the mechanisms and the physical principles of the real system in the range of interest. By simulating the interactive mechanisms, an adequate model will have the generality necessary to behave as the real system over a range of conditions. Note also that these models are not inherently "kinetic." Their solution gives an equilibrium, or in some cases a steady-state distribution of chemical species, and not the trajectory of a dynamic system. This condition occurs because the equations of the models are essentially algebraic and give a point solution. Kinetic models require expression in time-based differential equations.

Valid models of statistically normal data for average adult male blood have been developed and tested by RAND [6-8]. The construction of these models was accomplished prior to this research; however, the modeling of a unique patient's blood has not been demonstrated. Therefore, the first research objective is to demonstrate that, given the unique laboratory data, a valid model of an individual person's blood can be created. The second objective is to automate the construction of such models. The value of an individual model is increased if it can be made readily available; several days or weeks delay for a model limits its practical application.

The third research area is concerned with the use of a model in predicting the response of a unique blood to various chemical loads or stresses such as acid, base, water, electrolytes, or proteins.

The laboratory experiments on human blood have the following overall organization (detailed in Sec. III and Appendix B). Four hundred cc of blood are drawn into a 500-cc heparinized vacutainer. The blood is initially oxygenated and then separated into two 200-cc pairs, of which one is stressed and the other serves as a control. Sixty-cc aliquots of blood from each bottle are then placed in parallel tonometers at 37^{o}C and equilibrated with a known gas mixture for one hour. At the end of this time, three 20-cc syringes are filled from each tonometer, one pair of syringes from each tonometer for duplicate red cell and plasma analyses, and the third for whole blood analysis. The pairs of stress and control syringes are centrifuged at 37^{o}C to separate the plasma from the red cells. The whole procedure is then repeated, filling the tonometers with the remaining blood (that has been stored cold but not frozen) until the 400 cc of blood is exhausted.

The control blood is used as a test for the capabilities of modeling arbitrary bloods. The stressed half is used to test the predictive capabilities of the model.

II. THE DEVELOPMENT OF A NORMAL MODEL

THE CONSTRUCTION OF A MODEL OF BLOOD FOR A STATISTICALLY NORMAL HUMAN MALE

This section heuristically develops the construction of a model of the blood for a statistically normal human male. Since the detailed procedure is quite long, and the argument for its validity is fairly complicated, that argument is not recapitulated here. The reader is referred to earlier developments [6-8]. The following development is an overview of that work.

For purposes of this research, blood consists of two physiological compartments, the plasma and the interior of the red cells. Each is assumed homogeneous but not in chemical equilibrium owing to the continuous transport and consequent electrochemical gradients for certain species across the red cell membrane (notably the inorganic cations and possibly hydrogen ion). The assumption here is that the system is in steady-state (steady rate of passage of materials through the viable systems) for measurement and simulation. The distribution of species in the viable steady-state condition is slightly different for each individual, or for the same individual at different times. It is the pattern of distribution at a given time that is important for this model.

Human Plasma

For purposes of modeling the respiratory functions of blood, i.e., gas transport and acid-base chemistry, human plasma may be viewed as a simple chemical system consisting essentially of a water solution of gases, electrolytes, and certain proteins. Other constituents, minor in amount but perhaps critical from other points of view, such as fibrinogen, certain lipids, and other miscellaneous materials, can be

safely ignored here except for their total amount, which may contribute to the osmolarity of the solution. It is necessary, however, to account for the usual cations and the inorganic anions such as chloride, sulfate, and phosphate, plus amino acids, ammonia, and organic acids, such as lactic. For other purposes, we also account for urea, glucose, and lipids. The important proteins in gas transport and buffering are the globulins and serum albumin, which are lumped together as plasma protein.

A mathematical model of the sort required for plasma may be built in stages. First, one might write down all the equations for the solubility of the respiratory gases in water (or plasma, since it has slightly different solubility constants), and add the reactions of CO_2 and H_2O to form the carbonic acid system. Such a model has been described in detail [1], but it is simple enough so that the equilibrium could be calculated by hand. Using the partial pressures of gases in the alveolar sacs and ignoring, temporarily, the Hamburger shift of bicarbonate and chloride into the red cells, this model is a first approximation to the bicarbonate-buffering system in plasma.

While adding the constituents to the model one at a time, it is necessary to determine the appropriate solubility constant, a, or chemical equilibrium constant, K, for the given temperature and pressure ($37^{o}C$ and 1.0 atm). A consistent terminology requires reference to such constants as the thermodynamic *free-energy parameters*, or simply the c_j's, where j is the number of the species computed in the equilibrium or steady-state.

Additional terminology required is that the amount in moles of a chemical *component* in the model (from which the computed output *species* are derived) is b_i, where i is the number of the component. The stoichiometric coefficients of chemical reactions are called a_{ij}, where i refers to

the component appearing in the species j. Various concentration units may be used: moles per liter of plasma, moles per liter of total water (in the plasma), or mole fractions, which are the moles of species j divided by the total moles in the chemical *phase* or physiological *compartment*.† Compartments refer to the effective or apparent compartment determined either by natural membrane boundaries or by apparent radioisotope space. Red cells' compartment, however, refers to the aggregated interiors of red cells, considered as a unit space, in a given volume of blood.

Finally, the c_j's are determined from the published literature or handbooks whenever possible [9-10]. However, free energy parameters for non-equilibrium, steady-state systems, particularly those parameters relating to the transport of species between compartments, must be derived empirically to satisfy observed distribution ratios or other steady-state conditions. Methods have been developed for computing such effective parameters within the context of the computer model. The mathematical model is constrained, by adding linear conditions, to give a particular result, and from this an equivalent effective parameter is determined, which may then be substituted for the constraint. This process is equivalent, in a chemical reaction, to specifying the quantities in the mass-action equation in order to compute the equilibrium constant. Once determined, the equilibrium constant may then be used to compute unknown quantities.

The step from the bicarbonate system to plasma requires the addition of various electrolytes, proteins, and other species to the model. The amount to be added is obtained from the literature [7].

---

†The natural unit for computation is the mole fraction. For example, the equilibrium constants K usually found in the literature must be converted to mole fraction units for use in the current model [1].

The next problem is to simulate the chemical behavior of the proteins. The following characteristics of proteins are required: the total moles of the species should agree with the literature; they should have the proper osmotic coefficient, binding properties for small ions; and they should have the proper buffering curve.

The total moles of albumin and globulin are determined from Geigy [11]. The buffering curve of the proteins is determined using the total number of each ionizing group and their apparent ionization constants [12]. The procedure for this calculation is given in Ref. 13. The correct ionization of the protein sites at a particular pH will utilize the proper amount of H^+ ions and create the proper fixed charge for the required Gibbs-Donnan phenomenon. Water, chloride, calcium, and other species can be bound to the proteins in accordance with values that are to be found in the literature [12-15].

In the process of building a model, it is impossible to name the hundreds of species that are found in the blood. There remains, then, some unnamed species called "X-MISC." The X-MISC species also have a net charge, but neither the moles nor valence of these species are well known. These parameters are implied, however, by the hematocrit and chloride distribution, and by the requirement that plasma have a zero net electrical charge [7].

Human Red Cells

Since the final goal is to have a model of 1 liter of human blood, the plasma compartment is scaled to 0.547 liter (1-hematocrit). Correct instructions will cause the program to do this automatically.

To form the red cell compartment, the various cations of interest and other species for the red cells are added and fixed in the new compartment in amounts corresponding to average values in the literature. Effective cation-

active pumps are simulated by computing the theoretical free-energy parameters, c_j, to give the observed cation ratios between plasma and red cells for each cation. A rudimentary phosphate system is incorporated, and the correct average amount of hemoglobin is inserted [8].

The buffering curve for the oxystable sites on hemoglobin is created in the same manner as the buffering curves of albumin. Certain of the sites on hemoglobin, however, are oxylabile, and computation must allow for this lability. Other sites on hemoglobin react with CO_2, the carbamino reactions, which also must be computed. The mathematical aspects of these reactions have been developed in Refs. 7-8.

The Adair equations [16], postulated to express the oxygen reactions with hemoglobin, are shown in Eqs. (1)-(5).

$$Hb_4 \rightleftarrows Hb_4 \tag{1}$$

$$Hb_4O_2 \rightleftarrows Hb_4 + O_2 \tag{2}$$

$$Hb_4O_4 \rightleftarrows Hb_4 + 2O_2 \tag{3}$$

$$Hb_4O_6 \rightleftarrows Hb_4 + 3O_2 \tag{4}$$

$$Hb_4O_8 \rightleftarrows Hb_4 + 4O_2 \tag{5}$$

The c_j values required to produce an oxygen saturation curve for hemoglobin that closely (less than one percent saturation error maximum) matches the Severinghaus curves [17] have been determined by a curve-fitting routine. The respiratory characteristics to be matched by the model are detailed in Refs. 18-22.

If the model has been constructed correctly (see p. 15 for treatment of this supposition), the red cells will have the correct cations, the correct titration curve, and the

correct hemoglobin reactions including oxygenation and the oxylabile and oxystable ionizing sites that, combined with the carbamino reactions, produce the Bohr effect. The charge on the proteins contributes to the Gibbs-Donnan effect. The red cell compartment is now scaled to 0.453 liters.

Plasma and Red Cells

The model now consists of a set of equations and constraints that give the correct distribution of cations and proteins. Table 1 is a listing of the components of a normal liter of human blood in moles. The components shown with zero amounts are ionization sites of the proteins. The number of sites are adjusted internally.

Table 2 is a list of the pertinent species and reactions in each compartment of simulated human blood. The left column names the species; the second is the thermodynamic parameter, c_j, for each species; the remaining columns show the stoichiometric coefficients of the components forming the species. The column to the far right is the valence of each species for charge-accounting purposes [8].

Table 3 is the computed distribution of species between plasma and red cells in a liter of blood in moles, mole fractions, and moles per liter of compartment water for the standard, statistically normal human male. The model shown is for venous gas pressures, 46 mm Hg CO_2 and 40 mm Hg O_2.

The anions are postulated not only to have inactive pumps (corresponding to the literature), but also to distribute according to their charge (Gibbs-Donnan gradient) and with respect to the osmotic properties of the system. We assume equal osmolarity for plasma and red cells for the distribution of water and other neutral molecules.

Computer experiments show that small changes in the impermeable miscellaneous anions (X-MISC) cause shifts in water (because of the osmotic effect), and changes in their charge cause a redistribution of the electrolytes (because of the neutral charge constraint). Also, the pH of the

Table 1

EXPERIMENTAL HUMAN BLOOD: BFFR4, FREE H_2O

	ROW NAME	LIQUID PHASE	VENOUS GAS	ARTERIAL GAS
1	O2	6.83000000-03	5.26300000-02	1.31500000-01
2	CO2	2.34900000-02	6.05000000-02	5.26300000-02
3	N2	4.37000000-04	8.25810000-01	7.54030000-01
4	H2O	4.73550000 01	6.10600000-02	6.10600000-02
5	H+	2.00628420-01	0.0	0.0
6	NA+	8.25000000-02	0.0	0.0
7	K+	4.86400000-02	0.0	0.0
8	CA++	1.54704000-03	0.0	0.0
9	MG++	1.64000000-03	0.0	0.0
10	CL-	8.43200000-02	0.0	0.0
11	LACTIC ACID	2.50000000-03	0.0	0.0
12	ORGANIC ACID	5.00000000-04	0.0	0.0
13	HPO4=	7.00600000-03	0.0	0.0
14	SULFATE	3.10000000-04	0.0	0.0
15	AMINO ACIDS	2.35300000-03	0.0	0.0
16	NH4+	2.30000000-05	0.0	0.0
17	UREA	3.14200000-03	0.0	0.0
18	GLUCOSE	4.54000000-03	0.0	0.0
19	CHOLESTEROL	3.08000000-03	0.0	0.0
20	PLA PROTEIN	4.10000000-04	0.0	0.0
21	BCARB SITES	0.0	0.0	0.0
22	IMID SITES	0.0	0.0	0.0
23	EAMINO SITES	0.0	0.0	0.0
24	PHENOL SITES	0.0	0.0	0.0
25	GUANID SITES	0.0	0.0	0.0
26	ADENOSINE	3.90000000-03	0.0	0.0
27	X-MISC	2.00000000-02	0.0	0.0
28	HB4	2.33000000-03	0.0	0.0
29	REDASPGLU SI	0.0	0.0	0.0
30	REDNH2 SITES	0.0	0.0	0.0
31	OXYASPGLU SI	0.0	0.0	0.0
32	OXYNH2 SITES	0.0	0.0	0.0
33	ASPGLU SITES	0.0	0.0	0.0
34	HMCOOH SITES	0.0	0.0	0.0
35	HISTIDINE SI	0.0	0.0	0.0
36	TYROSINE SIT	0.0	0.0	0.0
37	LYSINE SITES	0.0	0.0	0.0
38	ARGININE SIT	0.0	0.0	0.0
39	*PLASMA	0.0	0.0	0.0

Table 2

LISTING OF THE PERTINENT SPECIES AND REACTIONS FOR A MODEL OF HUMAN BLOOD (SEE TEXT FOR EXPLANATION)

	GAS PHASE								
1	O2	-10.940000	1.000	O2					
2	CO2	-7.740740	1.000	CO2					
3	N2	-11.520000	1.000	N2					
4	H2O	2.790000	1.000	H2O					
	PLASMA								
5	O2	0.0	1.000	O2					
6	CO2	0.0	1.000	CO2					
7	N2	0.0	1.000	N2					
8	H2O	0.0	1.000	H2O					
9	H+	0.0	1.000	H+	0.0		0.0	1.000	*PLASM
10	OH-	39.390000	1.000	H2O	-1.000	H+	0.0	-1.000	*PLASM
11	NA+	0.0	1.000	NA+	0.0		0.0	1.000	*PLASM
12	K+	0.0	1.000	K+	0.0		0.0	1.000	*PLASM
13	CA++	0.0	1.000	CA++	0.0		0.0	2.000	*PLASM
14	MG++	0.0	1.000	MG++	0.0		0.0	2.000	*PLASM
15	CL-	0.0	1.000	CL-	0.0		0.0	-1.000	*PLASM
16	LACTIC	0.0	1.000	LACTIC	0.0		0.0	-1.000	*PLASM
17	ORGAN-	0.0	1.000	ORGANI	0.0		0.0	-1.000	*PLASM
18	HCO3-	18.055600	1.000	CO2	1.000	H2O	-1.000 H+	-1.000	*PLASM
19	H2CO3	6.566000	1.000	CO2	1.000	H2O			
20	CO3=	45.661600	1.000	CO2	1.000	H2O	-2.000 H+	-2.000	*PLASM
21	HPO4=	0.0	1.000	HPO4=	0.0		0.0	-2.000	*PLASM
22	H2PO4-	-19.515400	1.000	HPO4=	1.000	H+	0.0	-1.000	*PLASM
23	SO4=	0.0	1.000	SULFAT	0.0		0.0	-2.000	*PLASM
24	SESTR	6.766750	1.000	SULFAT	1.000	X-MISC	-1.000 H+	-1.000	*PLASM
25	AMIN+	-8.846000	1.000	AMINO	1.000	H+	0.0	1.000	*PLASM
26	AMIN+-	0.0	1.000	AMINO					
27	AMIN-	24.734310	1.000	AMINO	-1.000	H+	0.0	-1.000	*PLASM
28	NH4+	0.0	1.000	NH4+	0.0		0.0	1.000	*PLASM
29	NH3	24.461000	1.000	NH4+	-1.000	H+			
30	UREA	0.0	1.000	UREA					
31	GLUCOS	0.0	1.000	GLUCOS					
32	CHOLES	0.0	1.000	CHOLES					
33	PLA PR	0.0	1.000	PLA PR	-105.000	BCARE	-17.000 IMID	-60.500	EAMINO
33	PLA PR	0.0	-20.900	PHENOL	-23.700	GUANID			
34	X-MISC	0.0	1.000	X-MISC	0.0		0.0	-0.547	*PLASM

Table 2--Continued

RED CELLS											
35	O2	-0.490000	1.000	O2							
36	CO2	-0.064251	1.000	CO2							
37	N2	-0.500000	1.000	N2							
38	H2O	0.0	1.000	H2O							
39	H+	-0.082562	1.000	H+							
40	OH-	39.472562	1.000	H2O	-1.000	H+					
41	NA+	2.832012	1.000	NA+							
41	NA+	0.0	1.000	NA+	R						
42	K+	-2.835618	1.000	K+							
42	K+	0.0	1.000	K+	R						
43	CA++	3.388667	1.000	CA++							
43	CA++	0.0	1.000	CA++	R						
44	MG++	0.126679	1.000	MG++							
44	MG++	0.0	1.000	MG++	R						
45	CL-	0.0	1.000	CL-							
46	LACTIC	0.0	1.000	LACTIC							
47	ORGAN-	0.0	1.000	ORGANI							
48	HCO3-	18.073910	1.000	CO2	1.000	H2O	-1.000	H+			
49	H2CO3	6.501749	1.000	CO2	1.000	H2O					
50	CO3=	45.762472	1.000	CO2	1.000	H2O	-2.000	H+			
51	HPO4=	0.0	1.000	HPO4=							
52	H2PO4-	-19.597962	1.000	HPO4=	1.000	H+					
53	PSTER=	-5.306692	2.000	HPO4=	1.000	ADENOS	-1.000	H+			
53	PSTER=	0.0	1.000	PESTRF							
54	PSTER-	-23.993192	2.000	HPO4=	1.000	ADENOS					
54	PSTER-	0.0	1.000	PESTRF							
55	SO4=	0.0	1.000	SULFAT							
56	SESTR	3.783025	1.000	SULFAT	1.000	ADENOS	-1.000	H+			
56	SESTR	0.0	1.000	SESTRF							
57	AMIN+	-8.928562	1.000	AMINO	1.000	H+					
58	AMIN+-	0.0	1.000	AMINO							
59	AMIN-	24.816872	1.000	AMINO	-1.000	H+					
60	NH4+	0.0	1.000	NH4+							
61	NH3	24.543562	1.000	NH4+	-1.000	H+					
62	UREA	0.0	1.000	UREA							
63	GLUCOS	0.0	1.000	GLUCOS							
64	CHOLES	0.0	1.000	CHOLES							
65	ADENOS	0.0	1.000	ADENOS							
66	X-MISC	-1.797592	1.000	X-MISC							
67	MG++ B	-11.479500	1.000	ADENOS	1.000	MG++					
67	MG++ B	0.0	1.000	MG++ B							
68	HB4	0.0	1.000	HB4	0.0		-8.000	HMCOOH	-12.000	TYROSI	
68	HB4	0.0	-12.000	ARGINI	-50.000	ASPGLU	-20.000	HISTID	-44.000	LYSINE	
68	HB4	0.0	-4.000	REDASP	-4.000	REDNH2					
69	HB402	-15.382572	1.000	HB4	1.000	O2	-8.000	HMCOOH	-12.000	TYROSI	
69	HB402	0.0	-12.000	ARGINI	-50.000	ASPGLU	-20.000	HISTID	-44.000	LYSINE	
69	HB402	0.0	-3.000	REDASP	-3.000	REDNH2	-1.000	OXYASP	-1.000	OXYNH2	
70	HB404	-31.476444	1.000	HB4	2.000	O2	-8.000	HMCOOH	-12.000	TYROSI	
70	HB404	0.0	-12.000	ARGINI	-50.000	ASPGLU	-20.000	HISTID	-44.000	LYSINE	
70	HB404	0.0	-2.000	REDASP	-2.000	REDNH2	-2.000	OXYASP	-2.000	OXYNH2	
71	HB406	-45.177920	1.000	HB4	3.000	O2	-8.000	HMCOOH	-12.000	TYROSI	
71	HB406	0.0	-12.000	ARGINI	-50.000	ASPGLU	-20.000	HISTID	-44.000	LYSINE	
71	HB406	0.0	-1.000	REDASP	-1.000	REDNH2	-3.000	OXYASP	-3.000	OXYNH2	
72	HB408	-63.967787	1.000	HB4	4.000	O2	-8.000	HMCOOH	-12.000	TYROSI	
72	HB408	0.0	-12.000	ARGINI	-50.000	ASPGLU	-20.000	HISTID	-44.000	LYSINE	
72	HB408	0.0	0.0		0.0		-4.000	OXYASP	-4.000	OXYNH2	

Table 3

EXPERIMENTAL HUMAN BLOOD BFFR4 FREE H2O

RMS MASS BALANCE ERROR= 4.101D-14 MAX. ERROR= 9.730D-14 ON ROW REDAPGLU SI
RMS EQUILIBRIUM ERROR= 6.502D-15 MAX. ERROR= 4.535D-14 IN HB40B U RED CELLS

VALID SOLUTION
OBJECTIVE= -1.1049642000 04 RT * OBJECTIVE= -6.809607408D 06

		GAS PHASE	PLASMA	RED CELLS
X-BAR		9.99996D 02	2.88943D 01	1.87277D 01
PH		0.0	7.36716D 00	7.16852D 00
O2	MOLES	5.26300D 01	2.69690D-05	2.85327D-05
	MFRAC	5.26302D-02	9.33369D-07	1.52355D-06
CO2	MOLES	6.05003D 01	7.59996D-04	5.25276D-04
	MFRAC	6.05006D-02	2.63026D-05	2.80480D-05
N2	MOLES	8.25810D 02	2.36931D-04	2.53187D-04
	MFRAC	8.25813D-01	8.19992D-06	1.35194D-05
H2O	MOLES	6.10558D 01	2.87225D 01	1.86164D 01
	MFRAC	6.10561D-02	9.94055D-01	9.94055D-01
H+	MOLES	0.0	2.23488D-08	2.28856D-08
	MFRAC	0.0	7.73468D-10	1.22202D-09
OH-	MOLES	0.0	2.90349D-07	1.19113D-07
	MFRAC	0.0	1.00487D-08	6.36023D-09
NA+	MOLES	0.0	7.81600D-02	4.34000D-03
	MFRAC	0.0	2.70503D-03	2.31742D-04
K+	MOLES	0.0	2.85000D-03	4.57900D-02
	MFRAC	0.0	9.86353D-05	2.44504D-03
CA++	MOLES	0.0	7.56040D-04	3.50000D-05
	MFRAC	0.0	2.61657D-05	1.86889D-06
MG++	MOLES	0.0	4.80000D-04	5.80000D-04
	MFRAC	0.0	1.66123D-05	3.09701D-05
CL-	MOLES	0.0	5.83309D-02	2.59891D-02
	MFRAC	0.0	2.01877D-03	1.38774D-03
LACTIC	MOLES	0.0	1.72945D-03	7.70551D-04
	MFRAC	0.0	5.98543D-05	4.11449D-05
ORGAN-	MOLES	0.0	3.45890D-04	1.54110D-04
	MFRAC	0.0	1.19709D-05	8.22898D-06
HCO3-	MOLES	0.0	1.40712D-02	6.15564D-03
	MFRAC	0.0	4.86989D-04	3.28691D-04
H2CO3	MOLES	0.0	1.06327D-06	7.34887D-07
	MFRAC	0.0	3.67986D-08	3.92406D-08
CO3=	MOLES	0.0	1.86533D-05	5.16489D-06
	MFRAC	0.0	6.45570D-07	2.75789D-07
HPO4=	MOLES	0.0	5.68739D-04	1.74192D-04
	MFRAC	0.0	1.96834D-05	9.30127D-06
H2PO4-	MOLES	0.0	1.31458D-04	6.36115D-05
	MFRAC	0.0	4.54961D-06	3.39665D-06
SO4=	MOLES	0.0	2.04534D-06	6.26441D-07
	MFRAC	0.0	7.07370D-08	3.34499D-08
SESTR	MOLES	0.0	1.91928D-04	1.15400D-04
	MFRAC	0.0	6.64242D-06	6.16199D-06

Table 3--Continued

AMIN+	MOLES	0.0	7.52001D-09	7.70063D-09
	MFRAC	0.0	2.60259D-10	4.11189D-10
AMIN+-	MOLES	0.0	1.39961D-03	9.07150D-04
	MFRAC	0.0	4.84389D-05	4.84389D-05
AMIN-	MOLES	0.0	3.27785D-05	1.34470D-05
	MFRAC	0.0	1.13443D-06	7.18029D-07
NH4+	MOLES	0.0	1.15461D-05	1.08864D-05
	MFRAC	0.0	3.99597D-07	5.81301D-07
NH3	MOLES	0.0	3.55397D-07	2.12095D-07
	MFRAC	0.0	1.22999D-08	1.13252D-08
UREA	MOLES	0.0	1.90638D-03	1.23562D-03
	MFRAC	0.0	6.59779D-05	6.59779D-05
GLUCOS	MOLES	0.0	2.75461D-03	1.78539D-03
	MFRAC	0.0	9.53340D-05	9.53340D-05
CHOLES	MOLES	0.0	1.86877D-03	1.21123D-03
	MFRAC	0.0	6.46759D-05	6.46759D-05
PLA PR	MOLES	0.0	4.10000D-04	0.0
	MFRAC	0.0	1.41896D-05	0.0
X-MISC	MOLES	0.0	4.73139D-03	1.50767D-02
	MFRAC	0.0	1.63748D-04	8.05046D-04
PSTER=	MOLES	0.0	0.0	2.64567D-03
	MFRAC	0.0	0.0	1.41270D-04
PSTER-	MOLES	0.0	0.0	3.88330D-04
	MFRAC	0.0	0.0	2.07356D-05
ADENOS	MOLES	0.0	0.0	1.70600D-04
	MFRAC	0.0	0.0	9.10949D-06
MG++ B	MOLES	0.0	0.0	5.80000D-04
	MFRAC	0.0	0.0	3.09701D-05
HB4	MOLES	0.0	0.0	2.65397D-04
	MFRAC	0.0	0.0	1.41713D-05
HB402	MOLES	0.0	0.0	2.19412D-04
	MFRAC	0.0	0.0	1.17159D-05
HB404	MOLES	0.0	0.0	3.69437D-04
	MFRAC	0.0	0.0	1.97267D-05
HB406	MOLES	0.0	0.0	5.68610D-05
	MFRAC	0.0	0.0	3.03620D-06
HB408	MOLES	0.0	0.0	1.41889D-03
	MFRAC	0.0	0.0	7.57643D-05

red cell can be altered relative to plasma by using a small H^+ gradient as required for the red cell.

As part of this research, a computer subroutine was developed, GOALN8, that allows the specification of a desired set of dependent quantities (goals) if a corresponding set of independent variables can be found to which the goals relate. Thus, for each dependent goal variable, a partial derivative is determined with respect to each independent variable. The routine then simultaneously adjusts the independent variables using the available partial derivatives to obtain the simultaneous set of goals. Using this routine, the plasma water, chloride, and pH values are obtained in relation to the values for the corresponding species in red cells.

THE VALIDATION OF THE NORMAL MODELS

The normal model represents 1 liter of whole blood in equilibrium with a relatively large and stable quantity of gas. Since this is a model of venous blood, the gas should have the partial pressure (mole-fraction composition) of gases of the mixed venous blood on return to the heart. Other gas compositions could be used. Table 1 also lists the arterial gas composition. By varying the gas composition and other inputs (simulating a fluid therapy, for example), or by varying any other datum of the model, the response to this variation can be studied. Thus, under a given set of conditions, the problem for the computer is to distribute the sum of the gas and liquids, b_i, into the gas phase, plasma, and red cells according to the equations of the model and the constraints.

The ability to construct and validate a model for one (normal) set of values is the first problem. A second and more difficult problem is to refine the model so that it has predictive capabilities. Some of the predictive capabilities of this model are demonstrated in Figs. 1-5,

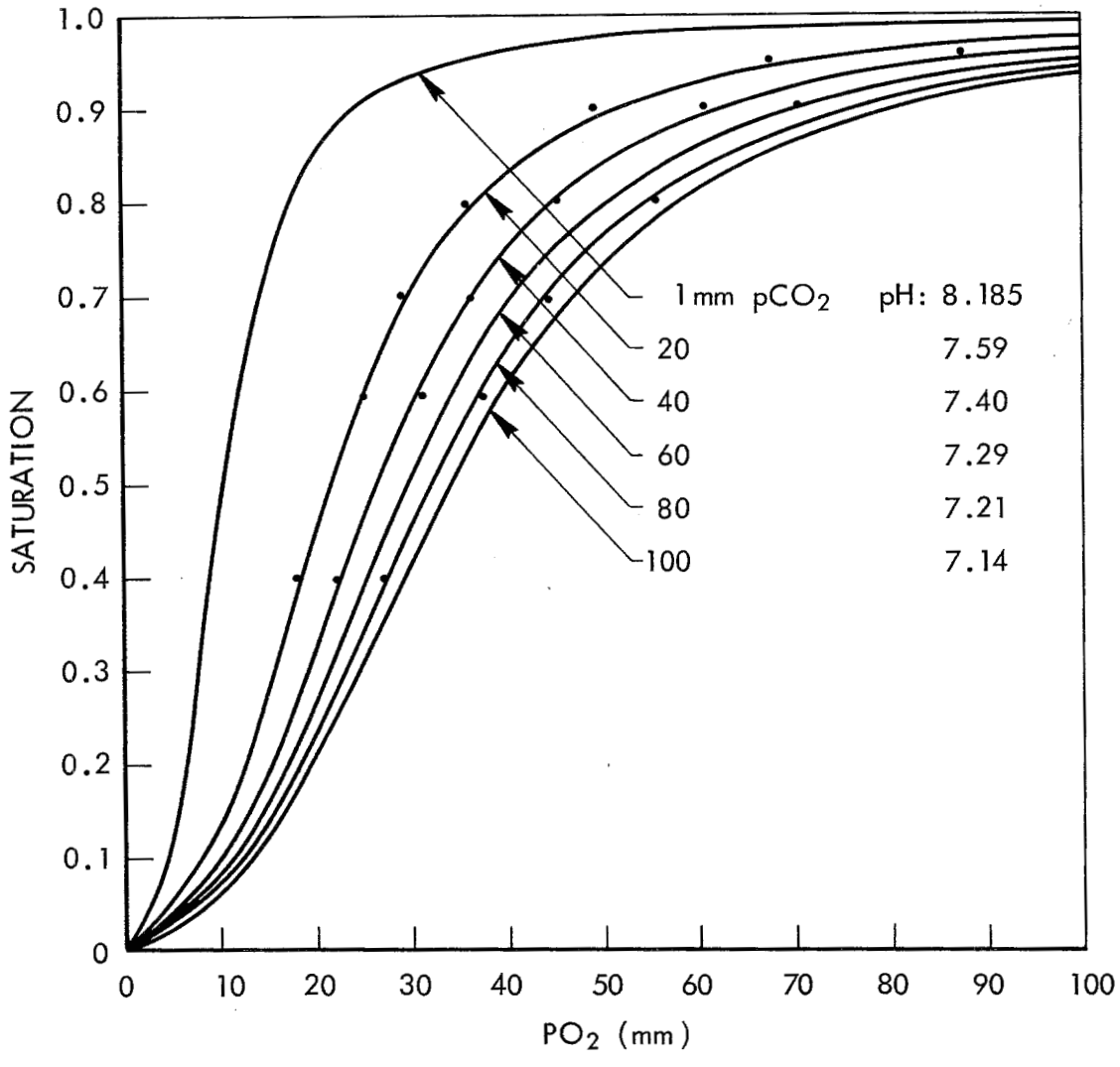

Fig. 1--Computed Oxygen Dissociation Curves at Various pCO_2 Values (Data Points from Dill)

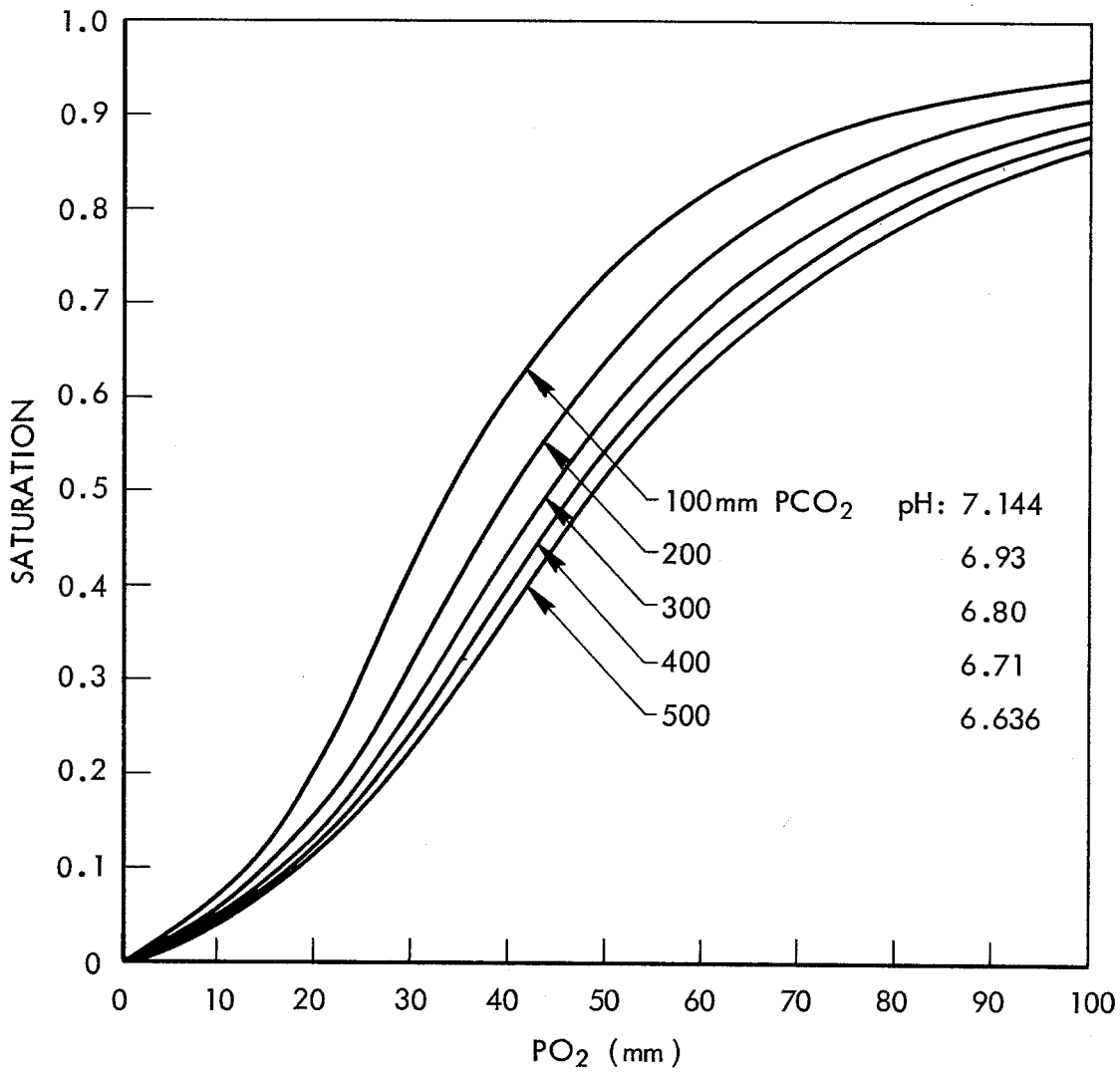

Fig. 2--Saturation Curves (Data from Severinghaus [17] versus Computations of the Normal Model)

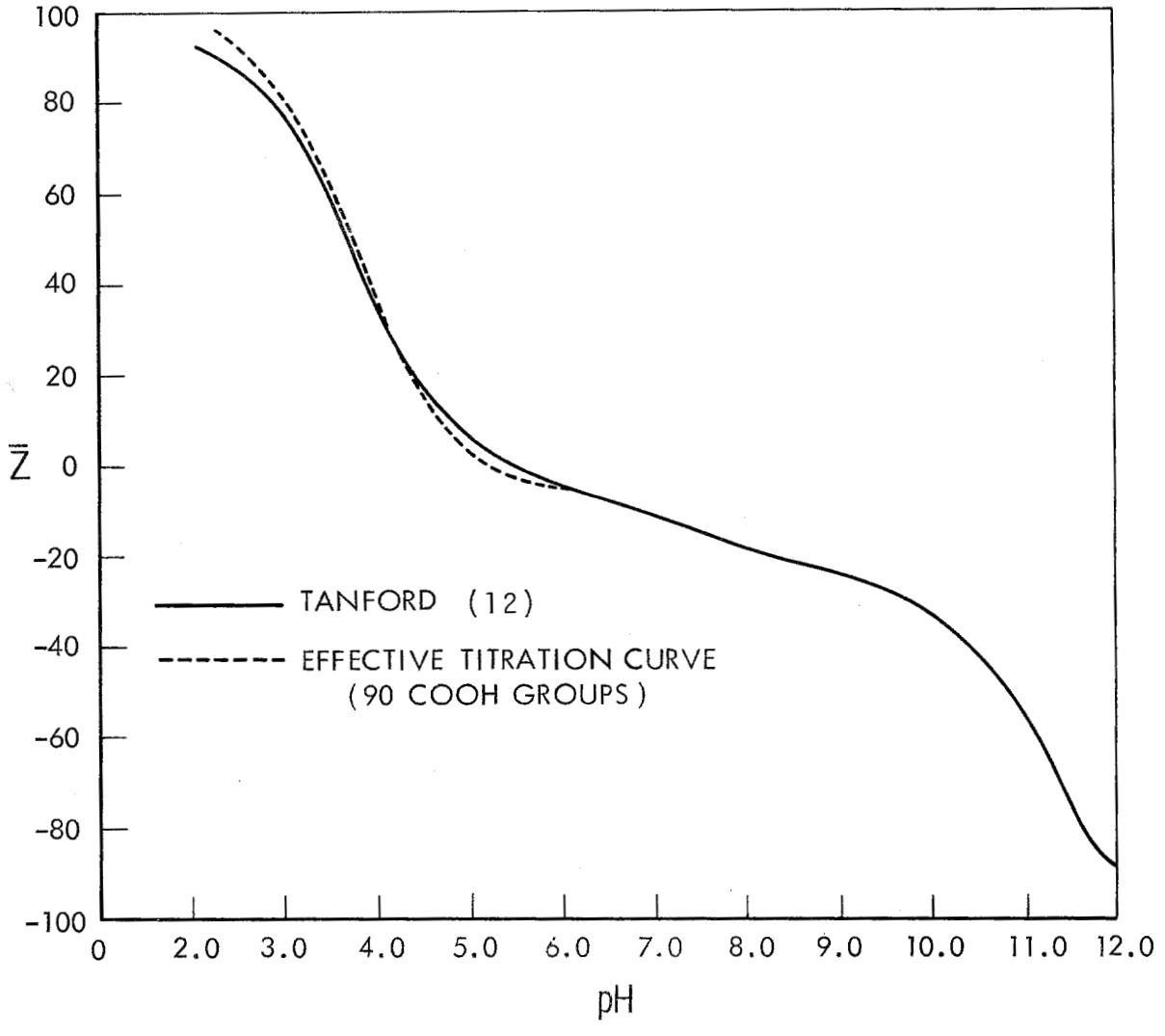

Fig. 3--Computed Titration of Bovine Serum Albumin
(25°C, Ionic Strength 0.15)

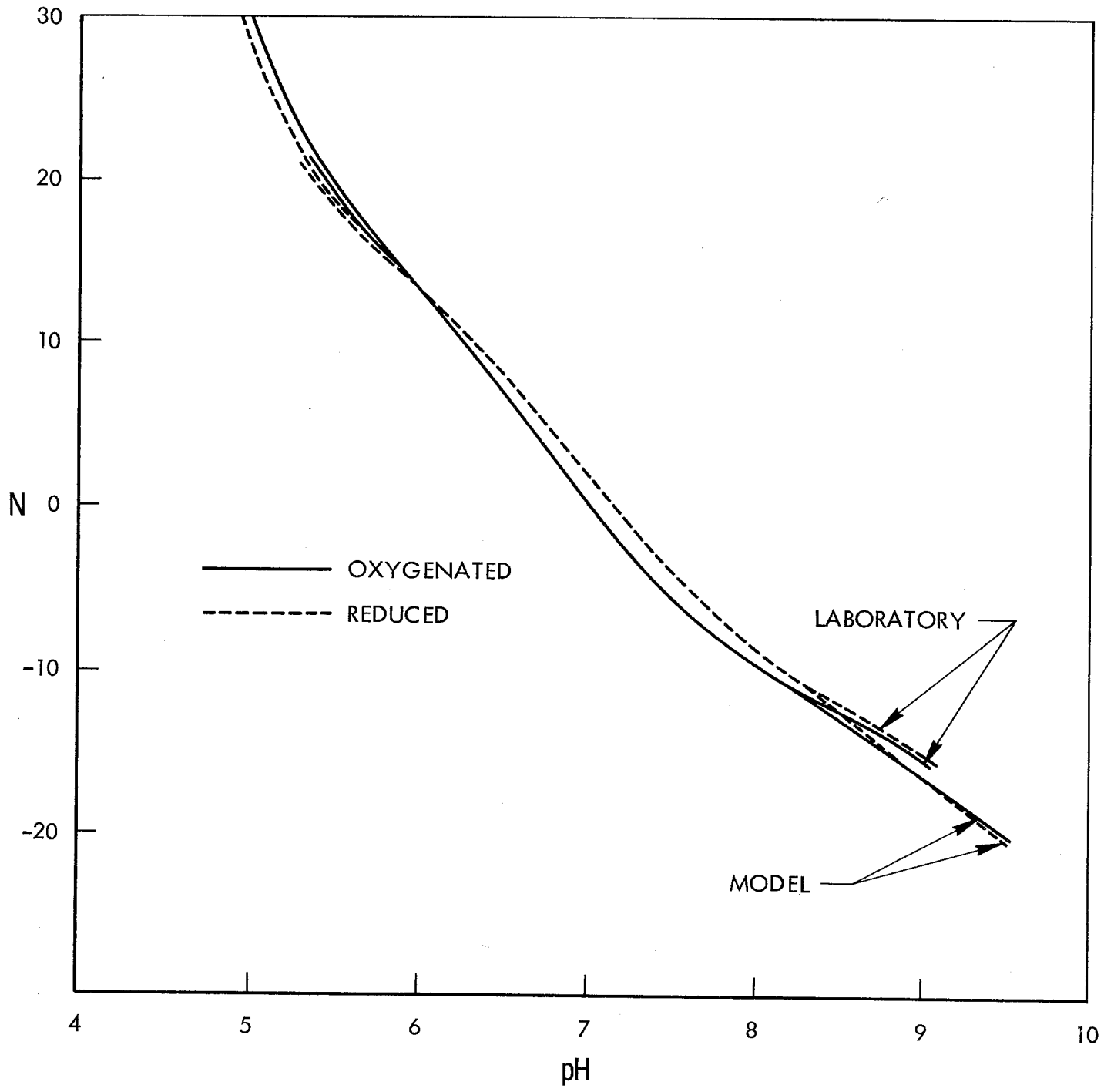

Fig. 4--Hemoglobin Ionization Curves; Computed and Laboratory Results

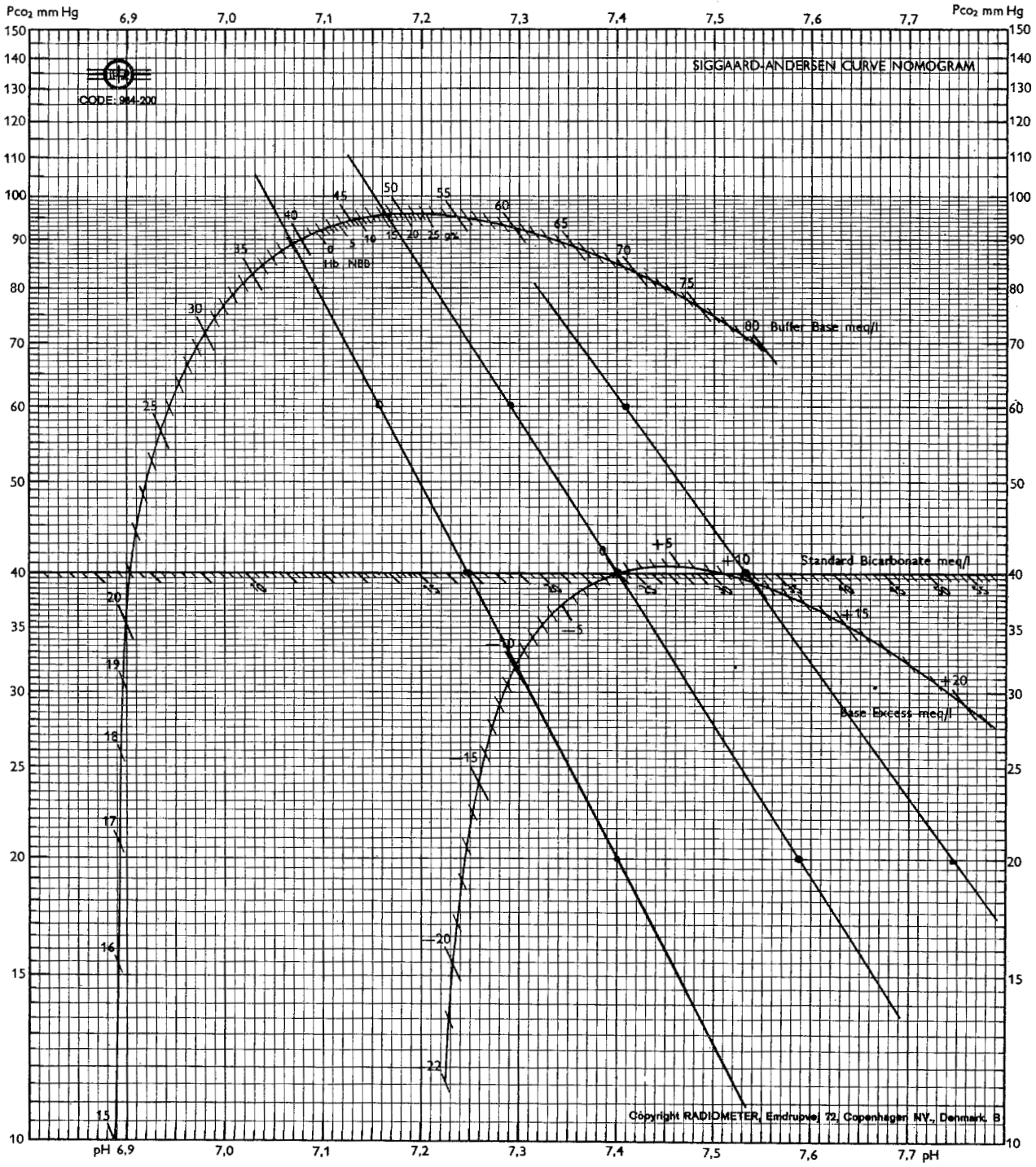

Fig. 5--Acid Base Data (Solid Lines from Siggaard-Andersen [19]; Data Points Computed)

generated from the model used in this report. For each case, data from the literature are overlaid on the computed curves.

Finally, in Ref. 23 it is shown that the Normal Model responds to chemical loading very much like the response of normal blood. This provides a base line for comparison of the response of the models to be constructed of individual blood.

III. MODELING AN INDIVIDUAL BLOOD

Given the statistically normal data and the equations for a model of normal blood, we now wish to convert this model to that of a particular blood so that the new model simulates the behavior of the particular blood over a range of conditions. First we require the laboratory analysis of the individual blood, gathered according to the following protocol.

LABORATORY PROCEDURES FOR OBTAINING DATA

Tonometry Protocol

A sterile venipuncture was performed using human male volunteers. 400.0 cm^3 of blood were drawn into a siliconized vacutainer containing 1.0 cm^3 of sodium heparin at 40,000 units per cm^3. The sodium content of this amount of heparin was 1490 meq/L, which represents the addition of approximately 3.73 mmoles of sodium per liter of blood. During three preliminary studies, the vacutainer also contained one-tenth (0.1) ml of radioactive albumin. The albumin was supplied by Isoserve, contained approximately 15 microcuries of I^{131} per ml. It was certified to contain less than 2-percent free iodine. These preliminary studies validated the literature values used for the trapped plasma in the red-cell samples [24].

The blood was then oxygenated in a 2000 ml^3 flask. Air saturated with water vapor was passed through this flask while it was rotated at approximately 200 rpm for 12 min. All containers and syringes that touched the blood throughout the course of these experiments were siliconized. The oxygenated blood was then separated into two weighted 250-cm^3 bottles and placed in a centrifuge of radius 20-cm measured at the bottom of the 8.0 cm column of blood. The bottles were spun at approximately 1000 rpm for 20 min.

For those samples receiving a chemical stress, the reagent was then gently stirred into the plasma layer or the plasma layer was removed, mixed with the stress and replaced, after which the blood was again mixed.

After a 10-40 min period for initial equilibrium, 65 cm^3 of stressed and control blood were placed in tonometers in a water bath regulated at 37°C. Gas was bubbled through distilled water into a "Y" that generated parallel streams, each of which passed into a tonometer. The gas remains under the 37°C water from the time it enters the saturation bottle until it leaves through a small exit in the top of the tonometers. For most experiments, the gas was compressed air with known (5 percent) CO_2 content.

The tonometers were checked for condensation by passing the gas through empty tonometers for 8 hr. 0.0 cm^3 of condensate formed. The tonometers were checked for evaporation by placing 50.0 grams of water in a weighed tonometer for 8 hr and re-weighing the tonometer at the end of this period. The amount of water lost was 0.1 grams.

An infrared lamp was used to heat the base of the tank the night prior to the experiment, and during the experiment to insure mixing within the gas tank and to avoid layering.

The tonometers were rotated through an arc of ninety degrees at 90 cycles per minute for 60 minutes. Each tonometer had a gas flow of approximately 1-1/2 liters per minute. During this time, the unused blood was stored in the refrigerator at approximately 5°C.

At the end of the hour, the two tonometers, stress and control, were removed from the bath and shaken to eliminate any preferential settling. The gas flow was reduced to a fraction of a liter per minute while three syringes from each tonometer were filled with 20 cm^3 of blood. The syringes were filled directly from a tonometer opening so that only gas that was in the tonometer entered the syringes. The syringes were labeled whole-blood control, whole-blood

stress, A control, A stress, B control, and B stress. One cm^3 of blood was removed from each whole-blood syringe for an oxymeter reading and lactic-acid analysis. A micro hematocrit capillary tube was filled from each syringe.

A pH, pCO_2, and pO_2 analysis was performed on each whole-blood sample. The last three readings were completed within 15 min after the samples were removed from the tonometer. The pH and pCO_2 readings received first priority. Except for the short period of sample removal, the syringes were capped with tightly fitting plastic tips.

Syringes A and B previously had their flanges trimmed. After filling, the plastic plunger was cut off and the syringes were placed bottom end out in the centrifuge cups. The centrifuge was operated at 3250 rpm for an hour, at $37^{\circ}C$. The middle of the red-cell mass was approximately 16 cm from the center of the centrifuge.

A series of I^{131} albumin experiments was performed to estimate the plasma trapping, found to be approximately 5 percent. This was similar to the amount found by Hlad [24] for the complete mass of red cells spun under similar conditions.

Plasma, red cells, and whole blood were frozen and stored for additional analysis.

As soon as one set of blood was removed from the tonometer, a new set of tonometers and blood were placed in the bath for another hour. This produced three sets of tonometer equilibrations for a single stress. As noted, each set of values was divided into duplicate pairs, A and B, upon removal from the tonometer.

Whole blood, red cells, and plasma were analyzed for the values marked with an X in Table 4.

A complete experimental set of values represented 252 separate tests. The specific details of the chemical analysis and the quality controls used are presented in Appendix B.

Table 4

SPECIES ANALYZED IN THE LABORATORY

	Whole Blood	Red Cells	Plasma
Na^+	X	X	X
K^+	X	X	X
Ca^{++}			X
Cl^-	X	X	X
H_2O	X	X	X
Hgb	X	X	
Protein			X
$Phos^=$	X	X	X
Urea			X
Glucose			X
Lactic acid	X		
pH	X	X	X

THE CONVERSION OF A MODEL OF A STATISTICALLY NORMAL BLOOD TO A MODEL OF AN INDIVIDUAL BLOOD

Generally, the process of particularizing a normal model consists of particularizing values of variables that can be measured, and of constraining the model to compute certain variables that can not be measured easily. The necessary chemical data for an accurate electrolyte model have not been firmly established on an experimental basis. But experience with the current blood model indicates that the composition of the gas phase, the pH, the plasma proteins, and the hemoglobin, in addition to the red cell and plasma sodium, potassium, chloride, and water, is sufficient for reasonable accuracy. These quantities, plus

several other species, are determined in the blood used for modeling in this research.

The following approach creates a model of an individual blood beginning with a statistically normal blood. Many of the fundamental parameters remain constant, e.g., the equilibrium constants for the buffering sites of albumin and hemoglobin, the reaction constants required to generate the oxygen saturation curve of hemoglobin, the c_j values for the carbamino reactions, and the ionization of carbonic acid. These quantities probably remain the same from blood to blood. It is primarily the quantity and distribution of the various species that will vary.

1) The quantities in the individual blood are determined and used to replace the amounts in the liter of normal whole blood, which include the total cations, chloride, protein, and water.
2) The moles of cations in plasma are determined from the laboratory data, and specified in the model in a way that initially constrains the plasma fraction of the cations to appear in plasma in the first computer solution.
3) The composition of the gas that is in equilibrium with the control blood is determined and specified.
4) The distribution of the chloride and water between red cells and plasma is determined by varying the amount of the miscellaneous fixed plasma protein and its charge. Simultaneously, the pH of the plasma compartment is adjusted by varying the total moles of H^+ ions as an input.
5) The cationic active pump levels are computed to create the proper cation gradients.
6) The equations are solved under these constraints, and the output compared with the laboratory data. If the fit is satisfactory, the constraints are

removed and replaced by simulated cation pumps. The model and blood are then stressed to compare their results at other points.

THE AUTOMATION PROCESS FOR MODELING AN INDIVIDUAL BLOOD

Earlier attempts at modeling various sets of laboratory data required many passes through the computer; a few sets of data generated several boxes of data cards. Although the desired objectives were obtained, it became apparent that if modeling individual blood was to be practical as a research procedure or as a tool for clinical investigation, the process must be automated.

Laboratory analysis provided a variety of units of measurement that must automatically be converted to moles. Some units for conversion are millimeters of mercury, milliequivalents, equivalents, milligrams, milligrams percent, and grams. Obviously, this procedure could be automated. In this Memorandum, however, we did not attempt to automate either the measurement or record of the laboratory data.

Corrections for trapped plasma in the hematocrit and in the red cells are necessary. Also, an estimation of the quality of laboratory data must be made. One criterion for evaluation of the chemistries measured for a particular species was to compute the ratio of the whole blood analysis divided by the sum of the analyses of plasma plus red cells. Analysis, a statistical routine, was developed to evaluate added components in a recovery study.

The red cell and whole blood analysis for a species such as urea is very poor. However, with certain assumptions, the red cell concentration can be computed. In the case of urea, following the literature we assume that there is no concentration gradient. Thus, if the concentration of urea in the plasma and the amount of water in the red cells are known, the amount of urea in red cells can be computed. Similarly,

if the chloride and water distribution between plasma and red cells is given, and we assume a normal value for a cationic pump, given a plasma value, the amount of that cation in red cells can be estimated. This type of approximation was used to estimate the free Ca^{++} in red cells. Finally, for other research interests, the concentration of each species in moles, grams per liter of water, and grams per 1000 gms of solids is desired.

To meet the preceding requirements, a set of computer subroutines called LAB DATA, written in FORTRAN, were generated. The LAB DATA routines will convert moles to arbitrary units, correct for trapped plasma, estimate certain red cell values, and compute whole blood values adding plasma and red cell values while comparing this result to a measured whole blood value. Thus, the only necessity is to insert the patient data properly into the blood model format. In the control phase of the modeling procedure, the cationic pumps can be specified by fixing the values in plasma that the laboratory has reported.

The pH of plasma and the distribution of chlorides and water between plasma and red cells are determined by automatically adjusting the magnitude of three independent variables, variables that are not known but can assume arbitrary (but reasonable) values. The pH is adjusted by varying the total moles of H^+ as an input. The chloride is adjusted using the charge on the miscellaneous plasma protein. The distribution of the water is adjusted by determining the proper distribution of the miscellaneous plasma protein. Since each dependent parameter is affected by all the independent parameters, a 3×3 matrix of partial derivatives is computed to adjust simultaneously all independent variables. This process is repeated until the desired goals of plasma Cl^-, pH, and hematocrit are reached. This adjustment of independent variables to reach a goal is performed by the subroutine GOALN8. The equations of the

model are then simultaneously solved using the constraints specified by the laboratory.

A set of statistical routines, STATISTICS, compares the laboratory data with the model in the units of moles, or moles per liter of water, at the end of the modeling procedure. The control and interrelationship of all programs described are achieved by a pair of subroutines called UNION1 and UNION2.

IV. RESULTS AND DISCUSSION

The criteria for evaluating a model of an individual blood will, in principle, be the same as the criteria for modeling and evaluating statistically normal blood. Given the steady-state laboratory patient data, it is not only desirable to model this steady-state, but also necessary that the model behave like the patient blood when subjected to stress. Therefore, we first compare the model with the initial steady-state blood, and then compare results after subjecting the model and the blood to chemical load. To this end, the 16 experiments of Table 5 were run. Table 4 lists the variables analyzed in each experiment.

Several of the experiments are now detailed, the remainder are listed in Appendix A. Each experiment resulted in 36 aliquots of blood for analysis in 252 separate tests, although the number varied somewhat depending upon the stress, which changed with each experiment. As noted in the protocol, all determinations were made in duplicate, many of which were blind and several of which were repeated at varying time intervals.

INITIAL MODEL

In all 16 experiments, the automated procedures produced a unique model of the individual blood in its unstressed steady-state. That is, the maximum deviation of any one species in the model, as compared to a measured species or parameter in the blood, was less than 0.1 percent. These data are, therefore, not shown, but are essentially identical to the initial blood analysis shown in each experiment.

In principle, this result is not surprising. Given sufficient consistent data from the laboratory, the mathematical model is completely determined. The interest in

Table 5

A LIST OF STRESSES USED IN TESTS

		Table
1.	Control (No Stress)	6
2.	Isotonic NaCl ..... 133 cc/L Blood; 21 meq NaCl	7
3.	NaCl plus Glucose ..... 102 cc/L Blood; 7.85 mmoles NaCl plus 1.28 mmoles Glucose	9
4.	Dry NaCl, 17 mmoles/L Blood	12
5.	Serum Albumin ..... 30 cc of 5 grams percent/L Blood	13
6.	NaCl plus KCl ..... (9 meq NaCl plus 7 meq KCl)/L Blood	14
7.	$NaHCO_3$ ..... 25 cc/L Blood; 22.5 meq $NaHCO_3$	15
8.	HCl ..... 20.50 meq/L Blood	16
9.	H_2O ..... 153.37 cc/L Blood	17
10.	Control pCO2 36 mmHG ..... Stress pCO2 76 mmHG	18
11.	Blood "A" Control pCO2 36 mmHG ..... Stress pCO2 1 mmHG	25
12.	Control pCO2 1 mmHG ..... Stress pCO2 76 mmHG	26
13.	KCl ..... 11.09 meq/L Blood	27
14.	Dried Plasma ..... 1.8 grams/L Blood	28
15.	H_2O ..... 120.0 cc/L Blood	29

the current work lies in the generality of such models and in the determination of the minimum information necessary to generate a model.

Under the assumptions of Sec. III, the principal constituent distributions to be determined are the cations, chloride, proteins, and water. The mathematical problem is to produce the proper distribution of the whole blood values into their respective red cell and plasma compartments, as well as the proper pH. The cationic gradients, the zero charge constraints in each compartment, and the assumption that each compartment has the same osmolarity, create the basic distribution of species. Finally, the chloride-ion distribution (which does not have an active pump), the pH of the blood, and the water distribution are determined by adjusting the Gibbs-Donnan gradient, the total protein condition, and the osmolarity of the individual blood.

H^+ ion, added with increments of protein to the inputs of the individual model, will affect the ionization of proteins and bicarbonate and hence the pH of plasma and red cells. Similarly, the miscellaneous, partially permeable substances (e.g., lipoids, enzymes, etc.) found in plasma and in red cells not specified by the laboratory analysis may be added within reasonable limits to give the measured water distribution. Finally, the chloride gradient is affected principally by the fixed charge on the proteins and miscellaneous substances. With both red cell and plasma data given, the gradients can be exactly adjusted.

CONTROL MODEL

In the control experiments, depicted in Table 6, the quantity of each of the species (Na, K, Cl, and H_2O) in both the plasma and red cells for the control portion of the experiment is shown on the first line. As indicated on p. 30, the initial quantities of the species considered

Table 6

CONTROL; NO STRESS ADDED (THE ERRORS IN THIS TABLE ARE THE RESULT OF LABORATORY VARIATION)

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	76.45	2.326	56.22	28.24
	STRESS LAB	77.94	2.391	56.29	28.15
	PCT CHANGE	1.963	2.715	.1370	-.3414
	STD DEV	1.711	3.643	1.396	.8815
	STRESS MODEL	76.51	2.351	55.88	28.20
*	PD LAB MODEL	-1.845	-1.582	-.7482	.1710
	STD DEV	1.550	2.053	1.972	.9265
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	7.028	49.58	24.82	19.73
	STRESS LAB	5.902	49.51	24.62	19.93
	PCT CHANGE	-15.82	-.1425	-.7848	1.152
	STD DEV	9.403	.4534	2.321	4.123
	STRESS MODEL	6.940	49.55	25.16	19.75
*	PD LAB MODEL	16.65	.0929	2.158	-.9522
	STD DEV	12.34	.4735	3.922	4.066
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

have the same control values for the laboratory and the model. The second line is the laboratory value of these species after stress, and the third line is the percent change. The fourth line is the standard deviation of the percent change in the laboratory values after stress. The mean of the percent difference between the laboratory and the model, that has resulted from the stress, is the sixth line. Its standard deviation is the seventh line of each compartment.

In this Memorandum, the percent difference (PD) will be the normalized difference (ND) expressed as a percent. The normalized difference is defined by

$$ND = (M - L)/((M + L)/2) \tag{6}$$

where M is the model value and L is the laboratory value, or

$$PD = (1.0 - ND) \times 100 \ . \tag{7}$$

The normalized difference assumes that the source of the difference can be attributed to either quantity. For each species considered, the results of the laboratory analysis of 30 pairs of samples are shown on line 8 (Table 6), expressed as the percent difference; its standard deviation is on line 9 (Table 6).

Since Table 6 is the result of a control experiment, no stress was added. The errors in the predictions, as expressed in the percent difference, are the result of laboratory variability and give a measure of the analytical accuracy as well as the precision with which the automatic computer routines can resolve discrepancies in the laboratory data and build a model of the particular blood. In Table 6, the stress model data, line 5, should compare with the control-laboratory blood since no stress was added. Errors are

uniformly less than half of one percent. After stress, which in this case consists of control blood in the usual tonometer, errors are greater since recovery of electrolytes and water is not perfect. In particular, however, Na^+ in the red-cell phase decreased 1.1 mmole while Na^+ in plasma increased 1.5 mmole in the laboratory analysis. This discrepancy is unaccountable, particularly because none of the other electrolytes changed appreciably. This result is probably due to the difficulty of measuring sodium in red cells. The consequence of this difficulty is that the percent change in red-cell sodium is quite large, as is the percent error in plasma potassium. On the whole, however, the variability of the modeling procedure is comparable to the variability of the analytic laboratory procedures.

ISOTONIC SALINE SOLUTION

Because the considerations involved are generally applicable, the first example chosen for detailed discussion is Table 7, which exemplifies the results of applying a stress of saline. Here, 133 cc of the isotonic solution was added to 1 liter of whole blood, resulting in the addition (to 1 liter of blood) of 21 mmoles of NaCl, and 7.33 moles of water. The Table displays 1 liter of the consequent blood and model results.

The sodium in the plasma was predicted with the greatest accuracy. Also, the laboratory analysis for 30 pairs of plasma sodium had minimum discrepancy, as might be expected. For this species, the model had a smaller standard deviation than the laboratory. Similarly, the red-cell potassium error is small, being only 0.5 mmole in 40.

The red-cell sodium has a larger percent difference between the laboratory and the model since it is a difficult species to analyze. The mean error of the model (-8.4%),

Table 7

ISOTONIC NACL: 133 CC/L BLOOD = 21 MEQ NACL/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	82.06	2.277	61.15	30.36
	STRESS LAB	93.00	2.136	74.62	33.92
	PCT CHANGE	13.33	-6.271	22.04	11.74
	STD DEV	1.154	1.994	1.316	1.549
	STRESS MODEL	93.39	2.666	75.05	34.07
*	PD LAB MODEL	4.171	22.17	.5631	.4437
	STD DEV	.3096	28.33	12.72	.6822
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	5.316	41.00	20.72	16.97
	STRESS LAB	4.865	35.74	20.28	14.61
	PCT CHANGE	-8.353	-12.92	-2.146	-13.88
	STD DEV	5.793	1.537	.9603	.8326
	STRESS MODEL	4.475	35.48	19.85	14.44
*	PD LAB MODEL	-8.404	.7266	-2.170	-1.189
	STD DEV	6.201	1.727	4.703	1.574
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

however, is less than one standard deviation of the reproducibility of laboratory analysis (8.47%). The adverse affects of random laboratory errors in the control and stress portion of blood analyses also can have an adverse effect in the recovery studies; the mathematical model has strict conservation of mass.

The plasma potassium errors are large, but the red-cell potassium prediction errors are small, as might be expected from the reproducibility of laboratory data.

The percent difference for plasma chloride was 0.56 ±12.72 percent, comparing the model prediction after stress and the laboratory. The laboratory error for plasma chloride, on the other hand, was 0.30 ±1.20 percent. Also, the model and the laboratory had a 2.17 ±4.70 percent difference in red-cell chlorides, whereas the laboratory error was 0.30 ±2.20 percent. Neither of the above errors are explainable on the basis of random laboratory errors. The analysis of whole-blood laboratory chlorides had an error of 0.20 ±1.30 percent for the 30 pairs. The measured whole-blood value compared to the calculated recovery whole-blood value (plasma chloride per liter of blood) for the 30 pairs was 0.3 ±1.2 percent error. These figures imply that any systematic error in the chloride measurements was small.

Chloride distribution is strongly influenced by the charge effects of cations and proteins. Table 8 indicates that the prediction of the chloride ion is the test most likely to be affected by errors in the laboratory analysis of other species. In addition, any errors in the representation of the cation pumps will greatly affect the distribution of the chloride ions. Finally, the binding of water and chloride ions to protein, as described in the literature, would theoretically improve the chloride predictability of the model. On the other hand, the error is only on the order of two percent.

Table 8

PREDICTED PERCENT ERROR IN THE MODEL OUTPUT SPECIES CAUSED BY A 1 PERCENT ERROR IN THE REPORTED INPUT

PLASMA

		NA+	K+	CL-	H2O	A ERR*
INPUT	NA+	1.036	.5410	.9565	.6667	0.7
	K+	-.0715	3.204	-.1011	-.2446	0.5
	CL-	.0394	.5496	.5057	-.2209	0.6
	H2O	.0039	.0544	-.0469	.9703	0.2
	PL PRT	.0222	.3093	-.3472	-.0145	0.4
	HGB	.0385	.5385	-.6238	-.0265	
	**ME	1.212	1.993	2.5812	2.144	

RED CELLS

	NA+	.4539	-.0442	-2.301	-1.255
	K+	1.099	1.082	.2433	.4686
	CL-	-.5556	-.0449	2.396	.4270
	H2O	-.0553	-.0044	.1128	1.027
	PL PRT	-.3131	-.0252	.8854	.0278
	HGB	-.0545	-.0440	1.501	.0505
	**ME	2.531	1.245	7.440	3.256

* AVERAGE PERCENT ERROR FOR PAIRS OF CALCULATED WHOLE BLOOD VALUES.

** MAXIMUM CUMULATIVE ERROR THAT COULD RESULT DUE TO THE ADDITIVE EFFECT OF THE INPUT ERRORS.

In Table 7, the water in the plasma had a 0.44 ±0.68 percent difference in the plasma and a 1.19 ±1.57 percent difference in the red cells; the laboratory had 0.0 ±1.01 percent difference in plasma and a 1.40 ±2.74 percent difference in the red cells.

Furthermore, the model could be improved by absorbing or binding water to the surface of protein by an amount indicated in the literature. Such a model has been developed as a result of this research, and tentatively, it does perform better than the free-water model in predicting results, but it was not used in this research. Table 20, Appendix A, illustrates the results of using a bound-water model for a single experiment. These results may be compared to the free-water model of Table 21, Appendix A.

NaCl PLUS GLUCOSE

Administration of standard saline and glucose solution (Table 9) produces larger discrepancies between real blood and the model than for saline alone, and illustrates the type of error one might expect under the hypotheses of the current modeling. For the model, it was assumed that glucose distributes uniformly between plasma and red cells (whereas in real blood it probably does not), and that during the time on the tonometer, some of the glucose may have been metabolized in the viable system.

In the real system, the red cell volume decreased by almost seven percent, whereas in the model there was essentially no change. For both, the plasma volume increased per liter of blood. An explanatory hypothesis is that in the real blood the glucose remained in the extracellular space. Because it is an osmotically active particle, the relative volume of that space increased.

Such a hypothesis could, of course, be built into the model, but there may be a simpler explanation. For example, if the uncharged glucose is uniformly distributed, then the

Table 9

NACL PLUS GLUCOSE: 102 CC/L BLOOD = 7.85 MMOLES NACL PLUS 12.8 MMOLES GLUCOSE

	PLASMA	NA+	K+	CL-	H2O
	CONTROL	85.95	2.591	62.30	29.81
	STRESS LAB	82.30	2.409	66.23	31.93
	PCT CHANGE	-4.248	-6.800	6.303	7.106
	STD DEV	2.014	6.814	.9448	1.503
	STRESS MODEL	82.59	2.581	62.95	30.66
*	PD LAB MODEL	.3576	6.820	-5.076	-4.043
	STD DEV	.3666	7.676	1.742	1.026
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL	4.236	45.45	24.68	17.68
	STRESS LAB	3.977	39.84	22.90	16.47
	PCT CHANGE	-6.094	12.32	7.165	6.821
	STD DEV	6.520	2.45	2.880	2.160
	STRESS MODEL	3.686	41.00	26.17	17.71
*	PD LAB MODEL	-7.407	2.874	13.26	7.290
	STD DEV	7.510	2.860	4.095	2.264
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

remaining effect of this fluid administration is one of hypotonic NaCl. Both the real system and the computer show increased dilution of the sodium ion due to hypotonic addition. Since both Na^+ and Cl^- are essentially extracellular ions, the result would be a slight reduction in hematocrit or in the relative volume of intracellular space, shown in both results.

LABORATORY ERROR IN SODIUM ANALYSIS

With errors in the laboratory data, a resulting model will not respond like the real blood under stress conditions. For example, in order to meet the steady-state conditions reported in error from the chemistry, the free-energy parameters simulating the cation pumps will be incorrectly set. Under stress, the model would behave like the blood reported in error, not like real blood. All of the species in the model relate to one another through their charges, their osmotic properties, or chemical reactions. Errors in the representation of any species will affect the distribution of those species as well as the distribution of chloride, water, the cations, and all other species. It will even affect remote data, like the oxygenation of hemoglobin.

By chance, the laboratory produced a set of data that illustrates the preceding concept. For an identical pair of whole blood, plasma, and red cell samples (parts 3A and 3B of the Hyland experiment), all the species of a pair had nearly the same values except for the red-cell sodiums, which had a discrepancy of 55.6 percent.

These two different red-cell sodiums, 3.59 and 7.74 mmoles/L blood, resulted in very different estimates for the c_j values of the red-cell sodium distribution. The dissimilar c_j values, which are a measure of the minimum work of the simulated sodium pump, resulted in a large variation after stress in the distribution of electrolytes and water in the two cases. Tables 10 and 11 will give the data

Table 10

REPORTED LABORATORY DATA FOR HYLAND EXPERIMENT WITH ERROR IN THE RED CELL NA+ PAIR, 3AC and 3BC
(C = CONTROL, S = STRESS)

	PLASMA				RED CELLS				C(J)	
	3AC	3BC	3AS	3BS	3AC	3BC	3AS	3BS	3AC	3BC
NA+	79.2	80.1	96.6	97.7	3.59	7.74	5.56	5.96	3.00	2.25
K+	2.69	2.66	3.16	3.27	43.1	44.0	43.5	43.1	-2.87	-2.81
CL-	55.2	55.3	69.5	69.5	22.6	22.2	22.0	22.0	0.0	0.0
H2O	29.9	29.8	30.7	30.9	18.0	17.8	15.5	15.6	0.0	0.0
HGB	0.0	0.0	0.0	0.0	2.27	2.30	2.29	2.24		
PL PRT	.554	.554	.661	.661	0.0	0.0	0.0	0.0		

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

Table 11

THE EFFECT OF THE RED CELL SODIUM ERROR OF TABLE 10 ON THE PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LABORATORY

PLASMA			NA+	K+	CL-	H2O
3AS:	PD	LAB MODEL	2.355	15.26	-5.411	-.4373
3BS:	PD	LAB MODEL	-7.813	8.988	-8.013	-1.647

RED CELLS			NA+	K+	CL-	H2O
3AS:	PD	LAB MODEL	-52.09	-1.206	14.96	1.133
3BS:	PD	LAB MODEL	11.99	-.7154	21.52	3.439

PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB
PD=PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.

for these two cases. The large differences in all species are primarily a result of the difference in reported red-cell sodium.

Unfortunately, species subject to the higher laboratory analytic errors, red-cell sodium and plasma potassium, have the largest effect on their respective c_j values and hence cause large errors in the computed prediction of all other species.

HYPERTONIC NaCl

Hypertonic NaCl was administered by adding dry salt to the plasma phase and then remixing. The red cells, of course, dehydrate. Adding 17 mmoles of NaCl per liter of blood caused the cells to lose 12 percent of their total water, and the plasma to gain 6 percent of its total water, as shown in Table 12. The model and the laboratory results coincide, except that the laboratory reports a loss of sodium and a gain in moles of potassium and chloride for the cell, which is unlikely. It is more likely that chloride and potassium are lost from the cell with the shift of water, as shown by the model.

SERUM ALBUMIN INCREASE

The administration of a concentrated solution of serum albumin is a test of the osmotic property of the plasma protein. The computer model and the blood system agree in all respects (see Table 13) except the plasma potassium. The computer predicted that K^+ ion would transfer out of the cell along with water, Na^+ and Cl^-. In the real system, however, K^+ did not appear in the plasma even though it decreased as predicted in the cell. Although this error is probably due to the difficulty of analysis of K^+ in the plasma phase, it may well be due to anomalous distribution of K^+ owing to the apparent impermeability of this species, illustrated in the next result.

Table 12

DRY NACL; 17 MMOLES/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	78.00	2.370	59.91	28.71
	STRESS LAB	98.26	2.494	75.99	30.59
	PCT CHANGE	25.98	5.104	26.85	6.568
	STD DEV	2.357	5.144	2.072	2.072
	STRESS MODEL	97.07	2.834	78.17	31.58
*	PD LAB MODEL	-1.221	12.91	2.823	3.194
	STD DEV	1.555	4.051	1.585	2.566
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	4.113	49.07	24.17	18.61
	STRESS LAB	3.055	49.20	24.80	16.41
	PCT CHANGE	-20.85	.2890	2.625	-11.82
	STD DEV	39.08	2.461	1.856	1.887
	STRESS MODEL	4.241	48.60	22.62	15.76
*	PD LAB MODEL	33.57	-1.211	-9.239	-4.017
	STD DEV	39.74	2.388	5.198	3.402
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 13

SERUM ALBUMIN; 30 CC of 5 GM PERCENT/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	79.98	3.154	64.90	30.33
	STRESS LAB	91.59	2.823	72.29	33.72
	PCT CHANGE	14.52	-10.45	11.39	11.78
	STD DEV	1.054	4.234	.7248	1.528
	STRESS MODEL	91.46	4.015	70.84	34.10
*	PD LAB MODEL	-.1646	34.73	-2.038	1.110
	STD DEV	1.618	5.974	1.338	1.036
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	8.081	39.57	16.33	16.40
	STRESS LAB	5.787	33.39	13.30	13.80
	PCT CHANGE	-26.12	-15.59	-18.45	-15.89
	STD DEV	19.17	1.629	5.287	.8895
	STRESS MODEL	5.919	32.20	14.75	13.40
*	PD LAB MODEL	1.667	-3.636	10.17	-2.946
	STD DEV	25.75	.7728	6.346	2.599
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

ADMINISTRATION OF NaCl PLUS KCl

Since the computer model of the particular blood is designed using a predetermined (from the unstressed steady-state values of the blood) and fixed value for each active cation pump parameter, it tends to distribute added cations in the same way that the normal steady-state cations were distributed. Thus, as in Table 14, when sodium and potassium chloride are added, the computer puts most of the Na^+ and Cl^- ions outside the cell, and the K^+ ion inside the cell. In the real system, however, the Na^+ and Cl^- ions agree quite well with the model prediction; the added K^+ remains in the plasma.

No doubt this indicates an error in the model since it is known (Hastings, 1956) that K^+ kinetics are slow, requiring several hours to attain equilibrium in these circumstances.

ADMINISTRATION OF ALKALI AND ACID

Table 15 gives the result of addition of hypertonic $NaHCO_3$, which, under the condition of constant pCO_2 = 40 mmHg, is equivalent to adding NaOH. The pH increases, HCO_3^- ion increases, and with addition of the external ion Na^+, the hematocrit goes down sharply. The model and the real system agree in all respects except for intracellular Na^+, a species difficult to analyze in the laboratory.

In Table 16, under the addition of HCl, the model and real blood agree in all respects. This time, however, in spite of the addition of Cl^-, an exterior ion, the relative red-cell volume increases. Approximately the same amount of HCl was added as $NaHCO_3$, but the volume gain was only one-third the volume loss under $NaHCO_3$. Evidently, red-cell volume decreases with increasing pH, but the exterior ions Na^+ and Cl^- mask this effect. One might ask if subtraction of HCl is equivalent to addition of $NaHCO_3$. This is probably

Table 14

NACL PLUS KCL; (9 MEQ NACL PLUS 7 MEQ KCL)/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	84.20	2.488	57.97	30.24
	STRESS LAB	93.61	7.323	72.47	31.86
	PCT CHANGE	11.20	195.0	25.03	5.361
	STD DEV	2.549	18.50	2.472	.8433
	STRESS MODEL	94.12	2.996	70.00	30.24
*	PD LAB MODEL	.5335	-83.87	-3.500	-4.629
	STD DEV	.6344	4.426	2.556	1.322
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H23
	CONTROL LAB	6.625	42.37	20.57	17.01
	STRESS LAB	7.278	42.48	23.89	15.32
	PCT CHANGE	10.41	.2650	16.12	-9.944
	STD DEV	11.51	1.332	3.014	1.631
	STRESS MODEL	6.774	46.80	26.36	16.85
*	PD LAB MODEL	-6.966	9.692	9.744	9.500
	STD DEV	9.038	.4755	6.781	3.499
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 15

NAHCO3; 25 CC/L BLOOD = 22.60 MEQ/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	79.54	2.172	58.80	28.82
	STRESS LAB	95.97	2.584	63.60	31.46
	PCT CHANGE	20.65	19.17	8.160	9.155
	STD DEV	.6938	4.465	.9393	.9743
	STRESS MODEL	97.38	2.441	62.82	31.79
*	PD LAB MODEL	1.445	-5.756	-1.235	1.044
	STD DEV	1.800	3.612	.9054	.9973
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	3.756	43.68	21.11	17.18
	STRESS LAB	5.358	42.01	15.51	14.68
	PCT CHANGE	44.88	-3.759	-26.54	-14.53
	STD DEV	52.09	3.514	-1.381	.8940
	STRESS MODEL	3.952	42.27	15.10	14.36
*	PD LAB MODEL	-26.41	.5915	-2.705	-2.211
	STD DEV	28.70	3.676	5.785	2.202
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 16

HCL; 20.50 MEQ/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	78.30	2.479	58.61	29.49
	STRESS LAB	82.96	2.910	68.16	28.70
	PCT CHANGE	5.974	17.41	16.30	-2.660
	STD DEV	2.346	3.060	1.246	.8741
	STRESS MODEL	78.60	2.764	67.09	28.56
*	PD LAB MODEL	-5.390	-5.139	-1.586	-.4896
	STD DEV	2.184	3.520	.5290	.8875
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	2.961	46.54	23.02	17.86
	STRESS LAB	2.581	44.91	33.86	18.46
	PCT CHANGE	-12.96	-3.364	47.25	3.367
	STD DEV	15.07	4.391	5.060	2.365
	STRESS MODEL	2.655	46.25	34.93	18.79
*	PD LAB MODEL	4.076	2.890	3.104	1.783
	STD DEV	18.94	4.679	.9685	2.065
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

not so. Although the effect of H^+ must be the negative of OH^-, Na^+ and Cl^- are not equivalent because considerably more Cl^- penetrates the cells than does Na^+, and therefore their effects are not reciprocal.

ADDITION OF H_2O

Added water distributes uniformly in red cells and plasma, as shown both by the model and blood in Table 17. This tends to confirm the equaosmotic hypothesis of the model.

INCREASING PCO_2

There were no anomalous results under increasing pCO_2 tension. The laboratory and model agree reasonably well, the maximum error being 8 percent in the red-cell potassium prediction (Table 18). Since this experiment was a pure CO_2 addition with no extraneous electrolytes, one might conjecture that this error is due to lack of equilibration. Since the CO_2 equilibrates immediately, and the Na^+ ion also equilibrates in one hour, the error may be due to K^+ kinetics. Unfortunately, in this experiment the error is not statistically significant. An experiment might be designed in this way to display the K^+ kinetic curves.

Table 17

H20; 153.37 CC/L BLOOD

	PLASMA	NA+	K+	CL-	H20
	CONTROL LAB	80.12	2.003	61.00	30.26
	STRESS LAB	68.25	1.768	53.78	31.16
	PCT CHANGE	-14.81	-11.71	-11.82	2.988
	STD DEV	1.199	2.811	1.414	.8044
	STRESS MODEL	69.44	1.748	52.68	31.09
*	PD LAB MODEL	1.732	-1.118	-2.063	-.2433
	STD DEV	1.141	3.046	1.452	.5039
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H20
	CONTROL LAB	5.772	45.26	22.13	17.38
	STRESS LAB	4.431	39.88	19.64	17.87
	PCT CHANGE	-22.88	-11.87	-11.23	2.828
	STD DEV	17.57	1.562	2.692	.9400
	STRESS MODEL	4.964	39.19	19.33	17.87
*	PD LAB MODEL	12.92	-1.739	-1.564	-.0369
	STD DEV	22.71	1.752	3.113	.8806
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H20 IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 18

CONTROL: PCO2 36 MMHG; STRESS: PCO2 76 MMHG

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	78.33	2.431	59.30	29.42
	STRESS LAB	78.24	2.368	56.98	28.96
	PCT CHANGE	-.1065	-2.574	-3.918	-1.554
	STD DEV	.9260	2.404	.4124	1.167
	STRESS MODEL	78.56	2.586	57.35	29.13
*	PD LAB MODEL	.4061	8.806	.6560	.5788
	STD DEV	.9306	2.460	.4111	1.215
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	3.855	46.80	22.14	18.06
	STRESS LAB	3.835	46.68	24.86	18.81
	PCT CHANCE	-.1073	-.2366	12.36	4.139
	STD DEV	8.437	1.670	3.052	2.157
	STRESS MODEL	3.623	46.64	24.08	18.33
*	PD LAB MODEL	-5.799	-8.331	-3.191	-2.559
	STD DEV	8.504	1.680	2.935	2.001
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

V. CONCLUSIONS

With consistent laboratory data, it has been possible, using automatic procedures, to reproduce particular laboratory blood at initial steady-state to arbitrary accuracy. The critical result is to show how well these models simulate the real blood over a range of stresses; that is, whether a particular model is sufficiently general. A model may simulate the blood at a point in the viable space of the blood where the model was constructed, but not emulate its response to normal chemical loads. Experiments were designed to show such models can emulate a variety of responses, and thereby explain their discrepancies.

The precision of the laboratory data in this series of experiments was such that, except for plasma K^+ and red-cell Na^+, the significant model discrepancies would be apparent. For the most part, discrepancies were inconsequential and of the same magnitude as the laboratory errors. In certain instances, however, the modeling errors direct attention to improvements required in the modeling procedure and theory.

Primarily, the discrepancies relate to the cation-pump assumptions that 1) the single parameter representing the active pump for each species is constant over all stresses and 2) all species equilibrate in one hour in the tonometer. Evidently, the second assumption is not true, particularly for K^+ ions. The first assumption may hold for a narrow viable range; we have no evidence that it does not. Under particular drugs, however, such as aldosterone or digitalis, even the first assumption is invalid [25].

For electrolyte and water stresses, the current models hold within satisfactory limits except for K^+ ion that equilibrates slowly when administered as KCl. Improved hypotheses for the cation pumps might relate the K^+ activity to protein concentration, to protein charge, to percent

solids in the cell (see Table 22, Appendix A), or to ATPase activity.

The importance of the cationic pumps can be illustrated by another computer experiment. If the stressed model is forced to acquire the laboratory cation gradients after stress, the distribution of the chloride ions and water should improve. Table 23, Appendix A, illustrates the results of forcing the cations to have the laboratory-measured distributions for the dry NaCl experiment. Compare Table 23 to Table 21, Appendix A. The error in plasma chloride changes from 2.98 percent difference to 0.7826 percent difference. The red-cell chloride change is -7.998, -2.11 percent difference. The improvement appears quite definite.

There are additional modifications of the model that also improve the fit of the mathematical models to the laboratory data. These include the binding of Cl^- ion to serum albumin, and the "binding" of H_2O to the surface of protein molecules. These improvements are being incorporated.

Results of this investigation have been encouraging. With relatively minor improvements in the modeling capability, one might consider experimental application in the clinical environment for certain well-defined situations. However, further research will be required to make the procedure generally applicable. First, it is necessary to discover whether such models can be reliably designed using only the minimal information now available from clinical-chemistry laboratories (where cell electrolytes are not measured). Second, additional information is required to determine the response to drug therapy.

The response to stresses by the blood of sick patients has not been sufficiently investigated. For example, preliminary investigations at UCLA have demonstrated that the response to stress of the blood of a rabbit with hyperaldosteronism is markedly different from the response of normal rabbit blood [26] even though both bloods appear

similar prior to stress. There is some evidence that the blood of a hypothyroid patient also responds differently to a stress than normal blood [25], and similarly for digitalis [25].

Although the usefulness of a stress as a diagnostic tool or a screening agent has not been sufficiently investigated, it would be helpful to have a mathematical tool that defines a normal response to stress. The ability to predict a normal response and to compare deviations from normal would represent a significantly greater degree of chemical comprehension of blood electrolytes than currently exists.

Finally, the ability of the mathematical program to model poor data may be useful as a tool for quality control of the large amount of information that comes from the automated analyses of laboratory data. At the University of Missouri, Columbia, preliminary investigations are being conducted for the application of this concept to clinical data [27].

Appendix A

COMPARISON OF MODEL AND LABORATORY RESULTS

Tables 19 through 29 give comparisons between model results and laboratory results for various chemical loads.

Table 19

CONTROL: NO STRESS ADDED (THE ERRORS IN THIS TABLE ARE THE RESULT OF LABORATORY VARIATION)

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	76.45	2.326	56.22	28.24
	STRESS LAB	77.94	2.391	56.29	28.15
	PCT CHANGE	1.963	2.715	.1370	-.3414
	STD DEV	1.711	3.643	1.396	.8815
	STRESS MODEL	76.51	2.351	55.88	28.20
*	PD LAB MODEL	-1.845	-1.582	-.7482	.1710
	STD DEV	1.550	2.053	1.972	.9265
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	7.028	49.58	24.82	19.73
	STRESS LAB	5.902	49.51	24.62	19.93
	PCT CHANGE	-15.82	-.1425	-.7848	1.152
	STD DEV	9.403	.4534	2.321	4.123
	STRESS MODEL	6.940	49.55	25.16	19.75
*	PD LAB MODEL	16.65	.0929	2.158	-.9522
	STD DEV	12.34	.4735	3.922	4.066
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 20

DRY NACL; 13 MMOLES/L BLOOD (THIS EXPERIMENT USES A STANDARD BLOOD MODEL THAT HAS BOUND WATER. THIS TABLE SHOULD BE COMPARED WITH TABLE 21 WHICH USES A FREE WATER MODEL)

	PLASMA	NA+	K+	CL-	H20
	CONTROL LAB	70.48	2.655	50.19	26.50
	STRESS LAB	91.31	2.940	66.34	30.49
	PCT CHANGE	29.55	10.98	32.20	15.04
	STD DEV	2.369	8.839	2.095	2.035
	STRESS MODEL	91.89	3.384	67.94	31.00
*	PD LAB MODEL	.6438	14.07	2.374	1.687
	STD DEV	1.113	6.642	.9240	1.722
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H20
	CONTROL LAB	5.319	53.46	27.37	20.48
	STRESS LAB	5.238	46.26	26.47	16.85
	PCT CHANGE	-1.035	-13.40	-3.254	-17.72
	STD DEV	24.29	3.025	2.227	2.367
	STRESS MODEL	4.658	45.82	24.88	16.35
*	PD LAB MODEL	-11.93	-.9628	-6.247	-2.998
	STD DEV	21.86	.4465	2.527	3.110
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H20 IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 21

DRY NACL; 13 MMOLES/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	70.48	2.655	50.19	26.50
	STRESS LAB	91.31	2.940	66.34	30.49
	PCT CHANGE	29.55	10.98	32.20	15.53
	STD DEV	1.369	7.839	2.095	2.035
	STRESS MODEL	91.05	3.282	68.36	30.15
*	PD LAB MODEL	-.2738	10.99	2.984	-1.091
	STD DEV	1.398	8.525	1.044	1.431
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	5.319	53.46	27.37	20.48
	STRESS LAB	5.238	46.26	26.47	16.85
	PCT CHANGE	-1.035	-13.41	-3.255	-17.72
	STD DEV	24.29	3.025	2.227	2.367
	STRESS MODEL	5.501	52.83	24.45	16.85
*	PD LAB MODEL	4.275	13.23	-7.988	.0319
	STD DEV	25.03	3.674	3.013	3.378
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 22

NACL PLUS KCL; 9 MEQ NACL PLUS 7 MEQ KCL/L BLOOD (THIS EXPERIMENT ASSUMED THE AMOUNT OF POTASSIUM IN THE RED CELLS WAS A CONSTANT PER UNIT OF DRY SOLIDS)

*** ERROR = 7.956 VS 19.32 FOR TABLE 14

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	84.20	2.488	57.97	30.24
	STRESS LAB	93.61	7.323	72.47	31.86
	PCT CHANGE	11.20	195.1	25.03	5.361
	STD DEV	2.549	18.50	2.472	.8434
	STRESS MODEL	94.79	7.432	73.44	32.01
*	PD LAB MODEL	1.249	1.193	1.297	.4792
	STD DEV	.6688	7.305	2.856	1.680
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	6.625	42.37	20.57	17.02
	STRESS LAB	7.278	42.48	23.89	15.32
	PCT CHANGE	10.41	.265	16.12	-9.944
	STD DEV	11.51	1.332	3.014	1.631
	STRESS MODEL	6.099	42.37	22.91	15.26
*	PD LAB MODEL	-17.44	-.2572	-4.352	-.4545
	STD DEV	9.614	1.322	8.908	4.386
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

*** ERROR = AVE. OF (ABS. MEAN VALUES + 1 STD. DEV.)

Table 23

DRY NACL; 13 MMOLES/L BLOOD (THE STRESS RED CELL AND PLASMA CATIONS EQUAL THE VALUES REPORTED BY THE LAB. COMPARE THE CHLORIDES AND WATER IN THIS TABLE WITH TABLE 21. THE WATER IS FREE)

*** ERROR = 2.10 VS 2.94 FOR TABLE 21

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	70.48	2.655	50.19	26.50
	STRESS LAB	91.31	2.940	66.34	30.49
	PCT CHANGE	29.55	10.98	32.20	15.04
	STD DEV	2.369	8.839	2.095	2.035
	STRESS MODEL	91.31	2.940	66.87	30.84
*	PD LAB MODEL	.0000	.0000	.7826	1.140
	STD DEV	.0000	.0000	1.372	1.883
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	5.319	53.46	27.37	20.48
	STRESS LAB	5.238	46.26	26.47	16.85
	PCT CHANGE	-1.035	-13.40	-3.254	-17.71
	STD DEV	24.29	3.025	2.227	2.367
	STRESS MODEL	5.238	46.26	25.94	16.51
*	PD LAB MODEL	.0000	.0000	-2.109	-2.093
	STD DEV	.0000	.0000	3.725	3.654
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

*** ERROR = ABSOLUTE MEAN + 1 STD. DEV.

Table 24

DRY NACL; 13 MMOLES/L BLOOD (THE WHOLE BLOOD STRESS VALUES EQUAL THE SUM OF THE STRESS RED CELL AND PLASMA)

	PLASMA	NA+	K+	CL-	H20
	CONTROL LAB	70.48	2.655	50.19	26.50
	STRESS LAB	91.31	2.940	66.34	30.49
	PCT CHANGE	29.55	10.98	32.20	15.04
	STD DEV	2.284	2.956	2.899	2.114
	STRESS MODEL	92.00	3.462	67.76	31.26
*	PD LAB MODEL	.7635	16.36	2.110	2.497
	STD DEV	1.059	6.103	.9417	1.836
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H20
	CONTROL LAB	5.319	53.46	27.37	20.48
	STRESS LAB	5.238	46.26	26.47	16.85
	PCT CHANGE	-1.035	-13.40	-3.254	-17.72
	STD DEV	2.670	2.735	2.890	1.084
	STRESS MODEL	4.548	45.74	25.05	16.10
*	PD LAB MODEL	-14.25	-1.134	-5.532	-4.597
	STD DEV	20.99	.4112	2.555	3.409
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H20 IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 25

CONTROL: PCO2 36 MMHG; STRESS: PCO2 1 MMHG

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	80.43	1.971	60.51	30.26
	STRESS LAB	82.40	2.699	68.40	30.65
	PCT CHANGE	2.469	36.96	13.05	1.301
	STD DEV	2.034	6.176	1.575	1.145
	STRESS MODEL	78.69	1.570	65.03	30.55
*	PD LAB MODEL	-4.610	-5.283	-5.060	-.3275
	STD DEV	2.058	6.283	.9728	1.273
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	7.226	43.49	23.04	17.61
	STRESS LAB	8.306	44.04	16.20	16.67
	PCT CHANGE	15.00	1.265	-29.70	-5.383
	STD DEV	2.754	.9462	.7452	.8258
	STRESS MODEL	8.966	43.89	18.52	17.29
*	PD LAB MODEL	7.561	-.3350	13.37	3.693
	STD DEV	4.027	.9212	2.202	.5726
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 26

CONTROL: PCO2 1 MMHG; STRESS: PCO2 76 MMHG

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	76.66	2.713	62.22	28.73
	STRESS LAB	75.50	2.035	54.88	27.90
	PCT CHANGE	-1.479	-25.06	-11.78	-2.652
	STD DEV	2.308	3.175	1.806	1.224
	STRESS MODEL	79.86	3.605	57.80	28.82
*	PD LAB MODEL	5.603	55.71	5.172	3.231
	STD DEV	1.923	2.591	2.539	1.525
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	13.96	48.32	18.75	17.88
	STRESS LAB	13.93	49.12	26.36	19.03
	PCT CHANGE	.9730	1.651	40.64	6.454
	STD DEV	18.10	2.827	2.654	1.580
	STRESS MODEL	10.76	47.43	23.17	17.78
*	PD LAB MODEL	-25.41	-3.467	-12.91	-6.785
	STD DEV	16.94	2.874	1.134	1.476
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 27

KCL: 11.09 MEQ/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	81.79	2.215	55.14	28.57
	STRESS LAB	82.23	11.13	66.81	30.21
	PCT CHANGE	.553	402.9	21.16	5.748
	STD DEV	1.922	23.65	0.0	1.539
	STRESS MODEL	81.32	2.448	59.41	26.54
*	PD LAB MODEL	-1.122	-127.9	-11.77	-12.96
	STD DEV	1.679	1.813	4.445	.1673
**	PD PAIRS	0.40	2.40	0.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	7.406	47.18	24.23	19.16
	STRESS LAB	5.308	47.17	22.51	16.44
	PCT CHANGE	-28.17	-.0331	-7.045	-14.07
	STD DEV	4.528	2.065	2.639	4.020
	STRESS MODEL	7.880	55.85	29.91	21.21
*	PD LAB MODEL	38.96	16.86	28.06	25.31
	STD DEV	3.302	.5902	9.033	1.673
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 28

DRIED PLASMA; 1.8 GRAMS/L BLOOD

	PLASMA	NA+	K+	CL-	H2O
	CONTROL LAB	79.31	2.600	55.44	29.54
	STRESS LAB	97.19	3.195	69.32	30.51
	PCT CHANGE	22.56	23.92	25.05	3.283
	STD DEV	1.807	11.44	1.907	1.095
	STRESS MODEL	98.92	3.576	65.67	30.53
*	PD LAB MODEL	1.758	10.77	-5.423	.0679
	STD DEV	1.545	9.356	2.293	1.637
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H2O
	CONTROL LAB	4.925	44.91	22.23	18.15
	STRESS LAB	6.149	44.95	22.33	15.77
	PCT CHANGE	32.78	.0910	.4740	-13.88
	STD DEV	36.51	1.277	1.811	1.518
	STRESS MODEL	4.412	44.57	25.98	15.80
*	PD LAB MODEL	-34.46	-.8552	14.99	.1370
	STD DEV	28.18	.8001	5.979	3.148
**	PD PAIRS	2.02	0.25	0.30	1.40
	STD DEV	8.47	1.42	2.20	2.74

UNITS H2O IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Table 29

H20; 120.00 CC/L BLOOD

	PLASMA	NA+	K+	CL-	H20
	CONTROL LAB	84.64	2.426	63.60	30.44
	STRESS LAB	74.05	2.261	56.69	31.27
	PCT CHANGE	-12.48	-6.560	-10.87	2.714
	STD DEV	2.000	6.089	.6372	1.385
	STRESS MODEL	75.55	2.178	56.62	31.05
*	PD LAB MODEL	1.990	-3.835	-.1095	-.6881
	STD DEV	2.288	5.920	.7453	.7175
**	PD PAIRS	.40	2.40	.30	0.00
	STD DEV	2.17	7.20	1.20	1.01

	RED CELLS	NA+	K+	CL-	H20
	CONTROL LAB	4.146	43.78	21.68	16.97
	STRESS LAB	4.255	38.10	19.80	17.16
	PCT CHANGE	13.60	-12.97	-8.642	1.160
	STD DEV	36.51	1.079	2.036	1.278
	STRESS MODEL	3.674	39.05	19.47	17.32
*	PD LAB MODEL	-19.88	2.470	-1.660	.8811
	STD DEV	31.62	1.241	1.883	1.292
**	PD PAIRS	2.02	.25	.300	1.4
	STD DEV	8.47	1.42	2.20	2.74

UNITS H20 IS MOLES, NA+, K+, CL- ARE MMOLES OF THE SPECIES IN PLASMA OR RED CELLS / LITER OF BLD

* PERCENT DIFFERENCE BETWEEN THE MODEL AND THE LAB.
PERCENT DIFFERENCE = (M-L)/((M+L)/2) M=MODEL L=LAB

** PERCENT DIFFERENCE FOR 30 PAIRS OF LAB ANALYSES
PERCENT DIFFERENCE = (A-B)/((A+B)/2)
A AND B ARE DUPLICATE ANALYSES OF A SAMPLE

Appendix B

DETAILS OF CHEMICAL ANALYSIS

I. GENERAL

All glassware was washed in a detergent solution and rinsed in distilled water. The glassware for red-cell sodium was rinsed in a distilled water and 5% HCl solution. The tonometers were given a final wash with 95% alcohol and water.

Red cells and whole blood were pipetted while the samples were constantly stirred. The stirring was done with a teflon coated magnet suspended in the specimen. All specimens were frozen at -19^{o} Centigrade for 12 hours or longer. All weighing was performed on a Mettler type analytic balance having an accuracy of 0.01 mgms [28].

II. QUALITY CONTROLS

A. Reproducibility

An evaluation of the reproducibility of the chemical techniques was obtained by taking 30 pairs (A & B) of randomly selected values and analyzed for the dispersion of normalized difference of these pairs. Formula 1 was used as the definition of the normalized difference.

$$ND = (A\_B)/((A+B)/2) \qquad (1)$$

The mean ($\underline{m}$) of the normalized difference is listed at the end of the chemical techniques. The mean multiplied by 100 would be the mean of the percent deviation of the 30 pairs from the theoretical average of these pairs. The standard deviation ($\underline{d}$) of the normalized difference also is listed at the end of the chemical techniques.

B. Precision

The method used to check the precision of the analytical techniques was to sum the number of moles of a species in red cells and plasma; this *calculated* whole blood value was compared with a *measured* whole blood value. The ratio of the measured/calculated represents a cumulative percentage error for all three analyses plus any error that may be present in the hematocrit and the correction for trapped plasma. The difference of this ratio for pairs of analyses has been determined for sodium, potassium, chloride, and water.

III. SPECIFIC CHEMISTRIES

The following analyses were performed in the research laboratory of the Division of Thoracic Surgery, and the Department of Surgery of the University of California at Los Angeles.

A. pH

All measurements were performed on whole blood within 10-15 minutes after the samples were drawn from the tonometer. The time of the completion of the pH measurements was within 15 minutes of the time the centrifuge reached its maximum rpm.

The pH readings were performed on a standard Astrup unit [29]. The unit uses a temperature regulated electrode set to 37°C, $\pm$0.1°C.

Two phosphate buffer standards were used, recommended by the National Bureau of Standards and supplied by Beckman Instrument Company. They have a specified pH of 6.84 and 7.38 units at 35°C [30]. Bradham *et al.* have shown that the pH of blood does not change with temperature variations of several degrees if the PCO_2 is maintained constant [31].

In a paper by J. W. Severinghaus dealing with an analysis of pH techniques, heparin was shown not to affect the pH of blood [32]. The concentrations used by Severinghaus were much less than those used in these experiments, but any errors that might occur were felt to be normalized since the control and stress blood were identically heparinized.

Technique

The unit was calibrated as recommended by the Radiometer manual. The electrode was flushed several times with distilled water. The sample to be measured was drawn into the electrode. Equilibrium was generally reached in less than one minute and the meter reading was observed for stability for two minutes. All determinations were repeated until at least 2 consecutive reproducible values were obtained.

To obtain the values for true plasma 0.01 pH units were added to the whole blood as suggested by Severinghaus [32].

(mean = 0.01, standard deviation = 0.09)

B. PO_2

The PO_2 of the whole blood w-s determined on a Beckman model 160 physiological gas analyzer [33]. Analyses were performed in less than 15 minutes after the specimen was removed from the

tonometer. The zero was determined using pure nitrogen. Two additional points were determined using different oxygen concentrations. The concentrations were determined via the Scholander technique [34]. The three separate gas samples were analyzed and found to contain approximately 0%, 8%, and 20% oxygen. During the calibration procedure, the gas was bubbled through water and a correction was made for the change in gas pressure due to its saturation with the water vapor. The PO_2 of the gas saturated with water vapor was measured. The unit was calibrated at 37°C. A polypropylene membrane was used that has been reported to give a fast response and an accurate reading [35]. Readings were repeated until two successive readings had a difference of less than 3 percent. Pairs of data were not statistically evaluated.

C. PCO_2

The PCO_2 was determined by an Astrup unit and chart. The blood is saturated at 2 different CO_2 pressures with their corresponding pH's; a straight line is drawn on the Astrup diagram and the actual PCO_2 is determined using the original pH reading of the specimen under consideration. This technique was occasionally checked with the Beckman direct reading PCO_2 electrode and the discrepancy was well within the error of the 2 techniques, i.e., 3% [33].

(m = -0.13, d = 1.53)

D. Hematocrit

The microhematocrit was determined by using an International Centrifuge model M.D. This centrifuge spins at 11,500 rpm for 5 minutes and generates a force of 25 x 10^{-9} dyne seconds at the base of the blood column. The trapped plasma in the capillary tubes was checked by counting lengths of plasma and packed cells in a Nuclear Chicago Auto-Gamma counter (Biospan model 4222). The trapped plasma in the packed cells was determined by dividing the red cell counts per unit length by the plasma counts per unit length. The amount of trapped plasma was approximately 2%, a consideration when computing the "true" hematocrit.

True hematocrit = reading - (0.02 x reading). This agreed with Hlad on the trapped plasma under these conditions [24].

(m = -0.18, d = 0.6)

E. Sodium and Potassium (whole blood, red cells, and plasma)

1. Plasma
   a. Take 0.2 ml plasma, pipette into 10.0 ml of solution (1:51 dilution).

   b. Using the sodium filter, set sodium reading (with working standard) to 75% transmittance.

c. Read sample and calculate from a graph.

d. Using the potassium filter and a standard, set the transmittance to 50%, then proceed as specified for the sodium.

2. Red Cells

a. While sample is being constantly stirred, pipette 0.5 ml of hemolized red cells (by freezing and thawing).

b. Rinse into 50.0 ml volumetric flash with triple distilled water.

c. Dilute 5.0 ml of the above with 5.0 ml of 0.02% Sterox. Final dilution (1/200).

d. Proceed as in the plasma protocol but use the red cell standards to set the sodium to 75% and the potassium to 50% transmittance.

3. Whole Blood

a. Use 2.0 ml of whole blood standard plus 8.0 ml of Sterox final dilution in 1:500.

Reagents and Standards

1. Plasma

a. Take 0.2496 gms of Iceland Spar (5.0 meq Ca), dissolve with 1.0 cc HCl.

b. Dilute to 1000.0 cc.

c. To the above add 8.7690 gms of NaCl (dried for 2 hours at 100°C) (150.0 meq NaCl).

d. To above add 0.3728 gms KCl (dried) (5.0 meq KCl).

e. Dilute the above mixture to 1.0 liter using triple distilled water.

f. Take 0.2 ml of the above concentrated standard and add 10.0 ml Sterox.

g. This is the plasma working standard (150.0 meq Na, 5.0 meq K).

2. Red Cells

   a. Using the same care and procedure as in plasma

   b. Use 2.0457 gms NaCl (dried) (35.0 meq NaCl),

   c. 8.94468 gms KCl (dried) (120 meq),

   d. 0.1726 gms $NH_4H_2PO_4$.

   e. Dilute 1 ml to 200.0 with Sterox.

   f. This is the red cell working standard (35.0 meq Na, 120.0 meq K).

3. Whole Blood

   a. Use 7.0140 gms NaCl (dried) (120 meq),

   b. 3.7274 gms KCl (dried) (50.0 meq),

   c. 0.1001 gms Iceland Spar in 5 ml HCl (2.0 meq),

   d. 0.1150 gms of $NH_4H_2PO_4$ (dried).

   e. Dilute to 1.0 liter (concentrated standard).

   f. Dilute 1 ml to 500.0 with Sterox.

   g. This is the whole blood working standard (1:500) (120 meq Na, 50 meq K).

Graph

1. Use semi-log paper.
2. Plot percent (%) T on the log scale.
3. Plot concentration on the linear scale.
4. Establish points for all six species by diluting the working standards.
5. Establish ten points using ten different dilutions to obtain ten different transmittances.
6. Use the six curves for the six unknowns.

Controls

1. Each day check three points from the standards for each of the six species.

2. After each of five readings pick any of the appropriate standards to check for electronic drift.

Coleman Technique

1. This facility uses city gas and an 0_2 mixture.

Sodium

	$\bar{m}$	$\bar{d}$
Wh. Bld. Meas.	0.3	2.5
Wh. Bld. Calc.	0.71	1.97
Meas/Calc.	1.10	3.38
Red Cells	-2.02	8.47
Plasma	-0.40	2.17

Potassium

Wh. Bld. Meas.	-0.7	3.04
Wh. Bld. Calc.	0.50	1.45
Meas/Calc.	-0.9	3.41
Red Cells	-0.25	1.42
Plasma	-2.40	7.2

F. Chloride (Whole blood, red cells, and plasma)

Technique

1. Pipette 1.0 ml of specimen into Erlemayer flask.

2. Rinse pipette until clean with triple distilled H_20.

3. Add 3.0 ml of 0.05 N $AgNO_3$.

4. Add 2.0 ml of concentrated HNO_3.

5. Boil over a hot plate, use washed glass beads to prevent loss of material; while boiling, saturated KMn04 is added to the mixture, a few drops periodically, until mixture becomes clear and colorless.

6. Rinse walls of flask until clean; cool in refrigerator.

7. Add 6 ml of 5% ferric Alum solution; back titrate the surplus of $AgNO_3$ with 0.02 N sulfocyanate.

Calculation

(7.54 - ml sulfocyanate) x 20.0 = meq Cl^- the factor is 1.0 when the solutions are properly prepared.

Reagents

1. 0.05 N $AgNO_3$
   a. Weigh 8.495 gms $AgNO_3$.
   b. Dissolve in minimum water.
   c. Dilute to 1.0 liters with concentrated HNO_3 (Solution I).

2. 0.02 N Na Sulfocyanate
   a. Take 1.6 gms of Na Sulfocyanate (SCN).
   b. Dilute with 900.0 ml water.
   c. Take 3.0 ml of $AgNO_3$ and titrate with SCN; do this to determine amount of H_2O to be added to SCN solution so that 3.0 ml of $AgNO_3$ uses 7.54 ml of SCN.

3. Saturated $KMNO_4$.

4. Ferric Alum 5% (5.0 gm/1000.0 cc H_2O) [40].

	$\bar{m}$	$\bar{d}$
Wh. Bld. Meas.	2.0	1.3
Wh. Bld. Calc.	0.3	1.2
Meas/Calc.	-0.1	2.1
Red Cells	0.3	2.2
Plasma	0.3	1.2

G. Water Content

Pyrex glass stoppered weighing bottles (diamater 20 mm, height 35 mm) are used for drying. An accurately calibrated .5 ml pipette is weighed, filled, and reweighed. The substance is then rinsed out of the pipette into the weighing bottle. The bottles are placed in an electric oven whose temperature is at least 100°C for 24 hours.

The completeness of drying was checked by continuing the drying for 48 and 72 hours with no appreciable change in weight (i.e., less than one percent mean change).

Leeuwen has also confirmed the thoroughness of this procedure [36].

	m	d
Wh. Bld. Meas.	-0.2	1.22
Wh. Bld. Calc.	0.19	1.05
Meas/Calc.	-0.31	1.70
Red Cells	0.0014	2.76
Plasma	0.00	1.01

H. Serum Protein (Biuret Method) Kingsley Modified Technique

1. Pipette 4 ml of biuret reagent to sufficient tubes (1/2 x 5) including standard and blank.
2. Put 9.5 ml of 23% sodium sulfate in 15 ml centrifuge tubes.
3. Add 0.5 ml of serum.
4. Cover with parafilm and mix by several inversions.
5. Immediately pipette a 2 ml portion of the uniform suspension into one of the tubes containing biuret reagent. (These are for total protein.)
6. Add 3 ml of ether to the remaining suspension; cork and shake vigorously for 30 sec.
7. Centrifuge for 5 minutes.
8. Hold tubes in a slanting position and insert the tip of a 2 ml pipette along the side of the tube, past the white layer of packed globulin and into the clear fluid below.
9. Remove a 2 ml portion and put in a tube labeled "albumin".
10. Prepare a blank by adding 2 ml of 23% sodium sulfate to a tube.
11. Prepare a standard by adding 2 ml of protein standard to a tube.
12. Let stand at room temperature for 15 minutes.
13. Read at 520 mu with blank set at 100% T.

Calculation

$$\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times \text{Total protein in standard Gms. \%}$$

Reagents

1. 23% sodium sulfate.
   a. Dissolve 230 gms. of anhydrous grade sodium sulfate in 1 liter of distilled water. (If salt recrystallizes, put in warm water bath.)
2. Biuret.
   a. To a liter flask add:
      (1) 1.5 gms of $CuSO_4$.
      (2) 6.0 gms of potassium sodium tartrate (Rochelle salts).
      (3) 300 ml of 10% sodium hydroxide with swirling.
      (4) While swirling, add distilled water to mark.
3. Standard Protein Solution
   a. Determine the protein content on a pooled serum by the micro-Kjeldahl method, also running an N.P.N. by the same method. T.N. - N.P.N. x 6.25 TP
   b. Dilute 5 ml of the remaining serum to 100 ml with 15% NaCl solution.
   c. Label with the total protein content in gms percentage since this is the value used in the calculations.
   d. This should be stable for a month under refrigeration.
   e. Near the end of the 2-month period and while the O.D. of standard is constant, determine the TP of a new sample of serum diluted with NaCl as above.
   f. As an alternative, normal clinical chemistry control, as put out by Hyland Laboratories or Versatol, may be used as total protein. Proceed as from a (L-13) [37].

      (m = 0.4, d = 2.2)

I. Hemoglobin

Technique

1. 5 ml cyanmethomoglobin reagent (10 ml for red cells) x 2.
2. Add 0.02 ml of blood (Sahli pipette).
3. Mix.
4. Set 20 minute minimum.
5. Read at 540 mu using reagent as a blank.

Calculation

Graph.

Reagents

1. Cyanmethemoglobin reagent - Hycel. Make up per instructions.
2. Cyanmethemoglobin standard - Hycel. Prepare standard curve as per instructions.
3. Color of blood and reagent is stable for months.
4. Gross lipemia may cause positive error up to 3 gm hb percent.
5. This method determines the sum of oxyhemoglobin, hemoglobin, methemoglobin, and carboxyhemoglobin.
5. It does not include sulfhemoglobin [38].

	$\bar{m}$	$\bar{d}$
Wh. Bld. Meas.	0.21	2.41
Wh. Bld. Calc.	-0.38	4.01
Meas/Calc.	0.40	5.18
Red Cells	-0.81	3.46

J. Calcium

The reagents used are prepared by Dade Reagents, Inc. The technique is in their reagent brochure.

Technique

For Coleman, B. and L. and Evelyn Spectrophotometers.

1. Unknown: Pipette 2 ml of serum or filtered urine into a 16 x 125 mm test tube.
2. Standard: Pipette 2 ml standard in a second tube.
3. Control: Pipette 2 ml Lab-trol into third tube.
4. Add 1 ml reagent No. 1 to each tube. Agitate constantly by swirling to redissolve any precipitated protein.
5. Let tubes stand for at least 30 minutes.
6. Centrifuge tubes at 1800 rpm for 10 minutes.
7. Decant the supernatant and drain tubes on absorbent paper for 2-3 minutes. Wipe off mouth of tube with filter paper or gauze.
8. Wash each precipitate with 6 or 7 ml reagent No. 2 using a polyethylene wash bottle with fine stream. Break up precipitates and resuspend in reagent No. 2.

9. Centrifuge at 1800 rpm; decant supernatant, drain, wipe off mouth of tube (as in steps 6 and 7). (If supernatant is cloudy, it may be due to a small amount of protein. This will not affect results.)

10. Add 2 drops (0.1 ml) distilled water to each packed precipitate.

11. Break up precipitate by striking bottom of tube against palm of hand until mat breaks loose and is suspended completely in the water.

12. Add 6 ml Reagent No. 3 to each tube (for Leitz use 3 ml).

13. Stopper and invert tubes until precipitate is completely dissolved. Avoid vigorous shaking. The pink solutions can be read immediately. (Color is stable for at least 24 hours.)

14. Transfer to cuvette and read at 520 mu against a water blank set for 100% T. For greater accuracy, use same cuvette for all readings. Drain cuvette on piece of gauze between readings.

15. Estimate readings to the nearest 1/4 division on scale of spectrophotometer. Convert % transmittance to Optical density. Use Transmittance Density chart in Lab-trol manual or calculate directly using the clinical chemistry calculator.

Calculation

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times 10 = \text{mg Ca/100 ml}$$

$$\frac{\text{mg/100 ml}}{2} = \text{mEq/liter} .$$

Reagents

1. Spectro Calcium 1: A solution of chloranilic acid. Note: This reagent is stable for approximately 1 year when stored in refrigerator (5-10°C) and for 3 months at room temperature. If sediment forms in refrigerated solution use clear supernatant or remove sediment by filtration.

2. Spectro Calcium 2: A solution of isopropyl alcohol.

3. Spectro Calcium 3: A solution of EDTA.

4. Standard (10 mg calcium/100 ml) weigh exactly 0.2497 gm Iceland Spar (Calcite or reagent grade calcium carbonate may be used). Transfer to 1 liter volumetric flask and add 9 ml dilute HCl (1 ml concentrated HCl plus 8 ml distilled water or 9 ml HCl). Allow to stand until Iceland Spar is completely dissolved. Dilute to volume with distilled water and mix.

5. Lab-trol: The known specimen.

Notes and possible errors for specific steps of this method:

1. Step 1 - for urine samples, adjust pH between 5 and 7 with either 0.1N acetic acid or 0.1N sodium hydroxide. Use Nitrazine paper when adjusting pH.

2. Step 2 - Contaminated calcium standard (mold growth in solution).

3. Step 5 - Insufficient time. Tubes must stand for minimum of 30 minutes.

4. Step 6 - Use centrifuge having free swinging trunion cups. Button formed in bottom of tube must be packed tightly. Angle-head centrifuge does not pack precipitate firmly and some precipitate may be lost when tubes are inverted and drained.

5. Steps 8 and 11 - If necessary use small glass rods to break up precipitate. A melting point tube which is sealed at one end makes an ideal stirring rod.

6. Step 12 - Serums that contain abnormal globulins (such as cryoglobulin) sometimes develop turbidity.

7. Step 13 - Make sure that all precipitate is dissolved. If shaking produces foam, let foam settle before reading in colorimeter.

8. Step 14 - If solution is cloudy due to lipids, add 2.3 ml of ether and shake. Take reading again after ether separates [39].

(m = -1.5, d = 9.0)

K. Glucose (Glucostat Method, Peralta Modification)

Technique

1. 4 ml of 0.0125N NaOH.
2. 0.1 ml of blood.
3. 1 ml $ZnSO_4$ - invert twice.
4. Centrifuge for 2 minutes.
5. 2 ml of the supernatant (Blank of standard).
6. 3 ml Glucostat reagent (freeze when keeping overnight).
7. Mix well.
8. Let stand 10 minutes. Add one drop 4N HCl and read at 425 mu.
9. Set instrument to 0 transmittance with Blank.
10. Read standard and unknown.

Calculations

$$\frac{\text{Concen. of Standard}}{\text{Reading of Standard}} = \text{factor}$$

factor x unknown = concentration of unknown.

Reagents

1. a. Dissolve contents of the large vial of prepared reagent with 36 cc H_2O and 15 cc of buffer. (Solution I).
   b. Rinse vial twice and pour into container.
   c. To "Chromogen" vial, add a little H_2O.
   d. Invert once.
   e. Pour into beaker containing 51 cc of solution.
   f. Mix.
2. a. 0.0125N NaOH (1.25 cc 10N NaOH/liter).
   b. $ZnSO_4.7H_2O$ (15.62 gms/liter).
   c. 4N HCl (20 cc 10N Hcl/50 cc H_2O).
3. Standard
   a. Stock - 98 mgm of Dextrose in 100 ml of H_2O and a few Benzoic crystals.
   b. Working standard - 1cc stock standard to 50 cc H_2O 2 cc = 100 mgm percent.

4. Buffers
   a. Na_2HPO_4 -- 9.47 gms/liter.
   b. KH_2PO_4 -- 9.08 gms/liter.
   c. For pH 7:
      (1) Mix 61.1 ml Na_2HPO_4 - 38.9 ml KH_2PO_4.
      (2) Use 15 ml of this in 51 ml total (Solution I) [40].

(m = 1.60, d = 2.9)

L. BUN

Technique

1. Pipette 1 cc of ammonia-free H_2O into a test tube.
2. Add 10 lamda serum or standard.
3. Add 0.2cc of buffered urease.
4. Incubate for 15 minutes at 37°C.
5. Add 5 cc of Phenol reagent.
6. Add 5 cc of Alkaline hypochlorite reagent.
7. Invert.
8. Incubate for 20 minutes at 37°C.
9. Read at 625 mu in 10 or 12 mm cuvettes against a reagent blank.
10. Color is stable for 24 hours.
11. Blank should read less than .08 O.D. against distilled H_2O.
12. If specimen is too dark dilute blank 1:5 and set at 100% T. Dilute specimen 1:5 with distilled water and read against diluted blank. (Can be diluted after reading.)

Reagents

1. Standard
   a. Stock standard 100 mgm % N_2.
   b. Make 10, 20, 40 and 60 mg % standards from 100% mgm % stock standard.
   c. Always pour small amount into beaker or tube before pipetting, for determination.

d. 100 mgm % stock standard - use either 214.25 mg urea/100 or 471 mg ammonium sulfate/100 cc.

2. Buffered urease

a. 150 mg urease (Sigma Chem. Co. Type II) or activity of 1000 U gm and 1.0 gm. EDTA (Disidium ethylene-diamine tetra acetate) in 100 ml ammonia free distilled water.

b. Adjust pH to 6.5.

c. Stability is 1 month.

d. Aliquots can be frozen indefinitely.

3. Phenol Color Reagents

a. Solution I - Phenol 20 gms., Sodium nitroprusside 0.1 gm.

b. Make up to 2 liters.

c. Refrigerate.

d. Stability approximately 60 days.

4. Alkaline Hypochlorite Reagent

a. Solution II - NaOH 10 gms., Sodium hypochlorite 0.84 gm.

b. Make up to 2 liters.

c. Can use Clorox or Purex (use 16 cc) if 5.25% sodium hyposulfate.

d. Refrigerate.

e. Stability 2-6 months [41].

(m = -0.04, d = 8.9)

Technique

1. Plasma: 1 ml into 9 ml of 10 percent T.C.A.

2. Blood: 5 ml of 20 percent T.C.A. in a (Klett) tube marked at 10 ml.

a. Add 0.2 ml of blood.

b. Wash out pipette with distilled water to the 10 ml mark on the tube.

3. Red cells: 5 ml of 20 percent T.C.A. in a Klett tube.

a. Add 0.1 ml of cells

b. Wash out pipette with distilled water to the 10 ml mark on the tube.

4. Stopper.

5. Shake well.

6. Filter through What. #42 into a 20 ml beaker.

7. Standard: transfer 5 ml of stock standard (0.4 mg) to a 50 ml volume flask.

   a. Make up to volume with 10% T.C.A.

8. Unknown

   5 ml of filtrate

   Blank

   5 ml of 10 percent T.C.A.

   Standard

   5 ml of dilute standard (0.04 mg)

9. Add 1 ml of Molybdate to each tube and mix.

10. Add 0.5 ml of aminonaphtholsulfonic acid reagent to all tubes.

11. Add 3.5 ml of distilled water to all tubes.

12. Let stand 5 minutes.

13. Transfer to cuvettes and read at 660 mu with blank set at 100% T.

Calculations

$$\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times \begin{matrix} 8 \text{ (plasma)} \\ 40 \text{ (blood)} \\ 80 \text{ (red cells)} \end{matrix} = \text{Mgms. inorganic P\%}$$

Reagents

1. 10 and 20% T.C.A.

2. Molybdate reagent:

   a. Dissolve 25 g reagent grade ammonium molybdate in about 200 ml of distilled water.

   b. Put 300 ml of 10N H_2SO_4 in a liter flask.

   c. Add the molybdate solution and dilute with washing to 1 liter with distilled water.

   d. Mix.

   e. Stable indefinitely.

3. Aminonaphtholsulfonic: Use Brook's reagent, available through Aloe.

4. Phosphate standard: (0.4 mg in 5 ml)
   a. Dissolve exactly 0.351 gms of pure dry monopotassium phospate in distilled water and transfer to a liter flask.
   b. Add 10 ml N H_2SO_4 and dilute to 1 liter with distilled water.
   c. Stable indefinitely [42].

N. Lactic Acid

Technique

1. Pipette 0.5 ml in 9.5 ml 6% perchloric acid.
2. Shake vigorously.
3. Centrifuge and remove supernatant.
4. 1 ml of supernatant to centrifuge tube with 15 ml graduation.
5. Place 5 cc of standard into same type tube.
6. 5 ml of H_20 into 3rd tube for blank.
7. Add 1 cc 20% $CuSo_4$ to each tube.
8. Dilute all to 10 cc with distilled water.
9. Add 1 gm of powdered Ca $(OH)_2$ to each tube.
10. Stopper and shake vigorously.
11. Stand 1/2 hour.
12. Shake at least 2 times in the 1/2 hour.
13. Centrifuge.
14. Filtrate will give low results if left overnight.
15. Transfer 1 cc of filtrate to a 150 mm x 25 mm tube.
16. Add .05 cc (1 drop) of 4% $CuSO_4$.
17. Add 6 ml concentrated H_2SO_4 drop by drop mix while adding (automatic repipet).

18. Place upright in boiling water bath for 5 minutes.

19. Cool in running H_2O to 20°C or less.

20. Add 0.1 ml P. hydroxydiphenyl reagent drop by drop. Each drop should be in center of tube.

21. Rotate to disperse mixture.

22. Place in 30°C water bath for 30 minutes or longer.

23. Mix at least once.

24. Place in vigorously boiling water for exactly 90 sec.

25. Cool to room temperature in cold water.

26. Transfer to cuvette and read.

27. Read at 560 mu with blank set at 100 percent.

Calculation

$$\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times 0.05 \times 200.0 \times 100 \text{ mgm \%}$$

Pairs of data were not analyzed because the distribution of this ion is not a major consideration. The amount of this substance in whole blood is determined to approximate some of the negative ions [43].

0.1 Hemoglobin Saturation

The hemoglobin saturation was determined on an American Optical cuvette oximeter, Model 10800, which contains a previously established national standard. This instrument was occasionally checked against a similar instrument. Although this technique is not the most precise available, it was considered satisfactory because the hemoglobin saturation was not one of the prime objectives of this project. No pairs of data are included because this was used as a preliminary check to confirm the fact that the blood had spent sufficient time in the tonometer to reach a high degree of saturation.

REFERENCES

1. De Haven, J. C., *Prerequisites for Chemical Thermodynamic Models of Living Systems*, The RAND Corporation, RM-5691-PR, November 1968.
2. Henderson, L. J., "Das Gleichgewich zwischen Basen und Saurem in tierischen Organismus," *Ergebn. Physiol.*, Vol. 8, 1904, pp. 254-325.
3. Hasselbalch, K. A., and S. A. Gammanmeltoft, "Die Neutralitatsregulation des graviden Organismus," *Biochem. Z.*, Vol. 68, 1915, pp. 206-264.
4. Henderson, L. J., "Blood, A Study in General Physiology," Yale University Press, New Haven, Connecticut, 1928.
5. McLean, F. C., "Application of the Law of Chemical Equilibrium (Law of Mass Action) to Biological Problems," *Physiological Review*, Vol. 18, 1935, pp. 495-523.
6. Dantzig, G. B., J. C. De Haven, I. Cooper, S. M. Johnson, E. C. DeLand, H. E. Kanter, and C. F. Sams, "A Mathematical Model of the Human External Respiratory System," *Perspectives in Biology and Medicine*, Vol. IV, No. 3, Spring 1961, pp. 324-376.
7. De Haven, J. C., and E. C. DeLand, *Reactions of Hemoglobin and Steady-States in the Human Respiratory System: An Investigation Using Mathematical Models and an Electronic Computer*, The RAND Corporation, RM-3212-PR, December 1962.
8. DeLand, E. C., *The Classical Structure of Blood Biochemistry--A Mathematical Model*, The RAND Corporation, RM-4962-PR, July 1966.
9. Altman, P. L., J. F. Gibson, Jr., and C. C. Wang (Comps.), *Handbook of Respiration*, Aero Medical Laboratory, U.S. Air Force, WADC Technical Report, Vol. 58, August 1958, p. 352.
10. *Lange's Handbook of Chemistry*, Ninth ed., Handbook Publishers, Inc., New York, 1956, p. 1465.
11. Diem, K. (ed.), *Documenta Geigy: Scientific Tables*, Sixth ed., Geigy Pharmaceuticals, Division of Geigy Chemical Corporation, Ardsley, New York, 1962.
12. Tanford, C. S., S. A. Swanson, and W. S. Shove, "Hydrogen Ion Equilibria of Bovine Serum Albumin," *Journal America-Chemical Society*, December 20, 1955, pp. 6418-6421.

13. DeLand, E. C., and Rose Heirschfeldt, *Protein Binding of Small Ions--A Mathematical Model of Serum Albumin*, The RAND Corporation, RM-5254-PR, April 1967.

14. Edsall, J. T., and J. Wyman, *Biophysical Chemistry*, Vol. 1, Academic Press, New York, 1958.

15. Haurowitz, F., *The Chemistry and Function of Proteins*, Second ed., Academic Press, New York, 1963, pp. 1-455.

16. Adair, G. S., "The Hemoglobin System," *Journal of Biological Chemistry*, Vol. 63, 1925, pp. 529-545.

17. Severinghaus, J. W., "Blood Gas Calculator," *Journal of Applied Physiology*, Vol. 21, No. 3, May 1966.

18. Roughton, F.J.W., "Transport of Oxygen and Carbon Dioxide," Chap. 13, *Handbook of Physiology*, W. O. Fenn and H. Rohn (eds.), Sec. 3, Vol. 1, American Physiological Society, Washington, D.C., 1964, pp. 767-825.

19. Siggaard-Andersen, Ole, *The Acid-Base Status of the Blood*, trans. by H. Cowan, Second ed., Villadsen og Christensen, Copenhagen, Chap. 3, 1964.

20. Bard, P. (ed.), *Medical Physiology*, Tenth ed., C. V. Mosby Company, 1956, p. 324.

21. "The Effect of Temperature on the Oxygen-Linked Ionizations of Hemoglobin," *Journal of Biological Chemistry*, Vol. 242, No. 5, March 10, 1967, pp. 784-792.

22. Siggaard-Andersen, Ole, and K. Engel, "A New Acid-Base Nomogram," The Department of Clinical Chemistry, Rigshopitalet, Copenhagen, Denmark. (Manuscript accepted for publication by the *Scandinavian Journal of Clinical and Laboratory Investigation*.)

23. DeLand, E. C., *The Classical Structure of Blood Biochemistry, II. Behavioral Characteristics*, The RAND Corporation (to be published).

24. Hlad, C. J., and J. H. Holmes, "Factor Affecting Hematocrit Determinations: Trapped Plasma, Its Amount and Distribution," *Journal of Applied Physiology*, No. 5, 1953.

25. Rettori, O., J. V. Maloney, Jr., "Effect of Aldosterone on Ionic Distribution Across the Red Cell Membrane of Rabbits," *Federation Proceedings*, Vol. 26, April 1967, p. 545.

26. Swedenborg, J., J. V. Maloney, Jr., E. C. DeLand, *Erythrocyte Composition in Cirrhotic Patients with Secondary Hyperaldosteronism*, The RAND Corporation, P-4136, July 1969.

27. Lindberg, D., Private Conversation, University of Missouri, Columbia, Missouri, 1967.

28. *Instruction Manual for Mettler Balance HGT Digital*, Mattler Instrument Corporation, Princeton, New Jersey,

29. *Radiometer Type AMEIC Instruction Manual*, Fourth ed., Astrup Micrp Equipment, Copenhagen, Denmark,

30. Bates, R. G., *Journal of Research National Bureau of Standards*, Vol. 66A, No. 2, 1962, pp. 179-184.

31. Bradham, G. B., M. B. Wolf, J. C. De Haven, E. C. DeLand, and J. V. Maloney, Jr., "Laboratory and Digital Computer Study of pH of Blood at Hypothermic Levels," *Proceedings of the San Diego Symposium for Biomedical Engineering*, 1964.

32. Severinghaus, J. W., M. Stupfel, and A. F. Bradley, "Accuracy of Blood pH and pCO_2 Determinations," *American Journal of Physiology*, Vol. 9, 1956, pp. 189-196.

33. Beckman Instrument Company, Inc., *Model 160 Physiological Gas Analyzer*, Instruction Manual, PGIM-1, Palo Alto, California, November 1961.

34. Scholander, P. F., "Analyzer for Accurate Estimation of Respiratory Gases in One-Half Cubic Centimeter Samples," *Journal of Biological Chemistry*, Vol. 167, No. 1, January 1947, pp. 235-250.

35. Rhodes, P. G., and K. M. Moser, "Sources of Error in Oxygen Tension Measurement," *Journal of Applied Physiology*, March 1966, pp. 729-734.

36. Leeuwen, A. M., "Net Cation Equivalency (Base Binding Power) of the Plasma Protein," *Acta Medica Scandinavica*, Vol. 196, Supple., Vol. 422, 1964, p. 63.

37. Kingsley, G. R., "The Direct Biuret Method for the Determination of Serum Protein as Applied to Photoelectric and Visual Colorimetry," *Journal of Laboratory and Clinical Medicine*, Vol. 27, 1942, pp. 840-845.

38. Hamline, A., Jr., *Standard Methods of Clinical Chemistry*, Vol. 2, D. Seligson (ed.), Academic Press, 1958, p. 49.

39. Ferro, P. V., and A. B. Hamm, "A Simple Spectrophotometric Method for the Determination of Calcium," *American Journal of Clinical Pathology*, Vol. 28, 1957, pp. 689-693.

40. Washko, M. E., and E. W. Rice, "Determination of Glucose by an Improved Enzymatic Procedure," *Clinical Chemistry*, Vol. 7, 1961, pp. 542-545.

41. Chaney, A. L., and E. P. Marcache, "Modified Reagents for Determination of Urea and Ammonia," *Clinical Chemistry*, Vol. 8, 1962, p. 130.

42. Fiske, C. H., and Y. Sabbarow, "The Colorimetric Determination of Phosphorus," *Journal of Biological Chemistry*, Vol. 66, 1925, pp. 375-400.

43. Baker, S. B., and W. H. Summerson, "The Colorimetric Determination of Lactic Acid in Biological Material," *Journal of Biological Chemistry*, Vol. 138, 1941, pp. 535-554.

BIBLIOGRAPHY

Anfinsen, C. B., (ed.), *Advances in Protein Chemistry*, Vol. 17, Academic Press, New York, 1962.

-----, *Advances in Protein Chemistry*, Vol. 19, Academic Press, New York, 1964.

Arrhenius, S., "Uber die Dissociation der in Wasser gelosten Stoffe," *Ztschr. f. Physikal. Chemie.*, Vol. 1, 1887, pp. 631-648.

Battaglia, F. C., R. E. Behrman, A. E. Hellegers, and J. D. Battaglia, "Intracellular Hydrogen Ion Concentration Changes During Acute Respiratory Acidosis and Alkalosis," *Journal of Pediatrics*, Vol. 66, No. 4, April 1965, pp. 737-742.

Behrendt, H., M.D., *Chemistry of Erythrocytes*, Charles Thomas, Springfield, Illinois, 1957.

Bernstein, R. E., "Potassium and Sodium Balance in Mammalian Red Cells, *Science*, Vol. 120, September 17, 1954, pp. 459-460.

Bigelow, J., *Semi-narrow Channels*, Operations Research Center, University of California, Report 65-15, Berkeley, June 1965.

Bishop, C., and D. M. Surgenor (eds.), *The Red Blood Cell*, Academic Press, New York, 1964.

Bronsted, J. N., "Einige Bemerkungen uber den Begriff der Sauren und Basen," *Rec. Trav. Chim. Pays-Bas.*, Vol. 42, 1923, pp. 718-728.

Carr, C. W., "Studies on the Binding of Small Ions in Protein Solutions with the Use of Membrane Electrodes. I. The Binding of Chloride Ion and Other Inorganic Anions in Solution of Serum Albumin," *Archives of Biochemistry and Biological Physiology*, Vol. 55, 1955, pp. 1-8.

Christiansen, J., C. G. Douglas, and J. S. Haldane, "The Absorption and Dissociation of CO_2 by Human Blood," *J. Physiol.*, Vol. 48, 1914, pp. 244-271.

Clasen, R. J., *The Numerical Solution of the Chemical Equilibrium Problem*, The RAND Corporation, RM-4345-PR, January 1965.

Comar, C. L., and F. Bronner (eds.), *Mineral Metabolism*, Vols. I and II, Parts A and B, Academic Press, New York, 1960.

Dantzig, G. B., J. C. De Haven, I. Cooper, S. M. Johnson, E. C. DeLand, H. E. Kanter, and C. F. Sams, *A Mathematical Model of the Human External Respiratory System*, The RAND Corporation, RM-2519-PR, September 1959.

Dantzig, G. B., J. C. De Haven, I. Cooper, S. M. Johnson, E. C. Deland, H. E. Kanter, and C. F. Sams, "A Mathematical Model of the Human External Respiratory System," *Perspectives in Biology and Medicine*, Vol. IV, No. 3, Spring 1961, pp. 324-375.

Dantzig, G. B., S. M. Johnson, and W. B. White, "A Linear Programming Approach to the Chemical Equilibrium Problem," *Management Science*, Vol. 5, No. 1, October 1958, p. 38.

Davenport, H. W., *The ABC of Acid-Base Chemistry*, Fourth Ed., University of Chicago Press, Chicago, Illinois, 1958.

DeLand, E. C., and G. B. Bradham, *Fluid Balance and Electrolyte Distribution in the Human Body*, The RAND Corporation, RM-4347-PR, February 1965.

De Haven, J. C., E. C. DeLand, N. S. Assali, and W. Manson, "Physicochemical Characteristics of Placental Transfer," *San Diego Symposium for Biomedical Engineering*, Vol. 2, La Jolla, California, 1962, pp. 217-224.

De Vries, H., "Eine Methode zur Analyze der Turgorkraft," *Jb. Wiss. Bot.*, Vol. 14, 1884, p. 427.

Dick, D.A.T., *Cell Water*, Butterworth, Washington, 1966.

Donnan, F. G., "The Theory of Membrane Equilibria," *Chem. Rev.*, Vol. 1, 1924, pp. 73-90.

Funder, J., and J. O. Wieth, "Determination of Sodium, Potassium, and Water in Human Red Blood Cells," *Scandinav. J. Clin. and Lab. Investigation*, 1966, pp. 151-166.

Gibbs, J. W., *The Scientific Papers of J. Willard Gibbs, Vol. I, Thermodynamics*, Dover Publications, New York, 1961.

Guggenheim, E. A., *Thermodynamics, An Advanced Treatment for Chemists and Physicists*, North-Holland, Amsterdam, 1959.

Gurber, G., "Sitzber. Physik, med. Ges. Wurzburg," cited in E. Gellhorn, *Das Permeabilitatsproblem*, Springer, Berlin, 1929.

Harned, H. S., and B. O. Benton, *The Physical Chemistry of Electrolyte Solutions*, Third Ed., Reinhold Publishing Corporation, New York, 1958.

Hasselbalch, K. A., and S. A. Gammeltoft, "Die 'reduzierte' und die 'regulierte'," *Wasserstoffzahl Blutes*, Vol. 74, 1916, pp. 56-62.

Henderson, L. J., *Blood*, Yale University Press, New Haven, 1928.

Hill, A. V., "The Possible Effects of Aggregation of Molecules of Hemoglobin on Its Dissociation Curves," *J. Physiol.* (Proceedings of the Physiological Society), Vol. 40, January 22, 1910, pp. iv-vii.

Hodgman, C. (ed.), *Handbook of Chemistry and Physics*, The Chemical Rubber Publishing Company, Cleveland, Ohio, Forty-fourth Ed., 1965.

Hoffman, J. F. (ed.), *The Cellular Functions of Membrane Transport*, Prentice-Hall, New Jersey, September 1963.

Hoppe-Seyler, F., "Handbuch der physiologischund pathologisch-chemischen Analyze," A. Hirschwald (ed.), Second Ed., Springer, Berlin, 1958.

Huckabee, W. E., "Control of Concentration Gradients of Pyruvate and Lactate Across Cell Membranes in Blood," *American Journal of Physiology*, Vol. 9, pp. 163-170.

Humoller, F. L., A. J. Barak, and J. M. Holthaus, "Distribution of Ammonia in Cells and Plasma," *Clinical Chemistry*, Vol. 10, No. 7, 1964, pp. 589-596.

Kernan, R. P., *Cell K*, Butterworth, Washington, 1965.

Kleinzeller, A., and A. Kotyk (eds.), "Membrane Transport and Metabolism," *Proceedings of a Symposium Held in Prague*, Academic Press, New York, August 1960.

Meldrum, N. U., and F.J.W. Roughton, "The State of Carbon Dioxide in Blood," *Journal of Physiology*, London, Vol. 80, 1933, pp. 143-170.

Neurath, H., and K. Bailey (eds.), *The Proteins*, Vols. I and II, Parts A and B, Academic Press, New York, 1954.

Olmstead, E. G., *Mammalian Cell Water*, Lea and Febinger, Philadelphia, Pennsylvania, 1966.

Pauling, L., "The Oxygen Equilibrium of Hemoglobin and Its Structural Interpretation," *Proceedings of the National Academy of Science*, Vol. 21, No. 4, April 1935, pp. 186-191.

Peters, J. P., and D. D. van Slyke, *Quantitative Clinical Chemistry, Vol. I. Interpretation*, Williams and Wilkins, Baltimore, Maryland, 1931.

-----, *Quantitative Clinical Chemistry, Vol. II. Methods*, Williams and Wilkins, Baltimore, Maryland, 1932.

Pfeffer, W.F.P., "Osmotische untersuchungen," quoted in *The Modern Theory of Solutions*, H. C. Jones (ed.), Harper and Bros., New York, 1899.

Ponder, E. H., *Hemolysis and Related Phenomena*, Grune and Stralton, New York, 1948.

Prankerd, T.A.J., *The Red Cell*, Oxford Press, Blackwell, England, 1961.

Raker, J. W., I. M. Taylor, J. M. Weller, and A. B. Hastings, "Rate of Potassium Exchange of the Human Erythrocyte," *Journal of General Physiology*, Vol. 33, July 1950, pp. 691-702.

Roughton, F.J.W., "Respiratory Functions of Blood," Chap. 5 in Walter M. Boothby, M.D. (ed.), *Handbook of Respiratory Physiology*, U.S. Air Force School of Aviation Medicine, Randolph Air Force Base, September 1954, pp. 51-102.

Saroff, H. A., and M. S. Lewis, "The Binding of Calcium Ions to Serum Albumin," *Journal of Physical Chemistry*, Vol. 67, June 1963, pp. 1211-1216.

Shapiro, N. Z., *A Generalized Technique for Eliminating Species in Complex Chemical Equilibrium Calculations*, The RAND Corporation, RM-4205-PR, September 1964.

Shapiro, N. Z., and L. S. Shapley, *Mass Action Laws and the Gibbs Free Energy Function*, The RAND Corporation, RM-3935-1-PR, September 1964.

Tanford, C., *Physical Chemistry of Macromolecules*, John Wiley and Sons, Inc., New York, 1961.

Tomkins, F. C. (ed.), "Membrane Phenomena," *Discussions of the Faraday Society*, No. 21, Aberdeen University Press, Aberdeen, 1956.

Valberg, L. S., J. M. Holt, E. Paulson, and J. Szivek, "Spectrochemical Analysis of Sodium, Potassium, Calcium, Magnesium, Copper, and Zinc in Normal Human Erythrocytes," *Journal of Clinical Investigation*, Vol. 44, No. 3, 1965, pp. 379-389.

van Slyke, D. D., "On the Measurement of Buffer Values and on the Relationship of Buffer Value to the Dissociation Constant of the Buffer and Reaction of the Buffer Solution," *Journal of Biol. Chemistry*, Vo.. 52, 1922, pp. 525-570.

-----, "Studies of Acidosis. XVII. The Normal and Abnormal Variations in the Acid-Base Balance of the Blood," *Journal of Biol. Chemistry*, Vol. 48, 1921, pp. 153-176.

van Slyke, D. D., H. Wu, and F. C. McLean, "Studies of Gas and Electrolyte Equilibria in the Blood. V. Factors Controlling the Electrolyte and Water Distribution in the Blood," *Journal of Biol. Chemistry*, Vol. 56, 1923, pp. 765-849.

van't Hoff, J. H., "Die Rolle des osmotischen Druckes in der Analogie zwichen Losungen und Gasen," *Z. Phys. Chem.*, Vol. 1, 1887, p. 481.

Whipple, H. E. (ed.), "Current Concepts of Acid-Base Measurement," *Annals of the New York Academy of Sciences*, Vol. 133, Art. 1, April 1, 1966, pp. 1-274.

Whittam, R., *Transport and Diffusion in Red Blood Cells*, Williams and Wilkins Company, Baltimore, Maryland, 1964.