MEMORANDUM
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A MATHEMATICAL MODEL OF THE CHEMICAL DISTRIBUTION IN A DISEASE STATE: HYPOTHYROIDISM

M. B. Wolf, E. C. DeLand and J. V. Maloney, Jr.

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SANTA MONICA • CALIFORNIA

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PREFACE

The object of this research was the construction and validation of a mathematical model of fluid and electrolyte distribution in a disease state: hypothyroidism. The following steps were required for this study: 1) to develop and validate a model of a standard laboratory dog; and 2) to construct a model of a hypothyroid dog, using the standard model as a basis and then modifying it to conform to laboratory measurements taken on hypothyroid dogs.

The models presented in this report are in an interim stage of development awaiting further laboratory data for more complete validation. They help to further the continuing effort of a group of physicians, physiologists, and mathematicians to produce a complete description of body-fluid chemistry in both normal and abnormal states.

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SUMMARY

A mathematical model of the fluid and electrolyte distribution in a standard laboratory dog was developed. It adequately predicted measured changes in distribution of various chemical substances following administration of saline and alkaline solutions; however, the measured changes due to acid addition were not as well predicted.

A new fluid compartment was added to the standard dog model to simulate hypothyroidism as a result of a hypothesis of body chemistry changes in hypothyroid animals. Although most parameters of the new compartment were determined from the literature, some were chosen numerically to correlate most accurately the model prediction and the chemical data taken on hypothyroid dogs following an alkaline chemical infusion.

Both hypothyroid and euthyroid dogs received injections of acid solution to provide chemical data for comparing chemical distributional changes in these groups and for validation of the models simulating these states. The results proved statistically that hypothyroid dogs respond differently from normal ones to acid infusion. However, the hypothyroid model, like the normal model, did not predict these experimental results well. Laboratory technological problems accounted for many of these discrepancies.

The results of this research have suggested that additional experiments and other chemical perturbations are necessary to test the biochemical hypotheses formulated in this Memorandum. The ideas explored are applicable to studies of other disease states that exhibit gross fluid and electrolyte abnormalities.

CONTENTS

TABLES

I. INTRODUCTION

This Memorandum illustrates the use of mathematical modeling combined with laboratory verification in the study of the steady-state fluid and electrolyte distribution in a disease. Mathematical modeling in the field of fluid and electrolyte balance began with L. J. Henderson [1] in 1908. He studied the chemical equilibrium of the electrolyte buffers in the blood and derived mathematical relationships governing their behavior. In 1913 L. Michaelis and M. L. Menten [2] initiated the mathematical treatment of enzyme kinetics with their research on the interconversions of sugars. These works have been expanded many times and new models have been constructed for other chemical systems, but the field has attracted significant attention only in the last ten years. The advent of the large electronic computer, which can handle extremely complex problems, has inspired such pioneers as Mose Berman [3,4], Britton Chance, *et al.* [5], and David Garfinkle [6] to model the reactions of complex enzyme-kinetic problems and other individuals to construct models in almost all biological fields.

The present study stems from work originated at The RAND Corporation by G. B. Dantzig, *et al.* [7], who proposed a mathematical model of the steady-state chemistry of blood. For the first time a mathematical model of fluid and electrolyte distribution was put into a format suitable for solution by electronic computers. J. C. De Haven and E. C. DeLand [8] expanded the first model to simulate more completely the complex reactions of hemoglobin with oxygen, carbon dioxide, and hydrogen ions. Later Deland [9] further perfected the buffering simulation capabilities of the model; then G. B. Bradham, *et al.* [10], and DeLand [11] expanded it to include the electrolytes of all the body's fluids. Current efforts are oriented toward: 1) further perfection of the blood model; 2) simulation of an individual

patient's blood [12]; and 3) further development and validation of the whole body model.

Both past research and current efforts have dealt with the simulation and verification of the fluid and electrolyte distribution in the blood and other body fluids of the *normal* individual. The next step was to decide if a model of this distribution in disease states could be constructed; thus allowing old hypotheses to be tested or new ones formed concerning the physical description of these diseases. This Memorandum illustrates the first attempt to model the steady-state fluid and electrolyte distribution in a disease.

The original plan was to model the distribution of fluids and electrolytes in a standard (euthyroid) laboratory dog, using the data from the available literature [10,11,13,14,15]. Then the model would be modified according to current hypotheses to match the alkaline-infusion data for hypothyroid dogs obtained in previous studies [13,14]. Following this, the model would be tested, using the results from a study of acid loading [16] of hypothyroid dogs and comparing these to the predictions of the model.

The basis for the model of the hypothyroid state arose from evidence in the biological literature. One early research effort concerning this disease was by W. M. Ord and E. White [17], who studied *myxedema*, the chronic form of hypothyroidism. They observed that administration of thyroid extract caused myxedemic patients' body temperature, nitrogen excretion, and urine production to increase, while body weight decreased. Based on the research, they defined myxedema as an accumulation of mucoproteins in the tissues, and postulated that in myxedema this accumulation is a by-product of the fundamental change that thyroid insufficiency produces in the activity and chemical composition of tissue cells. W. O. Thompson [18] in 1925 found that plasma volume in myxedemic patients increased after thyroid administration, and that this change correlated with the altered metabolic

rate. In 1933 F. B. Byrom [19], who reported on the nature of myxedema, agreed completely with the hypothesis that in myxedema an abnormal protein aggregate exists in the extracellular-extravascular space, and that this substance will decrease in quantity if thyroid hormone is administered. Jerry Aikawa [20] in 1956 discovered a decreased plasma-sodium concentration, a high radiosodium space, and increased total exchangeable sodium in this disease; all were reversed when the individual was treated with thyroid extract. The results of these studies are compatible with the hypothesis that myxedema is associated with an excessive deposition in the extracellular-extravascular space of a macromolecular, osmotically-active substance.

As early as 1860 A. Rolett [21] demonstrated the existence of *mucin* (a macromolecular material) in connective tissue. Later mucin was extracted from the skin of many animals, and in 1940 hyaluronic acid was identified as the mucinous material. V. Horsely [22] in 1885 mentioned an accumulation of mucin in myxedematous skin, and in 1950 G. Asboe-Hansen [23] studied the effect of thyroid hormone on dermal connective tissue. He concluded that hypofunction of the thyroid gland (hypothyroidism) was accompanied by an increase of hyaluronic acid in this tissue. Sara Schiller [24] in 1961 showed that in the skin of hypothyroid rats the concentration of hyaluronic acid was increased, and that this change was prevented or even reversed by the administration of the thyroid hormone.

Hyaluronic acid is extremely effective in binding ions and water. J. H. Bland [25] described the configuration of the molecule as a tangled thread, resulting in a high, specific, hydrodynamic volume. Also the water content of tissues has been determined to be a function of their hyaluronic-acid content [25]. E. Kulonen [26], in dialysis experiments, showed that hyaluronic acid retained cations in the

dialysis bag, the quantities depending upon the degree of its polymerization. M. B. Engel [27,28], N. R. Joseph [29,30], and H. R. Catchpole [31] used electrometric techniques in a series of experiments to determine both the changes in electrical charge density and the ion-binding characteristics of the ground substance of connective tissue (hyaluronic acid being predominant) under hormonal influence. These men demonstrated that depolymerization of the tissue caused a fall in the anionic polyelectrolyte charge density and an increase in serum electrolytes. Thus a basis was laid for considering the ground substance of connective tissue as a heterogeneous system of ions, polyelectrolyte macromolecules, and water; the distribution of these is a function of the physical state and the degree of polymerization of the ground substance.

All the previous evidence--including the demonstration by Dietor Dörr [14] that showed that the chemical distribution in euthyroid and hypothyroid dogs differed in response to the infusion of hypertonic sodium bicarbonate (an alkalinizing solution), even though both groups had similar body chemical composition before infusion--made the following hypothesis plausible: In this disease the accumulation of hyaluronic acid in the extracellular-extravascular space leads to fluid and electrolyte distribution abnormalities. These result from the sensitivity of this macromolecule to the pH of its surroundings, as far as characteristics of its polymerization, ion, and water binding are concerned. This hypothesis was incorporated in the modeling of the chemical state of the hypothyroid.

The mathematical models used to study chemical exchange in the body generally rely upon the division of the biological system into individual homogeneous fluid spaces (compartments) between which chemical exchange occurs. Originally, on anatomic grounds, intracellular and extracellular were hypothesized to be the only body fluid compartments [32].

However, the introduction of the use of isotopic tracers by F. D. Moore [33] in 1946, which provided the technique to measure quantitatively the volumes of these individual body phases, has shown that more than two distinct compartments exist. Since then a constant search has been underway for suitable isotopes and carriers to measure the volumes of all the observed anatomical spaces in the body and, in particular, the total extracellular fluid volume [34]. The main result has been that even this fluid is heterogeneous and can be divided into a number of subcompartments, each with different chemical compositions.

Validating mathematical models of chemical exchange necessitates using multiple isotope dilution methods to measure volume changes in all body fluid compartments included in the model. Therefore, one must be quantitatively specific, relating observed anatomical spaces to isotope-measured spaces. In this Memorandum the fluid compartments considered were chosen from an anatomical description of the body and were assumed to be analogous to the volumes measured by specific isotope dilution techniques.

II. MODEL OF FLUID BALANCE AND ELECTROLYTE DISTRIBUTION IN THE DOG

This mathematical model simulates the steady-state fluid balance and the gas and electrolyte distribution in a standard (euthyroid) laboratory dog before and after such disturbances as fluid and electrolyte loading. The format, is general, however, to incorporate the effects of disease states upon the distribution. Consequently, the requirements of this initial study of hypothyroidism are: 1) an idealization of the extra-renal, steady-state fluid and electrolyte distribution in a standard animal; and 2) a provision for adaptation to empirical results and abnormal states.

Chemical species, chemical reactions, compartments, and intercompartmental relationships pertinent to a detailed description of the whole body fluid subsystem constitute this model of fluid and electrolyte distribution. The model is intended to represent the composition of those fluid spaces in a nephrectomized (kidneys removed) dog that equilibrate within two to three hours with a fluid and electrolyte load. These various fluid spaces supposedly exist in discrete compartments that are, however, in mutual chemical communication via semipermeable membranes. These compartments are:

1) The interior of the red cells taken together as a physiologic space;
2) The plasma;
3) The interstitial lymph and the rapidly equilibrating portions of the connective tissue and bone;
4) The intracellular fluid of the body cell mass as a whole, excluding red cells.

The chemical content of the slowly equilibrating parts of the bones, the dense connective tissue, and transcellular

fluids are assumed to remain constant during the experimental period.

Table I shows the reference state of this model. It is described by the volumes and the chemical composition of the various compartments in an anesthetized, spleenectomized, nephrectomized dog. The concentrations are in mM/liter of water. Data for total volumes of the compartments are taken from the control period measurements (Table X, p. 27), the concentrations of species in blood from R. E. Bernstein [35] and D. S. Dittmer [36], and the concentration of species in intracellular and interstitial fluid from I. S. Edelman [37]. The last column of the table is the calculated total amount of each input species, shown in mM for the standard 10 kg dog.

Table II is an idealized model of the dog body subdivided into the rapidly equilibrating fluid compartments. The idealized compartments of the mathematical dog "body" are considered to be chemically homogeneous and iso-osmotic, and to be at constant temperature ($37^{o}C$) and pressure (1 atm). The separating membranes are assumed to be impermeable to the macroscopic molecules--proteins and miscellaneous large molecules--present over the course of a five to six hour experiment. They are permeable, however, to the solvent and miscellaneous small species, charged and uncharged, whose concentrations are subject to certain constraints; e.g., cation "pumps" and Gibbs-Donnan gradients, which shall be defined.

Each compartment, as shown in Table II, contains a list of chemical reactions pertinent to the fluid and electrolyte distribution problem. Thus, the bicarbonate system is complete, the proteins buffer H^{+} ions, and hemoglobin oxygenates properly. The simulation is detailed then in the chemical sense; more detail can be achieved by adding pertinent chemical reactions or compartments, as will be required subsequently in the simulation of the hypothyroid state.

Table I

COMPOSITION OF STANDARD 10 kg DOG MODEL

CHEMICAL SPECIES	COMPARTMENT				TOTAL
	Plasma	Red Cells	Inter-stitial	Intra-cellular	
	Millimoles/Liter of Water[a]				Millimoles[a]
Na^+ (sodium)	154	134	145.2	10.	306.27
K^+ (potassium)	4.83	9.92	4.55	160.	656.07
Ca^{++} (calcium)	1.52	1.71	1.35	1.0	6.64
Mg^{++} (magnesium)	.226	.604	.202	13.	53.0
Cl^- (chloride)	111.1	88.8	117.9	3.0	218.46
HCO_3^- (bicarbonate)	21.9	18.6	23.2	6.07	63.59
$HPO_4^=$ (mono hydrogen phosphate)	.26	.165	.292	50.	202.80
$H_2PO_4^-$ (di hydrogen phosphate)	.202	.161	.213	0	
$SO_4^=$ (sulfate)	.26	.198	.35	10.	41.13
$ORGAN^-$ (organic acid)	1.85	1.5	2.0	.0508	3.7
Hb_4^- (hemoglobin)	0	6.7	0	0	1.559
GLUCOSE	5.27	5.27	5.27	5.27	30.93
UREA	3.65	3.65	3.65	3.65	21.42
NH_4^+ (ammonium)	.0223	.028	.0211	.829	3.41
H_2O (water in moles)	19.58	12.83	68.36	222.76	384.68
H_2O (water in liters)	.355	.233	1.24	4.04	5.868
MISC-- (miscellaneous protein)		35.	1.64	39.6	(b)
MYOSIN	0	0	0	3.5	14.14
SERUM PROTEIN	.802	0	0	0	.285
pH	7.30	7.20	7.32	6.74	

[a]Compositions are shown in mM/liter of water; totals are given in millimoles, except for water, which is given in liters and moles.

[b]The miscellaneous proteins are considered as individual species in each compartment.

Table II

COMPUTER INPUT

```
GAS PHASE
      O2     -10.94          1.0 O2
      CO2     -7.74074       1.0 CO2
      N2     -11.52          1.0 N2
      H2O      2.79          1.0 H2O
 PLASMA
      O2       0.            1.0 O2
      CO2      0.            1.0 CO2
      N2       0.            1.0 N2
      H2O                    1.0 H2O
      H+       0.            1.0 H+                                    1.0 *PLASM
      OH-     39.390         1.0 H2O      -1.0 H+                     -1.0 *PLASM
      NA+      0.            1.0 NA+                                   1.0 *PLASM
      K+       0.            1.0 K+                                    1.0 *PLASM
      CA++                   1.0 CA++                                  2.0 *PLASM
      MG++     0.            1.0 MG++                                  2.0 *PLASM
      CL-      0.            1.0 CL-                                  -1.0 *PLASM
      ORGAN-                 1.0 ORGANI                               -1.0 *PLASM
      HCO3-   18.0556        1.0 CO2       1.0 H2O     -1.0 H+       -1.0 *PLASM
      H2CO3    6.566C        1.0 CO2       1.0 H2O
      CO3=    45.6616        1.0 CO2       1.0 H2O     -2.0 H+       -2.0 *PLASM
      H2PO4-  -20.57         1.0 HPO4=     1.0 H+                    -1.0 *PLASM
      HPO4=    0.            1.0 HPO4=                               -2.0 *PLASM
      SO4=     0.            1.0 SULFAT                              -2.0 *PLASM
      NH4+     0.            1.0 NH4+                                 1.0 *PLASM
      NH3     24.461         1.0 NH4+     -1.0 H+
      UREA     0.            1.0 UREA
      GLUCOS   0.            1.0 GLUCOS
      PROTN    0.0           1.0 SERUM -105. BCARB -17.0 IMID  -60.5 EAMINC
      PROTN                -20.9 PHENOL-23.7 GUANID
 RED CELLS
      O2      -0.48999999    1.0 O2
      CO2     -0.C64251      1.0 CO2
      N2      -0.50000000    1.0 N2
      H2O                    1.0 H2O
      H+                     1.0 H+
      OH-      39.39C        1.0 H2O      -1.0 H+
      NA+      .377939       1.0 NA+
      K+      -.48088        1.0 K+
      CA++     .36264        1.0 CA++
      MG++    -.49491        1.0 MG++
      CL-      0.            1.0 CL-
      ORGAN-                 1.0 ORGANI
      HCO3-   17.99135       1.0 CO2       1.0 H2O     -1.0 H+
      H2CO3    6.491749      1.0 CO2       1.0 H2O
      CO3=    45.597349      1.0 CO2       1.0 H2O     -2.0 H+
      H2PO4-  -20.57         1.0 HPO4=     1.0 H+
      HPO4=                  1.0 HPO4=
      SO4=     0.            1.0 SULFAT
      NH4+     0.            1.0 NH4+
      NH3     24.461         1.0 NH4+     -1.0 H+
      UREA     0.            1.0 UREA
      GLUCOS   0.            1.0 GLUCOS
      X MISC  -19.6498       1.0 X- MIS    1.0 H+
      X-MISC                 1.0 X- MIS
      HB4                    1.0 HB4                   -8.0 HMCOOH -12. TYROSIN
      HB4                   -12. ARGINI -50. ASPGLU -20. HISTID -44. LYSINE
      HB4                   -4.0 REDASP -4.0 REDNH2
      HB4O2   -15.396836     1.0 HB4       1.0 O2      -8.0 HMCOOH -12. TYROSIN
      HB4O2                 -12. ARGINI -50. ASPGLU -20. HISTID -44. LYSINE
      HB4O2                 -3.0 REDASP -3.0 REDNH2 -1.0 OXYASP -1.0 OXYNH2
      HB4O4   -31.504982     1.0 HB4       2.0 O2      -8.0 HMCOOH -12. TYROSIN
      HB4O4                 -12. ARGINI -50. ASPGLU -20. HISTID -44. LYSINE
      HB4O4                 -2.0 REDASP -2.0 REDNH2 -2.0 OXYASP -2.0 OXYNH2
      HB4O6   -45.220728     1.0 HB4       3.0 O2      -8.0 HMCOOH -12. TYROSIN
      HB4O6                 -12. ARGINI -50. ASPGLU -20. HISTID -44. LYSINE
      HB4O6                 -1.0 REDASP -1.0 REDNH2 -3.0 OXYASP -3.0 OXYNH2
      HB4O8   -64.024875     1.0 HB4       4.0 O2      -8.0 HMCOOH -12. TYROSIN
      HB4O8                 -12. ARGINI -50. ASPGLU -20. HISTID -44. LYSINE
      HB4O8                                             -4.0 OXYASP -4.0 OXYNH2
```

Table II--Continued

INTERSTITIAL									
	O2	0.	1.0	O2					
	CO2	0.	1.0	CO2					
	N2	0.	1.0	N2					
	H2O		1.0	H2O					
	H+	0.	1.0	H+				1.0	*ISF
	OH-	39.390	1.0	H2O	-1.0	H+		-1.0	*ISF
	NA+	0.	1.0	NA+				1.0	*ISF
	K+	0.	1.0	K+				1.0	*ISF
	CA++		1.0	CA++				2.0	*ISF
	MG++	0.	1.0	MG++				2.0	*ISF
	CL-	0.	1.0	CL-				-1.0	*ISF
	ORGAN-		1.0	ORGANI				-1.0	*ISF
	HCO3-	18.0556	1.0	CO2	1.0	H2O	-1.0 H+	-1.0	*ISF
	H2CO3	6.5660	1.0	CO2	1.0	H2O			
	CO3=	45.6616	1.0	CO2	1.0	H2O	-2.0 H+	-2.0	*ISF
	H2PO4-	-20.57	1.0	HPO4=	1.0	H+		-1.0	*ISF
	HPO4=	0.	1.0	HPO4=				-2.0	*ISF
	SO4=	0.	1.0	SULFAT				-2.0	*ISF
	NH4+	0.	1.0	NH4+				1.0	*ISF
	NH3	24.461	1.0	NH4+	-1.0	H+			
	UREA	0.	1.0	UREA					
	GLUCOS	0.	1.0	GLUCOS					
	PROTN		1.0	ISFPRO				-2.646	*ISF
INTRACELL									
	O2	0.	1.0	O2					
	CO2	0.	1.0	CO2					
	N2	0.	1.0	N2					
	H2O		1.0	H2O					
	H+	2.330770	1.0	H+				1.0	*INTRA
	OH-	37.059232	1.0	H2O	-1.0	H+		-1.0	*INTRA
	NA+	6.348013	1.0	NA+				1.0	*INTRA
	K+	.112267	1.0	K+				1.0	*INTRA
	CA++	7.875	1.0	CA++				2.0	*INTRA
	MG++	3.176687	1.0	MG++				2.0	*INTRA
	CL-	0.	1.0	CL-				-1.0	*INTRA
	ORGAN-		1.0	ORGANI				-1.0	*INTRA
	HCO3-	15.724832	1.0	CO2	1.0	H2O	-1.0 H+	-1.0	*INTRA
	H2CO3	6.5660	1.0	CO2	1.0	H2O			
	CO3=	41.000064	1.0	CO2	1.0	H2O	-2.0 H+	-2.0	*INTRA
	HPO4=	-12.450454	1.0	HPO4=				-2.0	*INTRA
	SO4=	-10.7	1.0	SULFAT				-2.0	*INTRA
	NH4+	0.	1.0	NH4+				1.0	*INTRA
	NH3	22.130232	1.0	NH4+	-1.0	H+			
	UREA	0.	1.0	UREA					
	GLUCOS	0.	1.0	GLUCOS					
	PROTN		1.0	MYOSIN	-165.	ACARB	-16. IMIDAZ	- 91.	EPAMIN
	PROTN				-43.	IGUANI			
	X MISC		1.0	MISINT					

Table II illustrates that the four liquid compartments--plasma, red cells, interstitial fluid, and intracellular fluid--contain essentially the same species, with the exception of proteins. The principal proteins of plasma--serum albumin and the globulins--are considered here as the species PROTN. The interstitial fluid--derived from plasma, connective tissue, and intracellular fluid--has a collection of miscellaneous impermeable species also named PROTN. In the red cells, the miscellaneous impermeable species are called XMISC; their charge and amount are variable quantities, usually determined to match the computer prediction of blood chemistry to the desired composition (Table I). Hemoglobin, however, has been entered in the red cells separately to show its detailed reactions with oxygen, hydrogen, and carbon dioxide.

In the intracellular compartment, myosin has been the only protein singled out from the miscellaneous impermeable species since it is a principal buffer in the cells. The myosin buffering, which has been detailed for this mathematical model, as well as the serum albumin buffering, is incorporated following the detailed discussion in DeLand [38]. These proteins are impermeable and have a fixed charge that is dependent upon the pH of the compartment. Adjacent fluid compartments consequently have Gibbs-Donnan gradients across intervening membranes for charged species; e.g., see DeLand [9]. In addition, the red-cell plasma and the intracell-interstitial relationships include active cation pumps that effectively establish the empirically measured gradients of Na^+, K^+, Ca^{++}, and Mg^{++} across the cellular membranes.

Dog blood is different chemically from human blood, principally in the cation and Gibbs-Donnan gradients (see Table I, p. 8). For example, Bernstein [35] shows that the Na^+ ion gradient for human blood from plasma to red-cell milieu is about 7:1, while for a dog it is only 1.1:1.

Frequently the ratio is less than 1:1; i.e., for a dog, sodium may be more concentrated inside the red cell than in plasma. Similarly, the concentration ratio of K^+ from red cells to plasma is only 2:1, while in man it is nearly 30:1. The chloride gradient in human blood is about 1.45:1, while in a dog it is only 1.25:1.

However, active cation pumps seem to be present in the dog. If the Gibbs-Donnan gradient is measured by the Cl^- ion-concentration gradient (an assumption implying that the Cl^- ion is not actively pumped but is distributed passively, and that both plasma and red cells are electrically neutral), then neither Na^+ nor K^+ conform to this gradient and such additional phenomena as active pumps must be invoked to explain the cation gradients. In this work the H^+ ion concentration in blood is assumed to exhibit the Gibbs-Donnan gradient; hence, for venous blood the pH difference between plasma and red cells is 7.36-7.24 or 0.12 (as shown in Table III). In the human blood, with a slight hydrogen ion "pump," the difference in pH is 0.20.

The interstitial compartment is assumed to be an ultrafiltrate of plasma with a Gibbs-Donnan gradient of 0.95 for monovalent ions.

The chemical composition of the intracellular space (less the red cells) of the dog is not well known. This composition--which may be approximately measured by subtracting the measured amount of a chemical species in the extracellular space from the amount in the total body measured by radioisotope dilution--consists of an average composition of a wide variety of cells. However, the principal cells in number are muscle cells. The chemical data for these cells are taken from Edelman [37] and Moore [33], and are essentially the same as those used for the human intracellular compartment [11]. The cation gradients, with respect to interstitial fluid, are very large: Na^+, 14.5:1 outside to inside; K^+, 35:1 inside to outside.

Table III

COMPUTED DISTRIBUTION OF SPECIES FOR THE STANDARD DOG USING THE MODEL OF TABLE II

		GAS PHASE	PLASMA	RED CELLS	INTERSTITIAL	INTRACELL
X-BAR		9.99877E 02	2.50408E 01	1.22685E 01	1.14303E 02	2.17946E 02
PH		-0.	7.35705E 00	7.23842E 00	7.37960E 00	6.79777E 00
O2	MOLES	5.26020E 01	2.33627E-05	1.86839E-05	1.06643E-04	2.03340E-04
	MFRAC	5.26084E-02	9.32983E-07	1.52292E-06	9.32983E-07	9.32983E-07
CO2	MOLES	6.04102E 01	6.57735E-04	3.43634E-04	3.00235E-03	5.72468E-03
	MFRAC	6.04176E-02	2.62665E-05	2.80096E-05	2.62665E-05	2.62665E-05
N2	MOLES	8.25797E 02	2.05354E-04	1.65879E-04	9.37375E-04	1.78732E-03
	MFRAC	8.25899E-01	8.20077E-06	1.35208E-05	8.20077E-06	8.20077E-06
H2O	MOLES	6.10675E 01	2.48997E 01	1.21993E 01	1.13659E 02	2.16717E 02
	MFRAC	6.10750E-02	9.94364E-01	9.94364E-01	9.94364E-01	9.94364E-01
H+	MOLES	-0.	1.98245E-08	1.27636E-08	8.59129E-08	6.25425E-07
	MFRAC	-0.	7.91687E-10	1.04036E-09	7.51622E-10	2.86963E-09
OH-	MOLES	-0.	2.45913E-07	9.16836E-08	1.18235E-06	5.90482E-07
	MFRAC	-0.	9.82047E-09	7.47311E-09	1.03439E-08	2.70931E-09
NA+	MOLES	-0.	6.90567E-02	3.04678E-02	2.99269E-01	3.92204E-02
	MFRAC	-0.	2.75776E-03	2.48343E-03	2.61820E-03	1.79955E-04
K+	MOLES	-0.	2.16831E-03	2.25807E-03	9.39675E-03	6.28894E-01
	MFRAC	-0.	8.65910E-05	1.84055E-04	8.22089E-05	2.88555E-03
CA++	MOLES	-0.	7.54897E-04	4.44425E-04	3.10592E-03	3.47172E-03
	MFRAC	-0.	3.01466E-05	3.62250E-05	2.71726E-05	1.59293E-05
MG++	MOLES	-0.	1.01225E-04	1.40484E-04	4.16475E-04	5.10970E-02
	MFRAC	-0.	4.04239E-06	1.14508E-05	3.64360E-06	2.34448E-04
CL-	MOLES	-0.	5.06419E-02	1.88808E-02	2.43486E-01	1.18222E-02
	MFRAC	-0.	2.02237E-03	1.53897E-03	2.13017E-03	5.42435E-05
ORGAN-	MOLES	-0.	8.57364E-04	3.19651E-04	4.12220E-03	2.00148E-04
	MFRAC	-0.	3.42387E-05	2.60547E-05	3.60637E-05	9.18340E-07
HCO3-	MOLES	-0.	1.19013E-02	4.73162E-03	5.72215E-02	2.85773E-02
	MFRAC	-0.	4.75277E-04	3.85673E-04	5.00612E-04	1.31121E-04
H2CO3	MOLES	-0.	9.20490E-07	4.85744E-07	4.20174E-06	8.01160E-06
	MFRAC	-0.	3.67596E-08	3.95929E-08	3.67596E-08	3.67596E-08
CO3=	MOLES	-0.	1.54138E-05	4.66329E-06	7.80596E-05	1.02108E-05
	MFRAC	-0.	6.15545E-07	3.80104E-07	6.82917E-07	4.68503E-08
H2PO4-	MOLES	-0.	8.34910E-05	3.11279E-05	4.01424E-04	-0.
	MFRAC	-0.	3.33420E-06	2.53723E-06	3.51192E-06	-0.
HPO4=	MOLES	-0.	1.22927E-04	3.48761E-05	6.22538E-04	1.96555E-01
	MFRAC	-0.	4.90908E-06	2.84275E-06	5.44637E-06	9.01854E-04
SO4=	MOLES	-0.	1.41743E-04	4.02144E-05	7.17826E-04	3.93664E-02
	MFRAC	-0.	5.66047E-06	3.27787E-06	6.28001E-06	1.80625E-04
NH4+	MOLES	-0.	1.00337E-05	6.46000E-06	4.34827E-05	3.25592E-03
	MFRAC	-0.	4.00693E-07	5.26554E-07	3.80415E-07	1.49391E-05
NH3	MOLES	-0.	3.01738E-07	1.47833E-07	1.37734E-06	2.70128E-05
	MFRAC	-0.	1.20498E-08	1.20498E-08	1.20498E-08	1.23943E-07
UREA	MOLES	-0.	1.64856E-03	8.07693E-04	7.52515E-03	1.43484E-02
	MFRAC	-0.	6.58349E-05	6.58349E-05	6.58349E-05	6.58349E-05
GLUCOS	MOLES	-0.	2.38024E-03	1.16617E-03	1.08650E-02	2.07167E-02
	MFRAC	-0.	9.50545E-05	9.50545E-05	9.50545E-05	9.50545E-05
PROTN	MOLES	-0.	3.61100E-04	-0.	2.90446E-03	1.67264E-02
	MFRAC	-0.	1.44205E-05	-0.	2.54101E-05	7.67457E-05
X MISC	MOLES	-0.	-0.	2.05149E-03	-0.	1.66354E-01
	MFRAC	-0.	-0.	1.67217E-04	-0.	7.63281E-04
X-MISC	MOLES	-0.	-0.	5.76881E-03	-0.	-0.
	MFRAC	-0.	-0.	4.70214E-04	-0.	-0.
HB4	MOLES	-0.	-0.	1.31939E-04	-0.	-0.
	MFRAC	-0.	-0.	1.07543E-05	-0.	-0.
HB4O2	MOLES	-0.	-0.	1.17913E-04	-0.	-0.
	MFRAC	-0.	-0.	9.61108E-06	-0.	-0.
HB4O4	MOLES	-0.	-0.	2.14618E-04	-0.	-0.
	MFRAC	-0.	-0.	1.74935E-05	-0.	-0.
HB4O6	MOLES	-0.	-0.	3.57079E-05	-0.	-0.
	MFRAC	-0.	-0.	2.91054E-06	-0.	-0.
HB4O8	MOLES	-0.	-0.	9.63221E-04	-0.	-0.
	MFRAC	-0.	-0.	7.85119E-05	-0.	-0.

But the Cl^- gradient is nearly 40:1, illustrating that the Cl^- ion is essentially extracellular.

In addition to the fluid compartments in Table I, the mathematical model has a gas-phase compartment containing gases of various mixtures under the control of the experimenter. Normally, the gas phase is maintained in an overwhelming amount (say 1000 moles) so that reactions in the viable range of the liquid phase will not alter appreciably the partial pressures of the gas phase. This is equivalent, in the wet laboratory, to equilibration of blood in a tonometer or placing an animal in a respirator for a long period with gas of fixed composition.

A similar "whole body" mathematical system for human chemistry has been described by DeLand and Bradham [11]; the system has been described for blood alone by DeLand [9]. These two papers contain bibliographical references to earlier models and more detailed discussion of the construction of steady-state models that will be abbreviated here since the interest is in the simulation of a disease state.

A model, as described here, consists of a list of chemical components from which product species are obtained by chemical reactions; each species has a free-energy parameter (i.e., the mass-action equation equilibrium constant), the mole numbers (mass) of each component, and such intercompartmental relationships as the solubility of gases. The mathematical problem is to compute the steady-state distribution of all species in the system subject to chemical and biological constraints; i.e.:

1) Conservation of mass;
2) Positive species mole numbers;
3) Simultaneous satisfaction of all mass-action equations;
4) Conservation of charge in each compartment;
5) Iso-osmolarity of compartments;

6) Satisfaction of active cation "pump" gradients;

7) Miscellaneous minor constraints.

A *solution* of the mathematical system is a list of the species mole numbers and their mole fraction concentrations in each compartment. The *normal* model merely reproduces Table I, but a valid model may then be used to compute the new distribution resulting from certain chemical loads or other perturbations. The computer can produce a solution for the system based upon many methods. The technique used here is based upon minimization of the Gibbs' free-energy function subject to constraints as described, for example, in N. Z. Shapiro and L. L. Shapley [39]. Both R. J. Clasen [40] and DeLand [41] discuss the detailed program.

Table III is the computed distribution of species for the standard 10 kg dog, using the input data of Table I and the model of Table II. Beginning with the totals' column (inputs to the computer program) from Table I, the program distributes the constituents in the various compartments so that a modified Gibbs' free-energy function will be minimized subject to the constraints listed above; the result is then printed. For example, Table III shows that the distribution of species follows the concentrations in Table I when these are multiplied by the appropriate volume factors for each compartment, as shown in Table I. The construction of a model that yields the desired composition of Table I involves specifying the magnitude of some parameters: 1) the amounts of miscellaneous proteins in each compartment to yield the desired volume and the fixed negative change on the miscellaneous species, thus establishing the correct Gibbs-Donnan gradient; 2) the free-energy parameter, associated with each cation pump (Table II), that gives the concentration gradient of Table I for these various cations; and 3) the amount of H^+ ion input needed by the model to yield the correct pH in the blood.

Validation of this standard dog model follows procedures described in more detail in Ref. 11. In addition to determining the standard distribution of species in the specified compartments of the 10 kg animal, the model must respond correctly to specified chemical additions. Tables IV and V are the computed distribution of species after additions of $NaHCO_3$ and HCl solutions to the standard model. The results of the laboratory experiments with the same perturbations are then compared (Table XI, p. 28) with these computed distributions as part of the model's validation procedure.

Table IV

STANDARD DOG MODEL, ADDITION OF 10 ml/kg OF .892M $NaHCO_3$

		GAS PHASE	PLASMA	RED CELLS	INTERSTITIAL	INTRACELL
X-BAR		9.99880E 02	2.81993E 01	1.23369E 01	1.30311E 02	2.05780E 02
PH		-0.	7.61571E 00	7.45908E 00	7.63619E 00	6.91500E 00
O2	MOLES	5.26020E 01	2.63094E-05	1.87881E-05	1.21578E-04	1.91988E-04
	MFRAC	5.26083E-02	9.32981E-07	1.52292E-06	9.32981E-07	9.32981E-07
CO2	MOLES	6.04382E 01	7.41040E-04	3.45711E-04	3.42440E-03	5.40761E-03
	MFRAC	6.04455E-02	2.62787E-05	2.80225E-05	2.62787E-05	2.62787E-05
N2	MOLES	8.25797E 02	2.31255E-04	1.66804E-04	1.06865E-03	1.68755E-03
	MFRAC	8.25897E-01	8.20074E-06	1.35207E-05	8.20074E-06	8.20074E-06
H2O	MOLES	6.10423E 01	2.80287E 01	1.22623E 01	1.29523E 02	2.04535E 02
	MFRAC	6.10496E-02	9.93951E-01	9.93951E-01	9.93951E-01	9.93951E-01
H+	MOLES	-0.	1.23064E-08	7.72193E-09	5.42493E-08	4.50823E-07
	MFRAC	-0.	4.36409E-10	6.25922E-10	4.16306E-10	2.19081E-09
OH-	MOLES	-0.	5.02169E-07	1.53176E-07	2.43262E-06	7.29965E-07
	MFRAC	-0.	1.78078E-08	1.24161E-08	1.86678E-08	3.54732E-09
NA+	MOLES	-0.	8.46632E-02	3.64042E-02	3.73212E-01	5.58344E-02
	MFRAC	-0.	3.00231E-03	2.95084E-03	2.86401E-03	2.71331E-04
K+	MOLES	-0.	1.87266E-03	1.90062E-03	8.25505E-03	6.30689E-01
	MFRAC	-0.	6.64079E-05	1.54060E-04	6.33488E-05	3.06488E-03
CA++	MOLES	-0.	5.87946E-04	3.68184E-04	2.47240E-03	4.34844E-03
	MFRAC	-0.	2.08497E-05	2.98441E-05	1.89730E-05	2.11315E-05
MG++	MOLES	-0.	6.32335E-05	9.33476E-05	2.65906E-04	5.13327E-02
	MFRAC	-0.	2.24237E-06	7.56654E-06	2.04054E-06	2.49455E-04
CL-	MOLES	-0.	5.16377E-02	1.57510E-02	2.50144E-01	7.29759E-03
	MFRAC	-0.	1.83117E-03	1.27674E-03	1.91959E-03	3.54632E-05
ORGAN-	MOLES	-0.	8.74223E-04	2.66663E-04	4.23493E-03	1.23548E-04
	MFRAC	-0.	3.10016E-05	2.16151E-05	3.24986E-05	6.00389E-07
HCO3-	MOLES	-0.	2.43145E-02	7.90879E-03	1.17785E-01	3.53442E-02
	MFRAC	-0.	8.62237E-04	6.41068E-04	9.03874E-04	1.71758E-04
H2CO3	MOLES	-0.	1.03664E-06	4.88476E-07	4.79040E-06	7.56472E-06
	MFRAC	-0.	3.67613E-08	3.95947E-08	3.67613E-08	3.67613E-08
CO3=	MOLES	-0.	5.71266E-05	1.29556E-05	2.90097E-04	1.65417E-05
	MFRAC	-0.	2.02581E-06	1.05015E-06	2.22619E-06	8.03854E-08
H2PO4-	MOLES	-0.	1.04698E-04	3.19359E-05	5.07181E-04	-0.
	MFRAC	-0.	3.71279E-06	2.58865E-06	3.89208E-06	-0.
HPO4=	MOLES	-0.	2.79645E-04	5.94733E-05	1.42008E-03	1.95449E-01
	MFRAC	-0.	9.91673E-06	4.82077E-06	1.08976E-05	9.49797E-04
SO4=	MOLES	-0.	3.15345E-04	6.70657E-05	1.60137E-03	3.82824E-02
	MFRAC	-0.	1.11827E-05	5.43619E-06	1.22888E-05	1.86036E-04
NH4+	MOLES	-0.	8.63684E-06	5.41936E-06	3.80730E-05	3.25439E-03
	MFRAC	-0.	3.06278E-07	4.39281E-07	2.92170E-07	1.58149E-05
NH3	MOLES	-0.	4.71177E-07	2.06135E-07	2.17734E-06	3.53661E-05
	MFRAC	-0.	1.67088E-08	1.67088E-08	1.67088E-08	1.71864E-07
UREA	MOLES	-0.	1.82166E-03	7.96955E-04	8.41801E-03	1.32932E-02
	MFRAC	-0.	6.45993E-05	6.45993E-05	6.45993E-05	6.45993E-05
GLUCOS	MOLES	-0.	2.63017E-03	1.15067E-03	1.21542E-02	1.91932E-02
	MFRAC	-0.	9.32705E-05	9.32705E-05	9.32705E-05	9.32705E-05
PROTN	MOLES	-0.	3.61095E-04	-0.	2.90446E-03	1.67264E-02
	MFRAC	-0.	1.28051E-05	-0.	2.22886E-05	8.12831E-05
X MISC	MOLES	-0.	-0.	1.37829E-03	-0.	1.66354E-01
	MFRAC	-0.	-0.	1.11721E-04	-0.	8.08408E-04
X-MISC	MOLES	-0.	-0.	6.44201E-03	-0.	-0.
	MFRAC	-0.	-0.	5.22174E-04	-0.	-0.
HB4	MOLES	-0.	-0.	1.34867E-04	-0.	-0.
	MFRAC	-0.	-0.	1.09320E-05	-0.	-0.
HB4O2	MOLES	-0.	-0.	1.19671E-04	-0.	-0.
	MFRAC	-0.	-0.	9.70026E-06	-0.	-0.
HB4O4	MOLES	-0.	-0.	2.16268E-04	-0.	-0.
	MFRAC	-0.	-0.	1.75301E-05	-0.	-0.
HB4O6	MOLES	-0.	-0.	3.57262E-05	-0.	-0.
	MFRAC	-0.	-0.	2.89588E-06	-0.	-0.
HB4O8	MOLES	-0.	-0.	9.56861E-04	-0.	-0.
	MFRAC	-0.	-0.	7.75609E-05	-0.	-0.

Table V

STANDARD DOG MODEL, ADDITION OF 25 ml/kg OF .2N HCl

		GAS PHASE	PLASMA	RED CELLS	INTERSTITIAL	INTRACELL
X-BAR		9.99924E 02	2.57972E 01	1.23273E 01	1.27479E 02	2.21324E 02
PH		-0.	7.11444E 00	7.03773E 00	7.13483E 00	6.69312E 00
O2	MOLES	5.26023E 01	2.40673E-05	1.87728E-05	1.18931E-04	2.06483E-04
	MFRAC	5.26063E-02	9.32945E-07	1.52286E-06	9.32945E-07	9.32945E-07
CO2	MOLES	6.04434E 01	6.77943E-04	3.45457E-04	3.35013E-03	5.81635E-03
	MFRAC	6.04480E-02	2.62798E-05	2.80237E-05	2.62798E-05	2.62798E-05
N2	MOLES	8.25797E 02	2.11547E-04	1.66667E-04	1.04538E-03	1.81494E-03
	MFRAC	8.25860E-01	8.20038E-06	1.35201E-05	8.20038E-06	8.20038E-06
H2O	MOLES	6.10812E 01	2.56563E 01	1.22600E 01	1.26783E 02	2.20116E 02
	MFRAC	6.10858E-02	9.94539E-01	9.94539E-01	9.94539E-01	9.94539E-01
H+	MOLES	-0.	3.57050E-08	2.03580E-08	1.68350E-07	8.08174E-07
	MFRAC	-0.	1.38407E-09	1.65146E-09	1.32060E-09	3.65154E-09
OH-	MOLES	-0.	1.44936E-07	5.80448E-08	7.50637E-07	4.71319E-07
	MFRAC	-0.	5.61830E-09	4.70864E-09	5.88830E-09	2.12954E-09
NA+	MOLES	-0.	6.72500E-02	2.62762E-02	3.17085E-01	2.74032E-02
	MFRAC	-0.	2.60688E-03	2.13154E-03	2.48734E-03	1.23815E-04
K+	MOLES	-0.	2.99313E-03	2.76042E-03	1.41126E-02	6.22851E-01
	MFRAC	-0.	1.16026E-04	2.23927E-04	1.10705E-04	2.81420E-03
CA++	MOLES	-0.	9.28722E-04	4.39652E-04	4.17815E-03	2.23044E-03
	MFRAC	-0.	3.60009E-05	3.56649E-05	3.27751E-05	1.00777E-05
MG++	MOLES	-0.	1.91529E-04	2.13740E-04	8.61651E-04	5.04883E-02
	MFRAC	-0.	7.42440E-06	1.73388E-05	6.75914E-06	2.28119E-04
CL-	MOLES	-0.	5.62190E-02	2.25149E-02	2.91163E-01	1.77739E-02
	MFRAC	-0.	2.17927E-03	1.82642E-03	2.28400E-03	8.03069E-05
ORGAN-	MOLES	-0.	7.97504E-04	3.19388E-04	4.13034E-03	2.52134E-04
	MFRAC	-0.	3.09144E-05	2.59090E-05	3.24001E-05	1.13921E-06
HCO3-	MOLES	-0.	7.01795E-03	2.99709E-03	3.63466E-02	2.28217E-02
	MFRAC	-0.	2.72044E-04	2.43126E-04	2.85117E-04	1.03114E-04
H2CO3	MOLES	-0.	9.48937E-07	4.88406E-07	4.68927E-06	8.14132E-06
	MFRAC	-0.	3.67846E-08	3.96198E-08	3.67846E-08	3.67846E-08
CO3=	MOLES	-0.	5.19900E-06	1.86080E-06	2.82200E-05	6.40824E-06
	MFRAC	-0.	2.01534E-07	1.50950E-07	2.21369E-07	2.89541E-08
H2PO4-	MOLES	-0.	7.85794E-05	3.14699E-05	4.06969E-04	-0.
	MFRAC	-0.	3.04605E-06	2.55286E-06	3.19243E-06	-0.
HPO4=	MOLES	-0.	6.61780E-05	2.22121E-05	3.59213E-04	1.96887E-01
	MFRAC	-0.	2.56532E-06	1.80186E-06	2.81781E-06	8.89586E-04
SO4=	MOLES	-0.	7.69134E-05	2.58154E-05	4.17484E-04	3.97459E-02
	MFRAC	-0.	2.98147E-06	2.09416E-06	3.27491E-06	1.79582E-04
NH4+	MOLES	-0.	1.38941E-05	7.92202E-06	6.55107E-05	3.23478E-03
	MFRAC	-0.	5.38589E-07	6.42639E-07	5.13893E-07	1.46156E-05
NH3	MOLES	-0.	2.38998E-07	1.14207E-07	1.18103E-06	2.10907E-05
	MFRAC	-0.	9.26452E-09	9.26452E-09	9.26452E-09	9.52933E-08
UREA	MOLES	-0.	1.62211E-03	7.75135E-04	8.01584E-03	1.39168E-02
	MFRAC	-0.	6.28795E-05	6.28795E-05	6.28795E-05	6.28795E-05
GLUCOS	MOLES	-0.	2.34206E-03	1.11916E-03	1.15735E-02	2.00935E-02
	MFRAC	-0.	9.07874E-05	9.07874E-05	9.07874E-05	9.07874E-05
PROTN	MOLES	-0.	3.61090E-04	-0.	2.90446E-03	1.67262E-02
	MFRAC	-0.	1.39973E-05	-0.	2.27838E-05	7.55731E-05
X MISC	MOLES	-0.	-0.	2.82172E-03	-0.	1.66354E-01
	MFRAC	-0.	-0.	2.28900E-04	-0.	7.51629E-04
X-MISC	MOLES	-0.	-0.	4.99858E-03	-0.	-0.
	MFRAC	-0.	-0.	4.05488E-04	-0.	-0.
HB4	MOLES	-0.	-0.	1.83203E-04	-0.	-0.
	MFRAC	-0.	-0.	1.48615E-05	-0.	-0.
HB4O2	MOLES	-0.	-0.	1.46578E-04	-0.	-0.
	MFRAC	-0.	-0.	1.18905E-05	-0.	-0.
HB4O4	MOLES	-0.	-0.	2.38849E-04	-0.	-0.
	MFRAC	-0.	-0.	1.93756E-05	-0.	-0.
HB4O6	MOLES	-0.	-0.	3.55772E-05	-0.	-0.
	MFRAC	-0.	-0.	2.88605E-06	-0.	-0.
HB4O8	MOLES	-0.	-0.	8.59184E-04	-0.	-0.
	MFRAC	-0.	-0.	6.96976E-05	-0.	-0.

III. MODEL OF FLUID AND ELECTROLYTE DISTRIBUTION IN A HYPOTHYROID DOG

The hypothyroid dog model was constructed by modifying the standard dog model according to hypotheses for the biochemical differences between euthyroid and hypothyroid animals. Previous work detailing some of those differences was described by Dörr [13,14].

Dörr observed that hypothyroid dogs reacted to an infusion of hypertonic $NaHCO_3$ solution with a significantly greater increase of their extracellular fluid volume than the same infusion results in euthyroid dogs. This observation, coupled with the evidence in the literature that hyaluronic acid accumulates in the extracellular-extravascular space of the hypothyroid, leads to the following hypothesis for the changes in body-fluid chemistry seen in this disease state: In the hypothyroid state the metabolism of the connective tissue cell is altered to produce an excess of the gel-like, polyelectrolyte hyaluronic acid that accumulates on the surfaces and in the vicinity of connective tissue. The gel, which is assumed to be pH sensitive, partially depolymerizes into a sol as pH increases and reversibly repolymerizes as pH decreases. This substance binds small ions and entraps water and electrolytes in interstices [42].

From this hypothesis, a new compartment called Colloid was added to the standard model; it consists of polyelectrolyte macromolecules and their associated ions and water. Studies [27,29] of various tissues that contain mucopolysaccharides have demonstrated that ions are bound chemically to these macromolecules, and that they also exist in close proximity because of the Gibbs-Donnan effect [25] of this nondiffusible, highly-charged particle existing in a fluid space. Therefore, a portion of the calcium, magnesium, and hydrogen ions in this compartment is hypothesized to be

chemically bound while the remainder of these ions--plus sodium, potassium, and chloride--are assumed freely diffusible. Table VI shows the concentrations of the bound and unbound ions in MEq and mM/liter of water in this compartment. These data for the ionic composition of various connective tissues were derived primarily from Engel's [27], Joseph's [29,30], and Catchpole's [31] articles. Values in the table for the unbound ions were calculated assuming a Gibbs-Donnan concentration gradient of 1.5:1 for positive ions (with respect to plasma). Engel [27], who also used data from L. Eichelberger, *et al.* [42], calculated this ratio to vary from 1.1 to 1.7 for certain types of connective tissues. Neither the molecular weight nor the osmotic effect of hyaluronic acid was known, so the species colloid, which was hypothesized to be mainly hyaluronic acid, was assigned a concentration (osmolarity) that would allow the colloid compartment to be iso-osmotic with plasma. The free (electrical) charge (unbound ionic sites) on the colloid molecule (species COLL) was determined by adding the charge of the free cations and anions in the compartment and assigning the species a charge (in MEq/liter of water) that would yield electroneutrality in the compartment. The total charge per molecule was -410, a value close to that found by Joseph [29,30]. The bound calcium and magnesium values were computed from data of Eichelberger [42], and the bound hydrogen made up the difference between the free charge and the total charge minus the bound magnesium and calcium. The volume of the colloid compartment was set equal to the CTW volume (Table X, p. 27) that was measured in the acid-infusion experiments. This isotope-measured volume is hypothesized to be analogous to the volume of the colloid space.

Twenty fragments (species MUCO) were assumed to result from the depolymerization of one molecule of the colloid. These fragments, which were hypothesized to be subunits of

Table VI

COMPOSITION OF THE HYPOTHESIZED COLLOID COMPARTMENT FOR A 10 kg HYPOTHYROID DOG

CHEMICAL SPECIES	CONCENTRATION	
	MEq/liter H_2O	mM/liter H_2O
Na^+	216.3	216.3
K^+	6.7	6.7
Ca^{++} (total)	47.0	23.5
Ca^{++} (unbound)	6.94	3.47
Mg^{++} (total)	21.46	10.73
Mg^{++} (unbound)	1.308	.654
Cl^-	80.1	80.1
COLL	151.	.491
MUCO (in interstitial compartment)	.55	.03
MUCO (in plasma compartment)	.35	.019
H_2O	.579 liters	
H^+ (bound)	31.2	31.2

hyaluronic acid, existed in the interstitial and the plasma compartments in the model. Each fragment that bound calcium, magnesium, and hydrogen was assigned a fixed charge, one-twentieth of the parent molecule. The small quantity of the fragments in the interstitial fluid and plasma before infusion were numerically determined to match the model prediction with the laboratory data for $NaHCO_3$ infusion. The computer results were extremely sensitive to these initial quantities.

Table VII shows the steady-state fluid and electrolyte distribution of the hypothyroid dog model, and Tables VIII and IX show this distribution after simulated infusion of 10 ml/kg of 0.892M $NaHCO_3$ and 25 ml/kg of .2N HCl. These results will be discussed in Sec. V.

Table VII

10 kg HYPOTHYROID DOG MODEL

		GAS PHASE	PLASMA	RED CELLS	INTERSTITIAL	COLLOID	INTRACELL
K-BAR		9.99876E 02	2.50228E 01	1.27103E 01	1.14351E 02	4.40530E 01	2.17786E 02
PH		-0.	7.35991E 00	7.22619E 00	7.38780E 00	7.19433E 00	6.79738E 00
O2	MOLES	5.26020E 01	2.33459E-05	1.93568E-05	1.06688E-04	-0.	2.03190E-04
	MFRAC	5.26085E-02	9.32984E-07	1.52293E-06	9.32984E-07	-0.	9.32984E-07
CO2	MOLES	6.04097E 01	6.57258E-04	3.56008E-04	3.00358E-03	-0.	5.72043E-03
	MFRAC	6.04172E-02	2.62664E-05	2.80094E-05	2.62664E-05	-0.	2.62664E-05
N2	MOLES	8.25797E 02	2.05206E-04	1.71853E-04	9.37767E-04	-0.	1.78601E-03
	MFRAC	8.25900E-01	8.20077E-06	1.35208E-05	8.20077E-06	-0.	8.20077E-06
H2O	MOLES	6.10671E 01	2.48816E 01	1.26386E 01	1.13706E 02	4.38045E 01	2.16557E 02
	MFRAC	6.10747E-02	9.94358E-01	9.94358E-01	9.94358E-01	9.94358E-01	9.94358E-01
H+	MOLES	-0.	1.98145E-08	1.36937E-08	8.49168E-08	5.10738E-08	6.29806E-07
	MFRAC	-0.	7.91857E-10	1.07737E-09	7.42598E-10	1.15937E-09	2.89186E-09
OH-	MOLES	-0.	2.45681E-07	9.17219E-08	1.19721E-06	2.95418E-07	5.85509E-07
	MFRAC	-0.	9.81831E-09	7.21635E-09	1.04696E-08	6.70596E-09	2.68847E-09
NA+	MOLES	-0.	6.97737E-02	3.30441E-02	2.99022E-01	1.79849E-01	3.99255E-02
	MFRAC	-0.	2.78841E-03	2.59979E-03	2.61495E-03	4.08255E-03	1.83325E-04
K+	MOLES	-0.	2.15298E-03	2.40671E-03	9.22681E-03	5.54952E-03	6.29142E-01
	MFRAC	-0.	8.60408E-05	1.89351E-04	8.06885E-05	1.25974E-04	2.88881E-03
CA++	MOLES	-0.	5.64767E-04	3.69518E-04	2.26980E-03	2.13138E-03	2.63656E-03
	MFRAC	-0.	2.25701E-05	2.90723E-05	1.98494E-05	4.83821E-05	1.21062E-05
MG++	MOLES	-0.	9.91413E-05	1.52915E-04	3.98449E-04	3.74150E-04	5.08012E-02
	MFRAC	-0.	3.96204E-06	1.20308E-05	3.48444E-06	8.49318E-06	2.33263E-04
CL-	MOLES	-0.	5.00706E-02	1.86932E-02	2.43995E-01	6.02071E-02	1.16012E-02
	MFRAC	-0.	2.00100E-03	1.47071E-03	2.13373E-03	1.36670E-03	5.32691E-05
ORGAN-	MOLES	-0.	8.48924E-04	3.16934E-04	4.13681E-03	-0.	1.96694E-04
	MFRAC	-0.	3.39260E-05	2.49352E-05	3.61765E-05	-0.	9.03153E-07
HCO3-	MOLES	-0.	1.18901E-02	4.73357E-03	5.79404E-02	-0.	2.83365E-02
	MFRAC	-0.	4.75170E-04	3.72420E-04	5.06689E-04	-0.	1.30112E-04
H2CO3	MOLES	-0.	9.19816E-07	5.03231E-07	4.20344E-06	-0.	8.00561E-06
	MFRAC	-0.	3.67591E-08	3.95924E-08	3.67591E-08	-0.	3.67591E-08
CO3=	MOLES	-0.	1.53959E-05	4.50494E-06	8.00009E-05	-0.	1.00469E-05
	MFRAC	-0.	6.15274E-07	3.54433E-07	6.99608E-07	-0.	4.61322E-08
H2PO4-	MOLES	-0.	8.47553E-05	3.16422E-05	4.13013E-04	-0.	-0.
	MFRAC	-0.	3.38712E-06	2.48950E-06	3.61180E-06	-0.	-0.
HPO4=	MOLES	-0.	1.24762E-04	3.42345E-05	6.48295E-04	-0.	1.96515E-01
	MFRAC	-0.	4.98594E-06	2.69345E-06	5.66935E-06	-0.	9.02333E-04
SO4=	MOLES	-0.	1.43776E-04	3.94520E-05	7.47098E-04	-0.	3.93358E-02
	MFRAC	-0.	5.74582E-06	3.10394E-06	6.53338E-06	-0.	1.80617E-04
NH4+	MOLES	-0.	9.96083E-06	6.88390E-06	4.26881E-05	-0.	3.25657E-03
	MFRAC	-0.	3.98070E-07	5.41601E-07	3.73308E-07	-0.	1.49531E-05
NH3	MOLES	-0.	2.99483E-07	1.52122E-07	1.36860E-06	-0.	2.68105E-05
	MFRAC	-0.	1.19684E-08	1.19684E-08	1.19684E-08	-0.	1.23105E-07
UREA	MOLES	-0.	1.64599E-03	8.36077E-04	7.52196E-03	-0.	1.43258E-02
	MFRAC	-0.	6.57795E-05	6.57795E-05	6.57795E-05	-0.	6.57795E-05
GLUCOS	MOLES	-0.	2.37653E-03	1.20715E-03	1.08604E-02	-0.	2.06841E-02
	MFRAC	-0.	9.49745E-05	9.49745E-05	9.49745E-05	-0.	9.49745E-05
PROTN	MOLES	-0.	4.46174E-04	-0.	3.37911E-03	-0.	1.67265E-02
	MFRAC	-0.	1.78307E-05	-0.	2.95504E-05	-0.	7.68025E-05
MUCO	MOLES	-0.	3.72456E-05	-0.	4.05034E-04	-0.	-0.
	MFRAC	-0.	1.48847E-06	-0.	3.54203E-06	-0.	-0.
X MISC	MOLES	-0.	-0.	2.10483E-03	-0.	-0.	1.67445E-01
	MFRAC	-0.	-0.	1.65601E-04	-0.	-0.	7.68852E-04
X-MISC	MOLES	-0.	-0.	5.71547E-03	-0.	-0.	-0.
	MFRAC	-0.	-0.	4.49672E-04	-0.	-0.	-0.
HB4	MOLES	-0.	-0.	1.33901E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	1.05348E-05	-0.	-0.	-0.
HB4O2	MOLES	-0.	-0.	1.19093E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	9.36983E-06	-0.	-0.	-0.
HB4O4	MOLES	-0.	-0.	2.15729E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	1.69728E-05	-0.	-0.	-0.
HB4O6	MOLES	-0.	-0.	3.57208E-05	-0.	-0.	-0.
	MFRAC	-0.	-0.	2.81038E-06	-0.	-0.	-0.
HB4O8	MOLES	-0.	-0.	9.58961E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	7.54476E-05	-0.	-0.	-0.
COLL	MOLES	-0.	-0.	-0.	-0.	4.22724E-04	-0.
	MFRAC	-0.	-0.	-0.	-0.	9.59581E-06	-0.

Table VIII

10 kg HYPOTHYROID DOG MODEL AFTER ADDITION OF 10 ml/kg OF 0.892M $NaHCO_3$

		GAS PHASE	PLASMA	RED CELLS	INTERSTITIAL	COLLOID	INTRACELL
X-BAR		9.99885E 02	3.23078E 01	1.26770E 01	1.43336E 02	2.60962E 01	2.06574E 02
PH		-0.	7.57337E 00	7.41698E 00	7.59017E 00	7.39224E 00	6.90088E 00
O2	MOLES	5.26019E 01	3.01424E-05	1.93060E-05	1.33729E-04	-0.	1.92728E-04
	MFRAC	5.26080E-02	9.32975E-07	1.52291E-06	9.32975E-07	-0.	9.32975E-07
CO2	MOLES	6.04410E 01	8.49040E-04	3.55256E-04	3.76682E-03	-0.	5.42871E-03
	MFRAC	6.04479E-02	2.62797E-05	2.80237E-05	2.62797E-05	-0.	2.62797E-05
N2	MOLES	8.25797E 02	2.64946E-04	1.71402E-04	1.17545E-03	-0.	1.69405E-03
	MFRAC	8.25892E-01	8.20070E-06	1.35207E-05	8.20070E-06	-0.	8.20070E-06
H2O	MOLES	6.10451E 01	3.21136E 01	1.26008E 01	1.42474E 02	2.59394E 01	2.05333E 02
	MFRAC	6.10521E-02	9.93990E-01	9.93990E-01	9.93991E-01	9.93990E-01	9.93990E-01
H+	MOLES	-0.	1.56491E-08	8.80225E-09	6.67942E-08	1.91822E-08	4.70708E-07
	MFRAC	-0.	4.84376E-10	6.94347E-10	4.65998E-10	7.35055E-10	2.27864E-09
OH-	MOLES	-0.	5.18379E-07	1.41894E-07	2.39052E-06	2.75919E-07	7.04567E-07
	MFRAC	-0.	1.60450E-08	1.11930E-08	1.66778E-08	1.05731E-08	3.41072E-09
NA+	MOLES	-0.	9.88706E-02	3.81096E-02	4.22004E-01	1.21192E-01	5.35381E-02
	MFRAC	-0.	3.06027E-03	3.00619E-03	2.94416E-03	4.64405E-03	2.59171E-04
K+	MOLES	-0.	2.28389E-03	2.07789E-03	9.74818E-03	2.79951E-03	6.31568E-01
	MFRAC	-0.	7.06915E-05	1.63910E-04	6.80095E-05	1.07277E-04	3.05734E-03
CA++	MOLES	-0.	6.02251E-04	3.37896E-04	2.47303E-03	1.12027E-03	3.42724E-03
	MFRAC	-0.	1.86410E-05	2.66542E-05	1.72534E-05	4.29284E-05	1.65908E-05
MG++	MOLES	-0.	8.18847E-05	1.08302E-04	3.36244E-04	1.52317E-04	5.11471E-02
	MFRAC	-0.	2.53452E-06	8.54319E-06	2.34585E-06	5.83673E-06	2.47597E-04
CL-	MOLES	-0.	5.87155E-02	1.60720E-02	2.70768E-01	3.12526E-02	7.75868E-03
	MFRAC	-0.	1.81738E-03	1.26780E-03	1.88905E-03	1.19759E-03	3.75588E-05
ORGAN-	MOLES	-0.	9.13912E-04	2.50161E-04	4.21453E-03	-0.	1.20764E-04
	MFRAC	-0.	2.82877E-05	1.97334E-05	2.94032E-05	-0.	5.84606E-07
HCO3-	MOLES	-0.	2.51004E-02	7.32656E-03	1.15751E-01	-0.	3.41158E-02
	MFRAC	-0.	7.76915E-04	5.77940E-04	8.07553E-04	-0.	1.65150E-04
H2CO3	MOLES	-0.	1.18777E-06	5.01984E-07	5.26962E-06	-0.	7.59454E-06
	MFRAC	-0.	3.67642E-08	3.95979E-08	3.67642E-08	-0.	3.67642E-08
CO3=	MOLES	-0.	5.31331E-05	1.08191E-05	2.54687E-04	-0.	1.53513E-05
	MFRAC	-0.	1.64459E-06	8.53441E-07	1.77686E-06	-0.	7.43138E-08
H2PO4-	MOLES	-0.	1.16487E-04	3.18855E-05	5.37182E-04	-0.	-0.
	MFRAC	-0.	3.60554E-06	2.51522E-06	3.74772E-06	-0.	-0.
HPO4=	MOLES	-0.	2.80322E-04	5.35277E-05	1.34369E-03	-0.	1.95489E-01
	MFRAC	-0.	8.67661E-06	4.22242E-06	9.37444E-06	-0.	9.46337E-04
SO4=	MOLES	-0.	3.16771E-04	6.04877E-05	1.51841E-03	-0.	3.83705E-02
	MFRAC	-0.	9.80479E-06	4.77144E-06	1.05934E-05	-0.	1.85747E-04
NH4+	MOLES	-0.	1.04937E-05	5.90246E-06	4.47897E-05	-0.	3.24662E-03
	MFRAC	-0.	3.24804E-07	4.65603E-07	3.12481E-07	-0.	1.57165E-05
NH3	MOLES	-0.	5.15786E-07	2.02386E-07	2.28832E-06	-0.	3.39217E-05
	MFRAC	-0.	1.59648E-08	1.59648E-08	1.59648E-08	-0.	1.64211E-07
UREA	MOLES	-0.	1.99052E-03	7.81044E-04	8.83105E-03	-0.	1.27272E-02
	MFRAC	-0.	6.16110E-05	6.16110E-05	6.16110E-05	-0.	6.16110E-05
GLUCOS	MOLES	-0.	2.87397E-03	1.12770E-03	1.27505E-02	-0.	1.83760E-02
	MFRAC	-0.	8.89559E-05	8.89559E-05	8.89559E-05	-0.	8.89559E-05
PROTN	MOLES	-0.	4.46168E-04	-0.	3.37911E-03	-0.	1.67263E-02
	MFRAC	-0.	1.38099E-05	-0.	2.35748E-05	-0.	8.09699E-05
MUCO	MOLES	-0.	3.56082E-04	-0.	2.35360E-03	-0.	-0.
	MFRAC	-0.	1.10216E-05	-0.	1.64202E-05	-0.	-0.
X MISC	MOLES	-0.	-0.	1.50006E-03	-0.	-0.	1.67445E-01
	MFRAC	-0.	-0.	1.18329E-04	-0.	-0.	8.10580E-04
X-MISC	MOLES	-0.	-0.	6.32024E-03	-0.	-0.	-0.
	MFRAC	-0.	-0.	4.98559E-04	-0.	-0.	-0.
HB4	MOLES	-0.	-0.	1.29580E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	1.02216E-05	-0.	-0.	-0.
HB4O2	MOLES	-0.	-0.	1.16484E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	9.18861E-06	-0.	-0.	-0.
HB4O4	MOLES	-0.	-0.	2.13261E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	1.68226E-05	-0.	-0.	-0.
HB4O6	MOLES	-0.	-0.	3.56903E-05	-0.	-0.	-0.
	MFRAC	-0.	-0.	2.81535E-06	-0.	-0.	-0.
HB4O8	MOLES	-0.	-0.	9.68393E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	7.63896E-05	-0.	-0.	-0.
COLL	MOLES	-0.	-0.	-0.	-0.	3.09372E-04	-0.
	MFRAC	-0.	-0.	-0.	-0.	1.18550E-05	-0.

Table IX

10 kg HYPOTHYROID DOG MODEL AFTER ADDITION OF 25 ml/kg OF .2N HCl

		GAS PHASE	PLASMA	RED CELLS	INTERSTITIAL	COLLOID	INTRACELL
X-BAR		9.99923E 02	2.45695E 01	1.24963E 01	1.18778E 02	5.48327E 01	2.20616E 02
PH		-0.	7.14081E 00	7.04136E 00	7.16964E 00	6.99231E 00	6.69766E 00
O2	MOLES	5.26023E 01	2.29220E-05	1.90302E-05	1.10813E-04	-0.	2.05823E-04
	MFRAC	5.26064E-02	9.32946E-07	1.52286E-06	9.32946E-07	-0.	9.32946E-07
CO2	MOLES	6.04435E 01	6.45681E-04	3.50193E-04	3.12146E-03	-0.	5.79776E-03
	MFRAC	6.04482E-02	2.62798E-05	2.80238E-05	2.62798E-05	-0.	2.62798E-05
N2	MOLES	8.25797E 02	2.01479E-04	1.68952E-04	9.74024E-04	-0.	1.80914E-03
	MFRAC	8.25861E-01	8.20039E-06	1.35202E-05	8.20039E-06	-0.	8.20039E-06
H2O	MOLES	6.10798E 01	2.44348E 01	1.24278E 01	1.18127E 02	5.45321E 01	2.19407E 02
	MFRAC	6.10845E-02	9.94518E-01	9.94518E-01	9.94518E-01	9.94518E-01	9.94518E-01
H+	MOLES	-0.	3.22212E-08	2.06053E-08	1.45765E-07	1.01224E-07	8.02670E-07
	MFRAC	-0.	1.31143E-09	1.64892E-09	1.22721E-09	1.84606E-09	3.63830E-09
OH-	MOLES	-0.	1.45681E-07	5.89299E-08	7.52611E-07	2.30965E-07	4.71510E-07
	MFRAC	-0.	5.92935E-09	4.71579E-09	6.33629E-09	4.21218E-09	2.13724E-09
NA+	MOLES	-0.	6.50759E-02	2.85182E-02	2.94396E-01	2.04439E-01	2.91843E-02
	MFRAC	-0.	2.64865E-03	2.28213E-03	2.47855E-03	3.72842E-03	1.32285E-04
K+	MOLES	-0.	2.71645E-03	2.80985E-03	1.22889E-02	8.53385E-03	6.22129E-01
	MFRAC	-0.	1.10562E-04	2.24855E-04	1.03461E-04	1.55634E-04	2.81996E-03
CA++	MOLES	-0.	6.13636E-04	3.43329E-04	2.59774E-03	2.71366E-03	1.70558E-03
	MFRAC	-0.	2.49756E-05	2.74744E-05	2.18706E-05	4.94898E-05	7.73097E-06
MG++	MOLES	-0.	1.63978E-04	2.16279E-04	6.94177E-04	7.25154E-04	5.00263E-02
	MFRAC	-0.	6.67407E-06	1.73075E-05	5.84433E-06	1.32248E-05	2.26757E-04
CL-	MOLES	-0.	5.28178E-02	2.13655E-02	2.72865E-01	8.37383E-02	1.66199E-02
	MFRAC	-0.	2.14973E-03	1.70975E-03	2.29727E-03	1.52716E-03	7.53340E-05
ORGAN-	MOLES	-0.	7.98707E-04	3.23088E-04	4.12625E-03	-0.	2.51325E-04
	MFRAC	-0.	3.25081E-05	2.58547E-05	3.47392E-05	-0.	1.13920E-06
HCO3-	MOLES	-0.	7.05403E-03	3.04280E-03	3.64423E-02	-0.	2.28311E-02
	MFRAC	-0.	2.87106E-04	2.43496E-04	3.06810E-04	-0.	1.03488E-04
H2CO3	MOLES	-0.	9.03760E-07	4.95092E-07	4.36911E-06	-0.	8.11513E-06
	MFRAC	-0.	3.67839E-08	3.96191E-08	3.67839E-08	-0.	3.67839E-08
CO3=	MOLES	-0.	5.51516E-06	1.89209E-06	3.04477E-05	-0.	6.43417E-06
	MFRAC	-0.	2.24472E-07	1.51412E-07	2.56341E-07	-0.	2.91645E-08
H2PO4-	MOLES	-0.	7.86529E-05	3.18162E-05	4.06333E-04	-0.	-0.
	MFRAC	-0.	3.20125E-06	2.54605E-06	3.42095E-06	-0.	-0.
HPO4=	MOLES	-0.	6.99087E-05	2.24911E-05	3.85947E-04	-0.	1.96857E-01
	MFRAC	-0.	2.84535E-06	1.79982E-06	3.24931E-06	-0.	8.92303E-04
SO4=	MOLES	-0.	8.11896E-05	2.61205E-05	4.48225E-04	-0.	3.97106E-02
	MFRAC	-0.	3.30450E-06	2.09026E-06	3.77364E-06	-0.	1.79998E-04
NH4+	MOLES	-0.	1.26604E-05	8.09629E-06	5.72742E-05	-0.	3.24402E-03
	MFRAC	-0.	5.15290E-07	6.47895E-07	4.82197E-07	-0.	1.47043E-05
NH3	MOLES	-0.	2.29839E-07	1.16899E-07	1.11113E-06	-0.	2.12279E-05
	MFRAC	-0.	9.35468E-09	9.35468E-09	9.35468E-09	-0.	9.62207E-08
UREA	MOLES	-0.	1.58787E-03	8.07611E-04	7.67637E-03	-0.	1.42580E-02
	MFRAC	-0.	6.46280E-05	6.46280E-05	6.46280E-05	-0.	6.46280E-05
GLUCOS	MOLES	-0.	2.29262E-03	1.16605E-03	1.10834E-02	-0.	2.05861E-02
	MFRAC	-0.	9.33119E-05	9.33119E-05	9.33119E-05	-0.	9.33119E-05
PROTN	MOLES	-0.	4.46167E-04	-0.	3.37911E-03	-0.	1.67264E-02
	MFRAC	-0.	1.81594E-05	-0.	2.84490E-05	-0.	7.58168E-05
MUCO	MOLES	-0.	4.51125E-06	-0.	5.39866E-05	-0.	-0.
	MFRAC	-0.	1.83612E-07	-0.	4.54518E-07	-0.	-0.
X MISC	MOLES	-0.	-0.	2.81894E-03	-0.	-0.	1.67445E-01
	MFRAC	-0.	-0.	2.25582E-04	-0.	-0.	7.58986E-04
X-MISC	MOLES	-0.	-0.	5.00136E-03	-0.	-0.	-0.
	MFRAC	-0.	-0.	4.00227E-04	-0.	-0.	-0.
HB4	MOLES	-0.	-0.	1.82936E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	1.46392E-05	-0.	-0.	-0.
HB4O2	MOLES	-0.	-0.	1.46440E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	1.17186E-05	-0.	-0.	-0.
HB4O4	MOLES	-0.	-0.	2.38746E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	1.91053E-05	-0.	-0.	-0.
HB4O6	MOLES	-0.	-0.	3.55800E-05	-0.	-0.	-0.
	MFRAC	-0.	-0.	2.84724E-06	-0.	-0.	-0.
HB4O8	MOLES	-0.	-0.	8.59691E-04	-0.	-0.	-0.
	MFRAC	-0.	-0.	6.87957E-05	-0.	-0.	-0.
COLL	MOLES	-0.	-0.	-0.	-0.	4.41927E-04	-0.
	MFRAC	-0.	-0.	-0.	-0.	8.05955E-06	-0.

IV. LABORATORY RESULTS AND DISCUSSION OF VALIDATION

LABORATORY VALIDATION OF THE STANDARD DOG MODEL

Table X illustrates averaged laboratory results for standard (euthyroid) and hypothyroid dogs before infusion compared to the values used in the models (Tables III, VII, pp. 13,23). Table XI shows the percent changes of the critical variables due to fluid and electrolyte infusion as measured in the work of Bradham [10], Dörr [13,14], and Wolf [16] and in the laboratory data of R. C. Swan [15,43], compared to the standard dog model predictions. Percent changes were used in the comparison to avoid problems of normalizing volume changes for the weight of animals.

The comparative results of Table XI are encouraging considering the various measurement methodologies and calculations made by the individual investigators. The measurements that have the minimum variation because of technique are the plasma chemistries; these show good agreement with the model, especially for sodium and slightly less for chloride, for Dörr's, Swan's and Wolf's work. The discrepancies in potassium prediction could be due to the much longer time required for its intracellular-extracellular equilibration.

The extracellular (ECW) and intracellular water (ICW) volumes, as defined in this Memorandum, are the most important water distributions to examine since roughly 80 percent of the water in the body exists in these two compartments. Changes here agree excelently with Dörr's results for $NaHCO_3$ infusion. Agreement with Bradham for $NaHCO_3$ and NaCl infusions, while not as good, is reasonable considering the large standard deviation of his laboratory methods [10]. Part of the discrepancy between model prediction and experimental measurement for NaCl infusion resulted from Bradham's use of radiosulphate in the measurement of the ECW space instead of radiosucrose, on which the

Table X

SUMMARIZED MEASURED BODY COMPOSITION BEFORE INFUSION WITH COMPARISON TO COMPUTER MODELS

QUANTITY	LABORATORY DATA		COMPUTER MODELS	
	Standard	Hypothyroid	Standard	Hypothyroid
RCW	23.3±4.83[a]	18.9±3.77	23.2	18.9
PW	35.5±6.04	35.2±3.47	35.5	35.2
ISW	124.±18.1	121.±16.7	124.	121.
ECW	161.±19.0	156.±16.4	159.5	156.2
ECEVW	197.±17.9	180.±20.3	--	184.5
CTW	71.7±19.1	57.9±12.8	--	57.9
ICW	404.±53.5	373.±24.8	404.	373.
TBW	645.±46.7	605.±31.6	645.	605.
$[NA]_{RCW}$	153.±7.45[b]	159.±7.13	134.	134.
$[K]_{RCW}$	8.17±.658	9.09±.798	9.92	9.92
$[CL]_{RCW}$	83.9±9.38	81.4±8.33	88.8	88.8
$[NA]_{PW}$	154.±4.12	153.±4.38	154.	154.
$[K]_{PW}$	4.19±.528	4.26±.734	4.83	4.83
$[CL]_{PW}$	116.±3.37	118.±2.66	111.1	111.1
PH	7.30±.051	7.32±.105	7.30	7.32
PCO2	43.6±6.98[c]	36.6±6.55	43.6	36.6

[a]The body spaces are expressed as mean±one standard deviation with units of ml/kg of body weight.

[b]The blood chemistry data is expressed as mean±one standard deviation with units of MEq/liter of water.

[c]PCO2 is expressed in mm Hg.

Table XI

COMPARISON OF LABORATORY AND COMPUTER SIMULATION RESULTS FOR VARIOUS FLUID AND ELECTROLYTE INFUSIONS IN STANDARD LABORATORY DOGS

VARIABLE	FLUID AND ELECTROLYTE INFUSION										
	NaCl		$NaHCO_3$			$NaHCO_3$		HCl		HCl	
	10 ml/kg .892M		10 ml/kg .892M			76.8 ml/kg .303M		32.5 ml/kg .3M		25 ml/kg .2N	
	Bradham	Model	Bradham	Dörr	Model	Swan	Model	Swan	Model	Wolf	Model
	(5)[a]		(5)	(9)		(1)		(1)		(9)	
ECW[b]	+18.	+29.	+12.5	+17.4	+17.9	+32.	+60.	+15.5	+20		
ICW	-6.5	-8.7	-3.0	-4.8	-4.5				-0.2	+.06[c]	+1.7
ISW										+5.9[c]	+14.
RCV	(c)		-8.								
RCW		-7.6		-12.1	-0.8	+18[e]	+5.8	+17[e]	+10.0	+5.4	+8.1
PV	+22.[d]		+23.								
PW		+18.1		+30.	+15.2	+22.5[e]	+47.	+2[e]	+5.7	-6.1[c]	+6.8
$[NA]_{PW}$				+8.1	+7.8	+9.5	+10.	-7.5	-8.0	-2.1[c]	-5.1
$[K]_{PW}$						-21.	-34.5	+125.	+190.	+63	+36
$[CL]_{PW}$						-24.2	-31.	+12.5	+15.8	+6.3	+8.1

[a]The numbers in ( ) refer to the number of animals tested.

[b]The notation for variable names is given on p. 53.

[c]Laboratory measurement not significantly different from zero change.

[d]All values are expressed as percent change from preinfusion measurements.

[e]Calculated data from plasma volume and hematocrit.

compartment sizes in our model were based. Radiosulphate, as opposed to radiosucrose, penetrates dense connective tissue and cells in the time of measurement [34,44] and thus measures about a 25 percent greater volume for the ECW space. Thus, when the magnitude of the distributional changes predicted by the model due to NaCl infusion, for example, is converted to percentage change from preinfusion period, a greater value is predicted than the laboratory results would show. Swan also used radiosulphate for his measurements and, as expected, the model again predicted greater percentage changes in the ECW than his laboratory results indicated. This difference was especially evident for the alkaline $NaHCO_3$ infusion in which the magnitude of the changes was much greater than for acid infusion. If it were feasible to construct a model to compare the individual laboratory experiments, one would expect that these discrepancies would disappear since absolute changes would be compared. However, at present insufficient laboratory data constrains the modeling of individual animals.

Comparison of red-cell and plasma-volume measurements with the model predictions shows similar directional changes but poor quantitative agreement; however, a number of methodological problems exist in these measurements. Swan [15] has noted that infusion of highly concentrated $NaHCO_3$ is poorly tolerated in dogs, producing extensive physiological changes that include hypotension. P. H. Sherman [45] observed that in endotoxin shock and its resulting hypotension, red cells are sequestered in the splanchnic circulation, decreasing the large-vessel hematocrit. Thus, in hypotensive dogs an apparently lower red-cell volume as well as a higher plasma volume would be measured (shown in both Bradham's and Dörr's $NaHCO_3$ results when compared to the model). The results of Swan's PW measurement in only one dog is suspect since, for example, the Evans Blue dye measurements could have been either back-extrapolated

or taken from a single sample. The single-sample technique depends critically upon equilibration time being the same for the dye both before and after the infusion [46].

The quantities that Swan measured in his acid-infusion experiments showed reasonable agreement with the model, considering the criticism of his PW measuring technique and the limited nature of his experiment (only one dog was used). Quantities measured by Wolf (Table XIII, App. A, p. 41) that had changes of a large enough magnitude to be statistically significant also show general agreement with the model.

RESULTS OF INFUSION EXPERIMENTS IN HYPOTHYROID DOGS AND VALIDATION OF THE HYPOTHYROID MODEL

Table XII presents a summary of Dörr's and Wolf's laboratory results (cf. Table XIII, p. 41) for infusions in hypothyroid dogs and shows comparison to the model. While generally the agreement between model and laboratory data is quite good in this table for $NaHCO_3$ infusion, a direct comparison for HCl infusion is not possible because of the statistical nature of the laboratory data. However, it is conclusive that the two groups of animals respond differently to HCl infusion and that the models simulating the euthyroid and hypothyroid states predict these differences reasonably well.

The red-cell water data indicate clearly that the model does not yet contain an adequate mathematical description of dog red cells. The unique characteristics of dog red cells are not well known, but this cell type is known to vary considerably from animal to animal, at least as evidenced by the variation in cation gradients. A subsequent study will attempt to elucidate the comparative blood biochemistry.

The laboratory data seem to justify the hypothesis that the connective-tissue colloid, which is characteristic

Table XII

COMPARISON OF LABORATORY AND COMPUTER SIMULATION RESULTS FOR ALKALINE AND ACID FLUID AND ELECTROLYTE INFUSIONS IN HYPOTHYROID DOGS

VARIABLE	FLUID AND ELECTROLYTE INFUSION[a]					
	$NaHCO_3$		HCl		Difference Between Hypothyroid and Euthyroid Responses	
	10 ml/kg .892M		25 ml/kg .2N			
	Dörr	Model	Wolf	Model	Wolf	Model
RCW	-8.2	+7.0	+6.6	-3.0	(b)	-11.1
PW	+27	+25.4	-.5[b]	+1.9	+5.57[c]	+4.9
ISW		+26.1	-4.8[b]	+6.6	-10.72[c]	-7.4
ECW	+24.8	+27.0	-3.7[b]	+5.5	(d)	-6.5
CTW		-31.2	+53[b]	+23.5	+17.9[b]	+23.5
ICW	-7.4	-4.1	+.3[b]	+0.8	(b)	-0.9

[a]Values are expressed as percent change from preinfusion measurement or difference in percent change when two groups are compared.

[b]Percent change not statistically significant (see Table XIII, App. A, p. 41).

[c]Statistically significant ($p < .05$).

[d]Not computed.

of myxedema, is stable under decreasing pH but decomposes at alkaline pH. Further careful experiments will be required to test this conjecture. The movement of water in the mathematical model--primarily into the connective tissue space--under acid loading primarily occurs because of the changing charge on the colloid as the mucopolysaccaride titrates. Charge neutrality causes a simultaneous shift of electrolytes and, consequently, water. Possibly for the real colloid, as contrasted to the mathematical model of it, HCl loading does not significantly alter this fixed charge.

The experimental results of Table XII do not demonstrate conclusively the validity of the hypotheses upon which the mathematical model was built; conclusive experiments are difficult to design in this case. However, the model, which has been derived from the literature, appears to satisfy the qualitative, if not precisely the quantitative, character of the experimental data.

V. DISCUSSION

MODELING

The objectives of this research were to construct and validate a mathematical model of fluid and electrolyte distribution in a standard laboratory dog and then to add sufficient detail to the model to simulate a disease state: hypothyroidism. The construction of the standard dog model was based on previous techniques and published data. The hypothyroid dog model consisted of the standard dog model and an additional fluid compartment (colloid) in which the necessary chemical species and reactions were hypothesized to occur.

Validation of such models requires considerable laboratory testing. The model must not only predict changes in the chemical distribution quantitatively for every arbitrary perturbation or stress (such as fluid and electrolyte additions), but satisfy other criteria as well. This involves performing laboratory experiments on animals with each practical perturbation and comparing the results to those predicted by the model. Since no two animals react identically under similar experimental conditions, laboratory verification requires many experiments of the same type with subsequent statistical evaluation of the data. In the present research, the perturbations considered were $NaHCO_3$, NaCl, and HCl infusions.

The results of Table XI (p. 28) establish the validity of the normal dog model. Although the results of Table XII (p. 31) are not conclusive proof of the hypothyroid model's validity, they do establish some confidence in that conclusion.

ASSUMPTIONS IN CONSTRUCTING THE HYPOTHYROID MODEL

The following evidence argues for the existence of a sol-gel state in the extracellular space. T. C. Laurent [47]

recognized that hyaluronic acid solutions began to gel at concentrations as low as .01 percent and that the molecules began to fill the whole solution and entangle. At higher concentrations a three-dimensional polymer occurred. In the connective tissues the collagen of the ground substance stabilized this polymer network, and a stable gel was formed that inhibited the diffusion of colloidal material. S. Glasstone [48] discussed the changes of sol-gel equilibrium due to changes in electrolyte concentration in the solution --particularly of the lyophilic sol that was formed from a solution of starch or protein of high molecular weight and high viscosity. Thus, the fluid in the extracellular-extravascular space of the hypothyroid is conceivably in a sol-gel equilibrium, a state that may be sensitive to pH and to small changes in electrolyte concentrations.

Since the measured concentration of macromolecular material in the plasma (other than plasma proteins) is relatively low, the assumption is reasonable that depolymerized fragments of hyaluronic acid return to the plasma only by way of lymph; thus, a gradient for the fragments--opposite to the albumen gradient across the vascular membrane--may exist between plasma and interstitial fluid. This also means that the molecular weight of the fragments must be at least 50,000 to 75,000 or they would diffuse into the plasma directly through the vascular membrane, achieving equal concentration on both sides. Therefore, the factor 20 was chosen for the degree of fragmentation so that the parent molecule would have a molecular weight approximately that of a molecule of hyaluronic acid (about one million) [25]; however, this factor was reasonably arbitrary since the molecular weight of hyaluronic acid was not precisely known.

The fragments formed were assumed to bind calcium and magnesium in the same proportion as the parent molecule. These tightly bound ions, being doubly valent, supposedly remained attached when the polymer split. The hydrogen-

binding coefficient of these fragments was one parameter adjusted to allow the model to fit laboratory data. The initial concentrations of the fragments in the interstitial fluid and plasma constituted the other parameters. The initial amounts of the fragments were taken to be much less than the amounts of protein in these compartments. Other combinations of the parameters, or additions of such phenomena as sodium binding by the macromolecule, could produce possibly another model that would be equally predictive of the changes induced by acid addition; therefore, uniqueness of the model is still open to question. The research suggests that additional experiments will be required for verification of the hypotheses.

This model research ignores the mechanism of thyroxine in changing the normal state to the hypothyroid state. In the hypothyroid model chemical reactions could be included in which an equilibrium constant is generated between thyroxine concentration in the extracellular fluid and the amount of the species colloid in the colloid compartment; however, little reason exists for hypothesizing a particular mechanism. Simulating the effect of thyroxine in this way introduces a kinetic phenomena--a time-dependent state--that could be simulated by a sequence of the system's new steady states. Since the polymerization is pH sensitive, then possibly thyroxine may operate by changing the pH in the environment of the macromolecule. This research supports suggestions in the literature that an important part of the acid-base regulatory system for the whole body resides with the extracellular fluid macromolecules and their ability to bind and release hydrogen and other ionic species.

DISCUSSION OF LABORATORY METHODOLOGY (SEE APPENDIX B)

The experimental results should be interpreted in terms of the methodology of the experiments, and the assumptions interpreted in terms of current thinking and research.

Comparison of Isotope-Measured and Anatomically-Observed Spaces

The validity of comparing anatomic and isotope spaces in this research presents interesting speculation. During the sampling period (1.5 to 2.5 hr), the sucrose molecule is assumed not to enter cells and to equilibrate only in interstitial fluid space, lymph, and portions of the connective tissue and bone. The following men have confirmed the validity of this assumption: 1) Cizek [49] showed that sucrose measures no gut water and very little of the cerebrospinal or other transcellular fluids [37]; 2) Keith [50] stated that sucrose does not enter red cells; and 3) E. Cotlove [51] and Nichols [34] demonstrated that sucrose slowly enters connective tissue. Thus, its plasma disappearance curve in the 1.5-to-2.5-hr period is primarily due to this slow penetration of the connective tissue. The extrapolation to injection time ideally gives a volume of distribution exclusive of this part of the tissue. In the hypothyroid, the gel-like hyaluronic acid polymer would impede diffusion of saccharides [47]; thus, the volume measured by sucrose probably includes little of this space.

The bromide ion would equilibrate in the gel-colloid space formed in hypothyroidism, since the ion rapidly penetrates dense connective tissue. Therefore, the CTW isotope space, which is the difference between the volumes measured by bromide and sucrose, is assumed to reflect volume changes of the anatomic gel-colloid space as a consequence of the acid infusion.

In addition, definite evidence exists that the bromide ion not only equilibrates rapidly with erythrocytes [52], but also with connective tissue, some transcellular fluids [44], and parts of bone. It equilibrates with the gut fluid [44] since it is selectively secreted into the stomach. The bromide ion distribution in the body essentially follows that of the chloride ion [53,54]; thus its concentration in

the body cells is very low. The bromide ion essentially distributes in the total extracellular space and part of the transcellular space in the 1.5-to-2.5-hr sampling period. Extrapolating the plasma disappearance curve to zero time compensates for the effect of its slow rate of entry into cells during this time period.

Hemolysis Due to Acid Infusion and the Effect on Radioactivity Determination

The acid infused was of sufficient concentration to produce a non-fatal acidosis in the normal animals. The pH decreased to dangerous levels in many of the hypothyroid dogs and the rate of infusion had to be decreased or stopped to prevent terminating the experiment. Longer infusion times, which would decrease the transient fall of pH, were not practical since the experiments already occupied 10 hr (including the surgery). A stronger acid concentration could also cause the aggravation of other problems; e.g., hemolysis of the blood. Hemolysis was not a physiological problem in the experiments described since the animals had to survive only about four hours after the infusion. Methodologically, hemolysis does affect the C^{14} counting because of increased quenching in the samples. Since the hemolysis increased significantly after the acid infusion, the quench corrections were correspondingly much larger and normal radioactivity measuring errors were magnified.

Problems of Isotope Radioactivity Counting and Methods of Measurement

Since no satisfactory method was found to count C^{14} in the presence of a gamma emitter, two problems emerged. First, the C^{14} could not be counted until the Br^{82} (γ emitter) had decayed sufficiently. Therefore, the samples were stored in a refrigerator for a minimum of eight days (about 5 half-lives of the Br^{82}) before they were counted for their C^{14}

activity. In addition, the plasma volume was not measurable by the standard technique of dilution of radioiodinated serum albumen, since radioiodine is also a gamma emitter. Thus the alternate technique was employed, which computes the plasma volume from the red-cell volume and the total-body hematocrit. The following question arises: Does the ratio of total-body hematocrit to large-vessel hematocrit remain constant during the experiments? The acid infusion may cause local vasoconstriction, resulting from trauma and general vasodilation. This is due to accumulation of metabolites, making it quite likely that this ratio may change after the infusion.

Definitions of Hypothyroidism

One may question whether such a disease state as hypothyroidism does exist in dogs. Some research has shown that apparent complete removal of the thyroid gland can produce no physiological effects thus; only minute amounts of the thyroid hormone are required for maintenance of a normal state [55]. If the gland is ablated with radioactive iodine, even a remaining small portion is sufficient to give normal function to the animal. Also, diet is very important to these thyroidectomized animals. If a normal iodine diet is given, the animal will also appear normal. R. C. Goldberg [55], however, has shown that dogs who have had their thyroid gland completely ablated became clinically myxedemic in a period of nine months to a year.

In the experiments upon which this Memorandum is based, producing myxedema was not desired; only the hypothyroid state was sought, since in myxedema the increase of weight due to fat accumulation might mask the primary physicochemical changes in body composition produced by this disease. In both current and historical literature, the terms *myxedema* and *hypothyroidism* have been used rather interchangeably. E. J. Wayne [56] gives a definition of the

difference between these two states. Myxedema is defined as a type of skin manifestation that occurs only in some cases of hypothyroidism--as opposed to hypothyroidism, in which an extracellular deposition exists in the connective tissue of the skin of a metachromatically stained material, probably consisting of combinations of a protein with mucopolysaccharides, hyaluronic acid, and chondroitin sulfuric acid. Proof that the animals in our experiments were hypothyroid was shown by decreased radioiodine uptake and protein-bound iodine, as well as increased concentration of serum cholesterol [57]. (See Table XVIII, App. A, p. 46.) In all the animals, after thyroidectomy, the protein-bound iodine decreased and serum cholesterol increased; in most, the radioactive iodine uptake decreased.

A model of a complex biological subsystem can be constructed and then used to simulate a disease state. The resulting model can never be fully validated because of its complexity, but even partial validation is sufficient to give some confidence and to indicate research directions and potentials. The present model, which combines old and new hypotheses for explanation of a disease state, suggests new ideas regarding both normal and abnormal body physiology.

Appendix A

ADDITIONAL LABORATORY RESULTS

Table XIII

PERCENT CHANGE DUE TO ACID INFUSION

	LABORATORY DATA	
	EUTHYROID	HYPOTHYROID
RCW[a]	5.42+3.69[b]	6.59+2.92
PW	-6.08+5.25	-0.51+2.89
ISW	5.91+8.99	-4.81+11.9
ECW	2.90+6.77	-3.74+9.43
ECEVW	13.6+12.3	13.7+11.3
CTW	34.8+39.1	52.7+25.5
ICW	0.06+5.67	0.32+5.03
$[NA]_{RCW}$	-1.00+5.50	-4.30+----
$[K]_{RCW}$	2.37+11.3	2.26+13.1
$[CL]_{RCW}$	17.1+15.4	12.4+9.99
$[NA]_{PW}$	-2.10+2.74	-2.26+1.39
$[K]_{PW}$	63.8+41.2	66.5+41.3
$[CL]_{PW}$	6.30+6.31	3.62+2.58
PH	-1.89+0.91	-1.48+1.22
PCO2	-24.8+19.5	-31.8+21.7

[a] For notation see p. 52.

[b] Values shown are mean±one standard deviation of the percent change from control measurements.

Table XIV

ISOTOPE DILUTION RESULTS FROM INDIVIDUAL EXPERIMENTS

EUTHYROID DOGS[a]

EXP NO.	WT	HCT	RCW	PW	ECW	ISW	ECEVW	CTW	ICW	TBW
1C[b,c]	24.0	.490	.496	.850	4.26	3.41	4.92	1.51	10.7	17.0
1S		.490	.500	.866	4.11	3.24	4.79	1.54	10.8	17.0
2C[b]	23.9	.548	.456	.643	3.49	2.85	4.16	1.32	8.07	13.3
2S		.557	.479	.641	3.48	2.84	4.20	1.36	8.04	13.3
3C	24.0	.590	.662	.763	2.69	1.93	4.09	2.16	7.32	12.8
3S		.628	.703	.709	2.85	2.14	4.02	1.88	7.91	13.3
4C	24.5	.472	.379	.692	3.85	3.15	5.13	1.98	9.80	16.0
4S		.502	.409	.668	3.87	3.20	5.41	2.21	10.1	16.6
5C	18.9	.572	.495	.645	3.36	2.71	3.71	1.00	6.29	11.1
5S		.615	.537	.576	3.18	2.61	4.48	1.88	6.02	11.6
6C	25.4	.462	.542	1.02	4.02	3.00	----	----	----	15.7
6S		.495	.539	.929	4.09	3.16	----	----	----	16.3
7C	22.7	.489	.489	.861	3.4	2.54	4.17	1.63	8.45	14.0
7S		.527	.516	.781	3.81	3.03	5.09	2.06	8.15	14.5
8C	19.0	.514	.384	.625	2.93	2.30	3.98	1.68	7.27	12.3
8S		.514	.515	.661	3.01	2.35	3.81	1.46	7.84	12.7
9C	14.5	.492	.301	.524	2.76	2.23	3.24	1.01	6.51	10.6
9S		.543	.318	.465	2.52	2.06	3.86	1.81	6.30	10.9
10C	20.6	.464	.525	.994	3.76	2.76	3.86	1.09	9.27	14.6
10S		.485	.521	.941	4.17	3.23	5.03	1.79	8.67	15.2
11C	16.2	.587	.425	.529	2.37	1.84	2.69	.844	6.31	9.95
11S		.620	.461	.506	2.51	2.00	3.15	1.15	6.24	10.4

[a] See Table XV for explanation of table.

[b] No acid infused.

[c] C refers to preinfusion; S to postinfusion.

Table XV

ISOTOPE DILUTION RESULTS FROM INDIVIDUAL EXPERIMENTS

HYPOTHYROID DOGS

EXP NO.	WT	HCT[a]	RCW	PW	ECW	ISW	ECEVW	CTW	ICW	TBW
12C	21.3	.507	.532[b]	.855	3.23	2.38	3.55	1.17	7.01	11.9
12S		.520	.564	.871	3.30	2.43	4.22	1.79	6.82	12.5
13C	19.5	.417	.327	.751	3.32	2.57	3.65	1.08	6.87	11.6
13S		.433	.332	.737	2.93	2.2	3.70	1.51	7.32	12.1
14C	21.1	.400	.256	.686	2.91	2.22	3.26	1.04	8.35	12.6
14S		.433	.276	.647	3.05	2.40	4.05	1.65	8.11	13.1
15C	19.5	.434	.335	.716	2.72	2.01	2.94	.939	7.29	11.3
15S		.448	.354	.717	2.53	1.81	3.77	1.96	6.93	11.3
16C	23.0	.499	.456	.788	3.22	1.84	4.28	1.84	8.33	13.8
16S		.513	.492	.792	2.98	2.33	4.52	2.33	8.61	14.4
17C	23.9	.513	.477	.763	3.80	3.04	4.16	1.12	9.24	14.6
17S		.534	.513	.746	3.58	2.83	4.60	1.77	9.38	15.2
18C	20.8	.491	.447	.779	3.47	2.69	4.28	1.59	7.79	13.3
18S		.513	.499	.768	2.89	2.12	4.20	2.08	8.35	13.8
19C	18.3	.484	.339	.604	3.34	2.74	3.70	.968	7.42	12.1
19S		.487	.335	.624	3.75	3.12	4.54	1.41	7.00	12.5
21C	21.8	.382	.733	1.40	4.57	3.18	4.64	1.47	7.02	13.8
21S		.407	.730	1.32	4.16	2.84	5.03	2.19	7.26	14.3
22C	22.2	.467	.479	.887	3.92	3.03	4.35	1.32	8.56	14.3
22S		.504	.505	.837	3.96	3.12	5.64	2.52	7.85	14.8

[a]See p. 52 for notation.

[b]Volumes expressed in liters.

Table XVI

BLOOD CHEMISTRY RESULTS FROM INDIVIDUAL EXPERIMENTS

EUTHYROID DOGS[a]

	PLASMA					RED CELLS		
EXP.	NA	K	CL	PH	PCO2	NA	K	CL
3C	153[b]	4.04	111	7.23	49.5[c]	144	7.92	83.8
3S	159	8.57	120	7.18	34.5	144	7.12	101.
4C	153	4.12	121	7.31	52.5	156	9.41	67.4
4S	150	8.57	131	7.11	34.5	147	11.2	102.
5C	152	3.83	115	7.32	38.0	154	7.98	89.5
5S	151	3.69	124	7.21	25.5	150	7.68	102.
6C	152	4.67	118	7.29	39.0	142	8.66	95.0
6S	150	6.74	119	7.20	44.0	158	8.11	94.6
7C	153	5.39	119	7.31	39.0	160	7.24	95.3
7S	145	7.58	124	7.22	17.3	150	8.78	101.
8C	151	4.02	116	7.24	42.0	156	7.74	71.8
8S	145	5.84	111	7.05	36.5	146	8.40	92.8
9C	153	3.71	117	7.31	41.5	155	8.04	84.2
9S	151	8.18	126	7.15	27.0	155	8.11	95.7
10C	158	3.82	115	7.26	55.0	146	7.76	83.9
10S	151	5.61	121	7.16	50.0	150	7.55	90.2
11C	164	4.10	111	7.40	35.5	164	8.76	92.8
11S	158	6.61	132	7.14	26.5	161	8.29	104.

[a] See Table XV for explanation of table.

[b] Electrolyte concentration in MEq/liter of water.

[c] Value expressed in mm Hg.

Table XVII

BLOOD CHEMISTRY RESULTS FROM INDIVIDUAL EXPERIMENTS

HYPOTHYROID DOGS[a]

	PLASMA					RED CELLS		
EXP.	NA	K	CL	PH	PCO2	NA	K	CL
12C	153	3.66	120	7.23	35.5	160	7.88	90.5
12S	150	7.87	123	7.22	25.0	148	7.85	98.2
13C	157	5.89	119	7.42	37.0	151	8.18	86.3
13S	152	5.94	118	7.45	15.5	160	8.75	86.6
14C	153	4.31	117	7.34	33.5	170	9.48	92.2
14S	150	8.84	119	7.14	27.0	160	10.3	107.
15C	159	4.40	121	7.31	51.0	167	10.0	69.3
15S	154	7.48	127	7.20	28.0	158	8.80	75.7
16C	154	4.22	117	7.46	39.0	158	8.92	77.3
16S	151	4.90	122	7.29	----	153	8.79	81.5
17C	149	3.99	113	7.40	30.0	150	8.84	81.3
17S	145	7.82	122	7.19	20.5	147	8.49	93.2
18C	145	4.50	118	7.14	31.0	163	9.36	71.6
18S	146	8.00	123	6.99	33.5	142	7.71	95.8
19C	154	4.17	121	7.29	35.5	156	10.1	83.0
19S	148	6.31	126	7.24	19.0	152	12.6	92.4
20C	146	4.35	113	7.21	51.0	153	8.95	95.6
20S	150	7.97	123	7.17	35.5	150	11.8	106.
21C	156	4.42	122	7.43	50.0	162	7.71	91.0
21S	163	6.59	125	7.16	44.0	158	9.03	94.8

[a]See Tables XV and XVI for explanation.

Table XVIII

HISTORY OF HYPOTHYROID DOGS

EXP. NO.	TIME BETWEEN THYRODECTOMY AND EXPERIMENT (DAYS)	WT. (KG.)	RADIOIODINE UPTAKE (%)	PBI (µGRAMS) %	SERUM CHOLESTEROL (MG. %)
12		23.5	7.0	1.1	106
	46	21.3	3.7	0.9	400
13		18.95	14.0	1.6	151
	49	19.5	3.0	0.4	296
14		21.5	22.0	3.0	154
	56	21.1	0.9	1.1	320
15		19.4	46.0	1.7	209
	42	19.5	2.0	1.6	378
16		19.6	9.0	2.2	158
	49	23.5	0.1	1.2	259
17		22.0	----	2.9	230
	42	24.35	0.4	1.4	392
18		18.0	----	1.8	140
	44	21.1	0.6	0.9	225
19		-		3.5	162
	40	18.7	8.0	1.6	207
20		20.9	29.0	---	---
	120	21.8	0.7	0.8	160
21		26.0	5.0	---	---
	263	22.5	2.5	---	---

Appendix B

LABORATORY MATERIALS AND METHODS

EXPERIMENTAL PLAN

Twenty-one mongrel dogs were divided into two groups. Group A, the normals or euthyroids, were spleenectomized and bilaterally nephrectomized the morning of the experiment. Group B, the hypothyroids, were surgically thyroidectomized. Six weeks post-thyroidectomy, the hypothyroids (Group B) were also bilaterally nephrectomized and spleenectomized, and the experiment was then performed. Group B was placed on a low-iodine diet approximately two weeks before the experiment. Each dog in this group was measured for protein-bound iodine, serum-cholesterol concentration, and radioactive-iodine uptake both before and five weeks after the removal of the thyroid gland.

In both groups measurements were made of red-cell volume, plasma volume, arterial blood hematocrit, pH and PCO_2, extracellular water, total body water, water content of plasma and erythrocytes, and sodium, potassium, and chloride concentrations; these were taken before and after an infusion of hypertonic hydrochloric acid. Two animals of Group A (controls) received no infusion.

EXPERIMENTAL PROCEDURE

The animals of both groups were given no food or water the day before the experiment. On the morning of the experiment they were lightly anesthetized with a sodium pentobarbital solution, weighed, bilaterally nephrectomized, and spleenectomized. Canulas were inserted in both femoral arteries and one femoral vein, and a gastric tube was inserted via the esophagus for collection of gastric juice.

At the beginning of the experiment a venous blood sample was taken to establish control values for sodium,

potassium, and chloride concentrations and water content of the blood; and an arterial sample was drawn for background determination of radioactivity. Radiosucrose (C^{14}), tritiated water (H^3), and radiobromide (Br^{82}) were injected into the venous system; and arterial blood samples were withdrawn after 100, 120, 140, and 160 minutes to determine plasma radioactivity. After the 120-minute point, isologous blood tagged with radiochromate ($Cr^{51}O_4$) was injected; samples of arterial blood were removed after the 160-minute point to determine the radioactivity, pH, PCO_2, and hematocrit. Following the sampling period, 0.2N HCl (25 ml/kg of body weight) were infused through a polyethyline canula into the femoral vein by a constant-speed infusion pump. The rate of infusion was adjusted for a total infusion time of 90 minutes.

Ten minutes after infusion of the HCl, an arterial blood sample was taken to determine the residual radioactivity. The radiosucrose and the radiobromide were reinjected into the animal and arterial blood samples were withdrawn 100, 120, 140, and 160 minutes after injection time. At 100 minutes a sample was taken for measurement of pH; PCO_2; hematocrit; sodium, potassium, and chloride concentrations; water content; and radioactivity of radiochromate in the red cells and plasma.

The gastric contents of the animals were collected in some experiments before and after infusion of the acid and at the end of the experiment. One ml was used to determine radioactivity and the remainder was replaced via the stomach tube.

Sixty ml of blood were withdrawn before the acid infusion, 5 ml during the infusion, and 40 ml in the period after the infusion. The animal received approximately 50 ml of normal saline before the infusion period, 10 ml during the infusion, and 40 ml in the period after the infusion. Following each experiment an autopsy was performed to determine if bleeding occurred at the operating sites.

ANALYTICAL METHODS

Plasma and erythrocyte water content were determined by weighing 0.5 ml amounts, drying them at 100°C for 24 hr, and then reweighing. The weighing was performed on a Mettler Balance to the nearest 0.1 mg. Plasma and erythrocyte sodium and potassium concentrations were determined in heparinized samples by internal, standard-flame photometry [58]. The sodium heparin used for collecting the blood samples changed the sodium concentration in the plasma by less than one percent. The chloride content of plasma and erythrocytes was measured by the method of D. D. Van Slyke and J. Sendroy, Jr. [59]. All analyses were performed in duplicate.

An International Microcapillary Ultracentrifuge determined the hematocrits. Samples were taken in quintuplicate, put into heparinized capillary tubes, spun at 11,000 RPM for five minutes, and then read on an optical reader. These hematocrits were corrected for two percent trapped plasma [60].

Samples for whole blood pH and PCO_2 were collected anaerobically in heparinized syringes and measured at 37°C within five minutes of sampling by an Astrup† pH meter. PCO_2 was calculated using the Astrup Diagram. The sodium heparin used did not change the pH of the sample.

RADIOISOTOPE METHODS

The volume of the red-blood cells in the animal was measured using sodium chromate -51. The procedure was to withdraw 20 ml of arterial blood from the dog before the injection of any isotopes. This blood was injected into a 100 ml siliconized bottle, prepared by Abbott Labs††, containing 4 ml of Strumia formula [61] ACD solution. Thirty

†Astrup, Radiometer, Copenhagen.

††Abbott Labs, N. Chicago, Illinois.

μ-curies of the isotope (in 0.1 to 0.15 ml of normal saline) were then injected into the bottle. This mixture was intermittently swirled over a period of 40 minutes at room temperature, and then fifty mg of ascorbic acid were added to halt the tagging process. After a ten-minute incubation period 15 ml of this mixture were injected into the femoral vein of the animal. Forty minutes later arterial blood was withdrawn to determine hematocrit and radiochromate radioactivity. Standards were prepared to determine the radioactivity of the whole tagged mixture injected and of the noncellular phase of the tagged mixture. These standards were counted to correct for the isotope that did not tag the red cells. A Nuclear Chicago Auto Gamma Counter determined the radioactivity. Approximately two hours after the infusion another blood sample was withdrawn to measure the red-cell volume. Since the animal was spleenectomized and minimal bleeding and sampling occurred between the two red-cell volume measurements, the isotope was not reinjected [60].

The measured red-cell volume of the animal and the total-body hematocrit determined the plasma volume (Eqs. 1,2). The ratio of total-body hematocrit to large-vessel hematocrit in normotensive, spleenectomized, anesthetized dogs was taken as 0.88.

Radiosucrose (C^{14}) was used to measure the volume of water in the plasma and interstitial-lymph compartments since sucrose equilibrates very slowly with dense connective tissue and does not enter cells [50,54]. Thirty μ-curies were injected in three ml of normal saline. The theoretical concentration of this isotope at the time of injection (assuming instantaneous mixing) was determined by fitting a single exponential through the four data points (taken after the mixing period) and extrapolating to injection time. The radiosucrose was counted in total darkness in a Packard Liquid Scintillation Counter Model 527 at $8^{\circ}C$. The scintillation fluid consisted of 17 ml of

a toluene, ethanol, POP, POPOP mixture [62] and 1 ml of Hyamine† hydroxide [63] to dissolve protein; 0.25 ml of plasma was counted. Quench correction was necessary in these experiments since the color of the solution, the Hyamine† hydroxide, and the water in the solution shifted the characteristic spectrum of C^{14}.

The correction was accomplished by using a set of quenched standards, made by adding 0.5 ml of a solution of radiohexadecane (C^{14}) and 0.25 ml of various concentrations of hemolyzed blood to the 17 ml of the scintillation fluid and the 1 ml of Hyamine† hydroxide. These samples were counted for their C^{14} activity and for activity of the external standard (a radium source). A curve was generated that showed the quenching of C^{14} activity as a function of the external standard activity [64]. For an unknown sample, the percent quenching of C^{14} could be determined by the activity of the external standard for that sample. The correction factor obtained was multiplied by the measured isotope concentration of each sample.

Sodium Bromide-82, which equilibrates rapidly with erythrocytes [52] but enters other body cells very slowly, was the isotope that measured the volume of water in the extracellular-extravascular space; 25μ curies were injected in 10 ml of sterile saline for each injection. The bromide ion distributed as the chloride ion [54], and thus the large outward gradient in muscle cells impeded entry. Mixing of this ion was essentially complete after 100 minutes [65], and the exponential disappearance curve remaining showed entry into cells. The four data points taken after mixing were fit by a single exponential and extrapolated to injection time to give the theoretical equilibrium concentration in plasma. The concentration of the bromide ion in red cells and in the interstitial space was computed from

†Packard Instruments.

the Gibbs-Donnan ratio for chloride ions in these fluids. The extracellular-extravascular water volume was then calculated by determining the activity of injected radiobromide existing in this space and dividing by its activity per ml of water in the interstitial space.

The total body water was measured by the dilution of tritiated water. The tritium and C^{14} were counted simultaneously in the liquid-scintillation counter using two separate channels. The measurement was made before the acid infusion, and the final body water volume was determined by the sum of the measured value plus the volume of acid infused; 300μ curies were injected in 3 ml of sterile saline.

The intracellular water was defined as the total body water less the sum of red-cell water, plasma water, and extracellular-extravascular water.

CALCULATIONS

The following notations are used in the formulas needed to calculate the various fluid volumes measured in the experiments:

$M(0)$	≡ measurements taken before first injection of isotope
$M(1)$	≡ measurements taken in control period after first injection of isotope
$M(2)$	≡ measurements taken after acid infusion
$M(1,2)$	≡ measurements taken immediately before second injection of isotope
I_n	≡ injected activity of isotope n
$C_{n,z}$	≡ extrapolated zero time concentration of isotope n in compartment z
$C_n(S,X)$	≡ concentration of isotope n in the internal standard per ml of fluid X
H	≡ large-vessel hematocrit corrected for trapped plasma

H_T	= total-body hematocrit (=.88·H)
RCV	= red-cell volume
RCW	= red-cell water volume
PV	= plasma volume
PW	= plasma-water volume
ECW	= volume of extracellular water as measured by radiosucrose
ISW	= volume of interstitial water as measured by radiosucrose
ECEVW	= volume of extracellular-extravascular water as measured by radiobromide
CTW	= volume of water measured by difference between radiosucrose volume and radiobromide volume
TBW	= total body water
ICW	= volume of intracellular water
WCP	= water content of plasma
WCR	= water content of red cells
GDR	= Gibbs-Donnan ratio
$[Y]_z$	= concentration of ion Y in MEq of Y/ml of compartment z
$[Y]_{zw}$	= concentration of ion Y in MEq of Y/ml of water in compartment z

General Notations

B	= refers to blood
P	= refers to plasma
RC	= refers to red cells
IS	= refers to interstitial fluid
IC	= refers to intracellular fluid
S	= refers to internal standard
W	= refers to water
D	= refers to dilution factor
V	= refers to volume injected or infused
·	= multiplication
L	= refers to fluid load added

Formulas:

1) Red Cell Volume ($Cr^{51}O_4$)

$$RCV(1) = \frac{I_{Cr}}{C_{Cr,RC}(1) - C_{Cr,RC}(0)} \tag{1}$$

$$RCV(2) = \frac{I_{Cr}}{C_{Cr,RC}(2) - C_{Cr,RC}(0)} \tag{2}$$

$$I_{Cr} = \left[C_{Cr}(S,B) - \left(C_{Cr}(S,P) \right) \left(1 - H(S) \right) \right] \cdot D_{Cr} \cdot V_{Cr} \tag{3}$$

$$C_{Cr,RC}(1) = \frac{C_{Cr,B}(1) - \left[C_{Cr,P}(1) \cdot \left(1 - H(1) \right) \right]}{H(1)} \tag{4}$$

$$C_{Cr,RC}(2) = \frac{C_{Cr,B}(2) - \left[C_{Cr,P}(2) \cdot \left(1 - H(2) \right) \right]}{H(2)} \tag{5}$$

2) Plasma Volume

$$PV(1) = RCV(1) \cdot \frac{1 - H_T(1)}{H_T(1)} \tag{6}$$

$$PV(2) = RCV(2) \cdot \frac{1 - H_T(2)}{H_T(2)} \tag{7}$$

3) Interstitial Water Volume (C^{14})

$$ISW(1) = ECW(1) - PW(1) \tag{8}$$

$$ECW(1) = \frac{I_C(1)/WCP(1)}{\left[C_{C,P}(1) - C_{C,P}(0)\right]} \tag{9}$$

$$PW(1) = PV(1)/WCP(1) \tag{10}$$

$$I_C(1) = C_C(S,W) \cdot D_C \cdot V_C(1) \tag{11}$$

$$ISW(2) = ECW(2) - PW(2) \tag{12}$$

$$ECW(2) = \frac{I_C(2)/WCP(2)}{\left[C_{C,P}(2) - C_{C,P}(1,2)\right]} \tag{13}$$

$$PW(2) = PV(2)/WCP(2) \tag{14}$$

$$I_C(2) = C_C(S,W) \cdot D_C \cdot V_C(2) \tag{15}$$

4) Extracellular-Extravascular Water Volume (Br^{82})

$$ECEVW(1) = \frac{I_{Br}(1) - \left(C_{Br,P}(1) \cdot PV(1)\right) - \left(C_{Br,RC}(1) \cdot RCV(1)\right)}{\left[C_{Br,P}(1) - C_{Br,P}(0)\right] \cdot \left[\frac{GDR_{Br,P,IS}}{WCP(1)}\right]} \tag{16}$$

where $GDR_{Br,P,IS} = 1.05$.

$$I_{Br}(1) = C_{Br}(S,W) \cdot D_{Br} \cdot V_{Br}(1) \tag{17}$$

$$C_{Br,RC}(1) = C_{Br,P}(1) \cdot \frac{[Cl]_{RC}(1)}{[Cl]_{P}(1)} \tag{18}$$

$$ECEVW(2) = \frac{I_{Br}(2) - \left(C_{Br,P}(2) \cdot PV(2)\right) - \left(C_{Br,RC}(2) \cdot RCV(2)\right)}{\left[C_{Br,P}(2) - C_{Br,P}(1,2)\right] \cdot \left[\frac{GDR_{Br,P,IS}}{WCP(2)}\right]} \tag{19}$$

$$I_{Br}(2) = C_{Br}(S,W) \cdot D_{Br} \cdot V_{Br}(2) \tag{20}$$

$$C_{Br,RC}(2) = C_{Br,P}(2) \cdot \frac{[Cl]_{RC}(2)}{[Cl]_{P}(2)} \tag{21}$$

5) Volume of water measured by difference between radiobromide volume and radiosucrose volume

$$CTW(1) = ECEVW(1) - ISW(1) \tag{22}$$

$$CTW(2) = ECEVW(2) - ISW(2) \tag{23}$$

6) Total Body Water (H^3)

$$TBW(1) = \frac{I_H}{C_{H,PW}(1) - C_{H,PW}(0)} \quad (24)$$

$$TBW(2) = TBW(1) + L \quad (25)$$

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