MEMORANDUM
RM-5392-PR
OCTOBER 1968

SPECULATIONS ON PHYSICOCHEMICAL FLUID PROPERTIES IN PHYSIOLOGICAL REGULATION

J. C. DeHaven and N. Z. Shapiro

PREPARED FOR:
UNITED STATES AIR FORCE PROJECT RAND

The RAND Corporation
SANTA MONICA • CALIFORNIA

MEMORANDUM
RM-5392-PR
OCTOBER 1968

SPECULATIONS ON PHYSICOCHEMICAL FLUID PROPERTIES IN PHYSIOLOGICAL REGULATION

J. C. DeHaven and N. Z. Shapiro

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

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

Published by The RAND Corporation

PREFACE

This study is related to a continuing Project RAND effort exploring the use of mathematical models to investigate the chemical aspects of physiological systems.

The objective of the overall project is to attempt the detailed quantification and synthesis of the chemistry of human physiological functions with the aid of advanced electronic computer and programming techniques. The emerging space age presents an especially difficult challenge to biological science and medical art. The results of stresses that may be placed on organisms by the new and stringent environments associated with space travel are largely unknown. Progress in understanding the effects of these environments on the human organism will be difficult and slow, if not impossible, unless the present quantitative aspects of the biological arts and sciences can be greatly advanced. It was decided that the techniques used in this project should be pushed to the biological or mathematical limit, whichever came first, in the hope that adequate mathematical models of increasingly large portions of the human system could be constructed.

The present Memorandum represents a survey of just such a region of biological and mathematical limitation. Previously, we constructed models of compartmented whole bodies in which simulated real time was introduced and in which renal and extrarenal excretory pathways were related to dietary inputs and to certain types of chemical stresses.

These models did a pretty good job of reflecting acute and chronic alterations in body fluid and electrolyte distributions for healthy, resting individuals subject only to moderate physiological stress. Such a state probably represents conditions under which most individuals exist much of the time. These models, however, are defective in that they cannot as yet respond properly to certain kinds of psychological and pharmaceutical stresses.

It is known, for example, that various classes of chemical substances have different steady-state concentration gradients between various intracellular and extracellular media. When relatively large amounts of some of these substances are added to the system, the concentration gradients of other substances may be altered in one way or another, or not at all. Fluxes of such substances into or out of cells, sometimes to regions of higher concentration, are similarly affected. Substances of greatest interest include metabolic fuels, intermediates, and products—various carbohydrates, amines, amides, and amino acids. The mutual antagonistic or augmentative influences of such substances are noted in many parts of the body: for example, between blood and gastrointestinal fluid, cerebrospinal fluid, urine, saliva, sweat, bile, and aqueous humor. The most widely accepted explanation for these phenomena is based entirely on the characteristics of the membranes separating the compartments. Such an explanation

postulates membrane carriers that supposedly combine differentially, or not at all, with the large number of substances involved. As many as 50 separate carriers need to be postulated for some systems. Inasmuch as few, if any, carriers have yet been identified, and hundreds or thousands of kinetic and diffusion constants (few now known and varying from system to system) must somehow be estimated in order to construct whole-body models incorporating the membrane-carrier hypothesis, this hypothesis entails grave difficulties for the model builder.

Whatever the present deficiencies of chemical solution theory for application to complex body fluids, physicochemical experience with somewhat similar types of nonbiological solutions enables one to state with a great deal of certainty that the properties of the fluids will affect fluxes and concentration gradients. The only question is whether their influence will be of a significant degree.

As so often happens, we found upon further investigation that our concern for the influence of body-fluid properties in physiological regulation was not unique. There are scientists in the Soviet Union who have been advocating the "phase" theory of regulation with emphasis on the characteristics of body fluids for a decade or more. As far as we can determine, the propositions of this school have not appeared in western literature nor have been accepted or even discussed by western physiologists. Although we have obtained

papers that describe the general hypotheses of this school, we have had difficulties (possibly because of distance and the language barrier) in obtaining papers that describe experiments in detail and contain data useful for corroborating the hypothesis. For this reason, it has been difficult to find appropriate data for our evaluation of body–fluid effects; the experiments described in the literature upon which we drew were mainly designed for other purposes.

Of all chemicals that influence body composition, the hormones are among the most potent. Although they exist in tiny amounts in the body, changes in their levels—as brought about by various psychological or pharmaceutical stresses or disease—can exert major acute and chronic chemical changes in the body. Hormones react both antagonistically and augmentatively with each other. There is no generalized theory of hormonal action; the science is still largely at the level of qualitative classification of hormonal effects. Any quantitative model of hormonal action must of necessity, then, be limited in nature, empirical, and inflexible. If, however, an important mode of hormonal action can be shown to occur through its effects on body fluids, then one might hope to call on solution theory to help provide the basis for a more generalized predictive model. The present study indicates that there may be some basis for this hope.

SUMMARY

The authors of this study examine the proposition that certain physicochemical properties of biological fluids contribute to maintaining the steady-state concentration gradients that occur in physiological systems. In particular, simple electrostatic solution theory is used to demonstrate that differences in ionic strength and dielectric constants of fluids separated by simple membranes can produce large concentration gradients for uncharged organic substances between the fluids. The magnitude of the gradients, and whether they are greater or less than one, is shown to depend on the electrostatic characteristics of the fluids and on the size and signs of the dielectric increments of the substances.

Examples selected from the literature of systems ranging in complexity from gels and aqueous media to plasma and urine are used to demonstrate that there is a statistically significant relationship between dielectric increments and concentration gradients for a variety of organic substances. Neither the lipoid solubilities of these substances nor their molecular weights are significantly related to their concentration gradients.

A model of species interaction is derived from simple solution electrostatics. The relevance of this model to the explanation of the known antagonistic and augmentative effects of certain chemical species upon each other's concentration gradients between

physiological compartments is examined in the context of the renal excretory system. The model is weakly supported by the rather sparse data available.

Hormones are among the most potent chemicals to affect the distribution of substances between body compartments. With anti–diuretic hormone as an example, computations are made that show that it is feasible for this hormone, by complexing with β globulin, to alter the dielectric constant of plasma so as to change the activity of water from that of diuretic urine to that of concentrated urine in the hydropenic state. This protein–interactive, bulk fluid mode of hormonal influence is suggested as a basis for explaining certain presently unexplained hormonal actions and interactions.

ACKNOWLEDGMENTS

The authors wish to acknowledge the expert computational assistance of M. L. Shapley and useful suggestions by N. S. Assali and T. H. Kirschbaum.

CONTENTS

SPECULATIONS ON PHYSICOCHEMICAL FLUID PROPERTIES IN PHYSIOLOGICAL REGULATION

1. THE POSSIBLE INFLUENCE OF THE BULK ELECTROSTATIC PROPERTIES OF BIOLOGICAL FLUIDS IN ESTABLISHING CONCENTRATION GRADIENTS BETWEEN BODY COMPARTMENTS.

In considering the mechanisms by which concentration gradients of various chemical species are maintained and controlled across membranes separating biological fluids in living systems, most biologists place great emphasis on the role of the membrane. However, little attention is paid to the possible influence upon these gradients of the properties of the separated fluids interacting with the substances. The study of membrane transport mechanisms occupies the attention of a great many biologists, who conduct investigations with different biological systems both *in vivo* and *in vitro*. Membrane transport phenomena for many different chemical species are hypothesized and investigated in diverse areas, including renal clearances, intestinal absorption, gall bladder function, cerebrospinal fluid clearance, cultured tissue–slice studies, toad bladder experiments, red–cell transport and plant–root exchanges.

The lack of concern with the bulk characteristics of biological fluids was brought to the authors' attention during some recent

studies of the physicochemical characteristics of certain physiological subsystems. It appeared to us that the very large gradients maintained across membranes must at least be influenced by the properties of the fluids bathing the membranes. We could find few investigations of this subject reported in the literature. Certain small, relatively simple, uncharged but diffusible highly water-soluble substances for which no satisfactory transport mechanisms have been formulated often have large concentration gradients between fluids separated by membranes. Urea and glucose are examples of compounds that frequently demonstrate large gradients of opposing sign.

1.1. A Basis for Evaluation

One might ask what physicochemical characteristics urea and glucose possess that might interact with the fluid properties to produce concentration gradients. An obvious property to examine in this connection is the dielectric increment for the two species in aqueous solution. The dielectric increment is defined as $\partial D/\partial m_i$, where D is the dielectric constant of the solution and m_i is the concentration of the solute in moles per kilogram of water. The values for the dielectric increment at 20°C for urea and glucose in aqueous solution computed from data in [1] (Vol. VI, pp. 100 and 101) are 2.6 kg mole^{-1} and –24.32 kg mole^{-1} respectively.

According to Harned and Owen (Ref. [2] pp. 531–534) and Edsall and Wyman (Ref. [3] pp. 265–275), the activity coefficients of neutral molecules having negative dielectric increments increase with increasing ionic strength of the solution and thereby tend to be "salted–out" of solution. The reverse is true for molecules having positive dielectric increments. Thus, if we imagine two saline solutions with differing ionic strengths (maintained by external processes) on opposite sides of a membrane permeable to both urea and glucose, urea will tend to be salted out of the solution having the lower ionic strength and salted into the solution having the higher ionic strength. Glucose will be partitioned in an opposite fashion, so that concentration gradients for the two species (the one greater than one, the other less) should be established between the two solutions.

Similar concentration gradients will occur between solutions having identical ionic strengths but different dielectric constants. Generally, substances having negative dielectric increments will be salted out of such electrolytic solutions (activity coefficients greater than one) and substances having positive dielectric increments will be salted–in (activity coefficients less than one). The manner in which this phenomena can cause concentration gradients is illustrated in the next section.

We wish first to demonstrate the qualitative relations that exist between the concentration gradients and the following factors: ionic strength, dielectric constant and dielectric increment. To this end we make use of the following expression (developed by Edsall and Wyman [3], page 270), that relates the activity coefficient of a neutral species in electrolytic solution to the electrostatic parameters

$$\ln f_2 = \frac{A\beta N_1^*}{RT}\left(\frac{N_3}{1-N_3}\right). \qquad (1)$$

Here f_2 denotes the activity coefficient (on the mole-fraction scale) for an uncharged species; R and T have their usual meanings as the gas constant and absolute temperature; N_1^* is the mole fraction of the solvent assuming that only the uncharged species but not the electrolyte are present; and N_3 is the mole fraction of the un-ionized electrolyte. A and β in (1) represent an aggregation of parameters which for a particular system may be assumed to be constant.

$$A = \left(\frac{-N\epsilon^2 \nu Z_+ Z_-}{2b}\right). \qquad (2)$$

In the above definition of A, N is Avogadro's number 0.6024×10^{24} mole^{-1}; ϵ is the proton charge, 4.8×10^{-10} franklins; ν is the number of ions produced by the ionization of the electrolyte; Z+ and Z- are the valences of the cation and anion respectively; and b is a "mean radius" associated with the ions of the electrolyte such that

$$\frac{1}{b} = \frac{2}{Z_+ - Z_-} \left(\frac{Z_+}{2b_c} - \frac{Z_-}{2b_a} \right) , \tag{3}$$

where b_c and b_a are the conceptualized "radii" of the cation and anion respectively, and the other symbols are as defined above.

β, the factor reflecting the dielectric increment of the uncharged species, is defined as

$$\beta N_2^* = \frac{1}{D} - \frac{1}{D_0} . \tag{4}$$

D_0 is the dielectric constant of the solvent (here, water in all cases), and D is the dielectric constant of a solution containing n_1 moles of water and n_2 moles of the uncharged species. Also, $N_2^* = 1 - N_1^*$.

The assumptions used in deriving (1) are technically inapplicable to the situations in which we will use it. However, it has been shown to yield qualitatively correct results in other situations to which it is formally inapplicable ([2] pp. 531–534, [3] pp. 265–275). Also, (1) may be rewritten in a form in which the various parameters that affect f_2 are more readily apparent. Thus ionic strength, ω, is usually defined by

$$\omega = \frac{m_3}{2} (\nu_+ Z_+^2 + \nu_- Z_-^2) = - \frac{1}{2} m_3 \nu Z_+ Z_- , \tag{5}$$

where m_3 is the concentration of salt in moles per kilogram of water. We have approximately from (4) ,

$$\beta = -\frac{D^2}{\theta}\frac{\partial D}{\partial m_2} , \tag{6}$$

where θ is the molecular weight of pure water in kilograms per mole and D is the dielectric constant of the solution, and, to a very good approximation,

$$\frac{N_1^*}{1-N_3} = 1 . \tag{7}$$

Finally, we may combine (1), (2), (5), (6), and (7) to obtain

$$\ln f_2 = -\left(\frac{N\epsilon^2}{RT}\right)\left(\frac{1}{D^2 b}\frac{\partial D}{\partial m_2}\right)\omega . \tag{8}$$

The first factor on the right of (8) is a constant at constant temperature. The second factor reflects the effect of the differing dielectric increments of the uncharged species, the dielectric constant of the system, and the "mean radius" of the electrolytes in the system.

Keeping in mind that (8) reflects only the electrostatic parameters affecting f_2, the activity coefficient of the uncharged species, and is at best an approximate model of real solutions, it is instructive to examine the directional effects indicated by (8). Thus, if $\partial D/\partial m_2$ is positive, f_2 is less than one; such a compound is said to be salted into solution. An example is HCN, which raises the dielectric constant of water. In this case, $\partial D/\partial m_2$ for HCN is therefore

positive, and the compound HCN is salted into solution by most electrolytes ([3], p. 271). If $\partial D/\partial m_2$ is negative, i.e., if it reduces the dielectric constant of the solution, f_2 is alway greater than one and the compound is said to be salted out of solution (see acetone in Ref. [3] p. 271).

If $\partial D/\partial m_2$ is positive, then f_2 is less than one and will decrease with D, the dielectric constant of the solution. When $\partial D/\partial m_2$ is negative, f_2 is greater than one and increases with decreasing D. The influence of b on f_2 is directionally the same as for D. The directional effects of ω, the ionic strength, are the opposite of those of D and b. Thus, for a negative $\partial D/\partial m_i$, f_2 is greater than one and increases in size with increasing ω. When $\partial D/\partial m_i$ is positive, f_2 is less than one and decreases with increasing ionic strength.

To obtain an appreciation for the numerical magnitude of the values of f_2 that result from the use of (8), we have prepared Table 1 using this equation for solutions having dielectric constants of 300, 80, 60, 40, and 25, and for uncharged species having dielectric increments of 3.4, 25, 60, −3.4, −25, and −60. In all cases shown in Table 1 the mean ionic radius was held constant at 2×10^{-8} cm, the temperature at 300°K, ionic strength at 0.166 moles per kilogram of H_2O (N_3 = 0.006 according to definition of [3], p. 266).

As we shall attempt to demonstrate by subsequent examples, the activity coefficients and concentration gradients listed in this table

Table 1

COMPUTED ACTIVITY COEFFICIENTS OF UNCHARGED SUBSTANCES HAVING DIFFERENT DIELECTRIC INCREMENTS IN ELECTROLYTIC SOLUTIONS OF DIFFERING DIELECTRIC CONSTANTS

Dielectric Constant of Standard Solution, D^I	Dielectric Constants of Differing Solutions, D^{II}	Dielectric Increment[a] of Added Substances in H_2O, kg-mole^{-1}	Activity Coefficient f_2^I of Substances in D^I	Activity Coefficients f_2^{II} of Substances in D^{II}	Equilibrium Concentration Gradients $c_2^{II}/c_2^I = f_2^I/f_2^{II}$ of Substances between Solutions D^{II} and D^I
80.0	300	3.4	0.95	1.00	0.95
80.0	80	3.4	0.95	0.95	1.00
80.0	60	3.4	0.95	0.91	1.05
80.0	40	3.4	0.95	0.79	1.21
80.0	25	3.4	0.95	0.52	1.83
80.0	300	25.0	0.70	0.98	0.71
80.0	80	25.0	0.70	.70	1.00
80.0	60	25.0	0.70	0.52	1.34
80.0	40	25.0	0.70	0.23	3.09
80.0	25	25.0	0.70	0.02	32.80
80.0	300	60.0	0.42	0.94	0.45
80.0	80	60.0	0.42	0.42	1.00
80.0	60	60.0	0.42	0.21	2.00
80.0	40	60.0	0.42	0.02	14.11
80.0	25	60.0	0.42	0.00012	353.80
80.0	300	−3.4	1.05	1.01	1.04
80.0	80	−3.4	1.05	1.05	1.00
80.0	60	−3.4	1.05	1.08	0.97
80.0	40	−3.4	1.05	1.17	0.90
80.0	25	−3.4	1.05	1.43	0.74
80.0	300	−25.0	1.44	1.03	1.39
80.0	80	−25.0	1.44	1.44	1.00
80.0	60	−25.0	1.44	1.89	0.76
80.0	40	−25.0	1.44	4.08	0.35
80.0	25	−25.0	1.44	35.1	0.04
80.0	300	−60.0	2.38	1.07	2.23
80.0	80	−60.0	2.38	2.38	1.00
80.0	60	−60.0	2.38	4.64	0.51
80.0	40	−60.0	2.38	30.90	0.08
80.0	25	−60.0	2.38	6290.00	0.00038

Note:

[a]The dielectric increments listed in this column were determined in aqueous solutions and are intended to be appropriate for solutions having a dielectric constant near that for water, as does the standard comparison solution of Column 1. The question arises as to what corresponding dielectric increment should be assumed for solutions having dielectric constants widely differing from the standard. A common assumption (in the absence of actual measurements) is that the dielectric increment of a substance is independent of the solution to which it is added. This assumption is obviously incorrect in the limit. Another possible assumption, and the one used in these computations, is that the dielectric constant of a mixture is the sum of the products of the dielectric constants of the individual substances in the mixture and their mole fractions, thus,

$$D = \sum_{i=1}^{n} D_i \, m_i .$$

Based on this assumption it can be shown that

$$\delta^{II} = (D^{I} - D^{II})\theta + \delta^{I}$$

and

$$\delta^{I} = (D^{II} - D^{I})\theta + \delta^{II} ,$$

where D^{I} is the standard dielectric constant of Column 1, D^{II} is the varying dielectric constant of Column 2, δ^{I} is the dielectric increment of Column 3 representing that in the standard solution, δ^{II} is the dielectric increment computed by the formula above. These values (not shown in the Table) were used in Equation (8) to compute f_2.

encompass those for uncharged species in biological systems with reasonable assumptions being made regarding dielectric constants and ionic strengths. There are more sophisticated formulations of the electrostatic effects on activity coefficients of substances in solutions.* However, the biological data pertinent for evaluating these formulations are so few and uncertain that the use of these more detailed formulations is not warranted at this time. For example, there are no data on the dielectric constants or ionic strengths of biological fluids suitable for directly evaluating the worth of the hypothesis that electrostatic solution effects contribute to concentration gradients. Measurement of the electrical properties (including dielectric constants) of biological materials has largely been directed at understanding the electrical potentials generated by the heart, the mode of action of clinical electrical treatments, diagnosis, and the structure of membranes and complex molecules [4]. The measured dielectric constant of whole blood, for example, gives no pertinent information as to the dielectric constants of plasma or of cell protoplasm because of the overriding influence of the suspended red cell in determining the values for blood.

A few sets of experiments are available, however, that permit evaluating f_2 (the activity coefficient) in terms of changes in $\partial D/\partial m_i$

---

*The reader interested in these developments, particularly as they involve complex polyelectrolytes like proteins, may consult references such as [2, 3, 69, 71, 72].

(the dielectric increment) without explicitly evaluating all of the other terms of equation (8).

1. 2. Experiments with Chemical Models

Troshin [5] reports experiments on the distribution of galactose and sucrose between an equilibrium aqueous solution and the water of a complex gel. The concentration gradients between external solution and intracoacervate water are within the limits predicted by (8) for substances having negative dielectric increments in an environment containing one-tenth normal $HC\ell$ as the electrolyte. See Table 2.

The gradients of galactose and sucrose change as the concentrations of these substances are changed in the mixture. Troshin explains the gradients as occurring because of a combination of two factors: the lowered solubility of the substance in the water of the coacervate as compared with its solubility in the water of the equilibrium solution, and the absorption of the nonelectrolyte on the surfaces of the colloidal particles. These combined effects, operating differentially, can explain the changes in gradients that occur with concentration. Troshin reports having conducted many experiments with muscle, erythrocytes and yeast in studying the distribution of various substances (sugars, amino acids, creatinine, urea, acid and basic dyes), and he maintains that the same pattern exists for these systems as for the coacervates.

Table 2

THE VARIATION IN CONCENTRATION (IN GM PER 100 GM H_2O) OF GALACTOSE AND SUCROSE IN COACERVATE AND IN EQUILIBRIUM SOLUTION WITH TIME

(After Troshin [5])

Duration of Experiment (in hr)	Number of Experiments	Concentration of Galactose		Number of Experiments	Concentration of Sucrose	
		In the Coacervate	In the Equilibrium Solution		In the Coacervate	In the Equilibrium Solution
15	3	0.40	0.36	5	0.22	0.19
15	2	2.10	2.80	2	2.13	3.14
18	4	0.38	0.34	2	0.26	0.21
18	3	2.02	2.93	3	2.08	3.07

In a later work [6] , Troshin says "at the present time the lower solubility of substances in protoplasm is not linked to the solvation of water. Its mechanism is still unclear. . . nevertheless, the actual fact of the lower solubility is indisputable."

We propose that the different solubilities in protoplasm and external fluid are importantly affected by the electrostatic properties of the protoplasm (or coacervate) and of the dissolved substances. In addition, we suggest that the variation in solubility with concentration is not necessarily due only to absorption but may be caused by changes in dielectric increments with concentration for the uncharged species. The dielectric increments for very few substances have been measured in the concentration range of typical biological occurrence. Thus, the least concentrated solution for which the dielectric constant is typically measured is 5 gm percent so that the increments of dielectric constant and concentration must be calculated over a wide range to establish $\partial D/\partial m_i$. The concentration range of biological interest is more typically 0 gm percent to 0. 10 or 0. 5 gm percent. Saccharin is one compound for which dielectric measurements are reported in this low range of concentration ([1], Vol. VI, p. 101). At 20°C the dielectric increment for saccharin computed between 0 gm percent and 0. 1 gm percent is +732 kg-mole^{-1}; between 0 gm percent and 2 gm percent the value is -745 kg-mole^{-1}. Calculated between 0 gm percent and

and 10 gm percent saccharin, a not unusual lower value reported in the literature for substances of interest, the dielectric increment is only -39 kg mole^{-1}. Here, then, is a compound whose dielectric increment changes sign between 0.25 gm percent and 0.50 gm percent saccharin.

The predicted large influence of such major changes in $\partial D/\partial m_i$ on concentration gradients in biological systems can be appreciated through examination of Eq. (8) and Table 1. Unfortunately, we could not locate reports of studies of saccharin concentration gradients in biological systems to determine if such gradients might actually be altered with changes in saccharin concentration.

Glucose is also a candidate to suspect of having anomalous dielectric increments in that the substance exhibits "threshold" concentrations in biological systems (suggesting large changes in $\partial D/\partial m_i$ with concentration) and its concentration gradients do not correspond to its reported dielectric increment as determined between 0 gm percent and 5 gm percent, the lowest concentration of glucose in solution for which the dielectric constant has been determined [7]. This feature of glucose will be examined subsequently.

1.3. Experiments With Biological Models

1.3.1. Dielectric Increments of Diffusible Organic Substances in Relation to Their Concentration Gradients in Yeast Cultures.

Lindenberg et al., have reported studies of the distribution of a number of organic substances between intracellular water and extracellular water in baker's yeast [8, 9]. The results are listed in Table 3. The intracellular–extracellular concentration ratios reported by Lindenberg et al., $c_2/c_1 = f_1/f_2$, have been converted to activity coefficients by assuming that the activity coefficients in the dilute bathing solutions are one ($f_1 = 1$); these are listed in column two of Table 3.

The results of statistical analysis, given in Table 3, indicate that the correlation coefficient between $\ln f_2$ and $\partial D/\partial m_i$ is extremely high. By the Student "t"–test there is less than one chance in a thousand that the estimate of $\ln f_{2(i)}$ from $\partial D/\partial m_i$ is accidental. Lindenberg et al., attribute the gradients to the electrostatic solution phenomena we have described. In contrast to our analysis these investigators treat thiourea and sulfanilamide separately from the other compounds and attribute a portion of their gradients to more specific chemical affinities of these compounds for certain cellular components. This latter effect is analogous to the absorption proposed by Troshin.

Table 3

ACTIVITY COEFFICIENTS AND DIELECTRIC INCREMENTS OF ORGANIC SUBSTANCES IN RELATION TO BAKER'S YEAST CULTURES (After Lindenberg et al.[9])

Substance	Intracellular[a] Activity Coefficient of Substance, f_2	$\ln f_2$[b]	Dielectric Increment ($kg\text{-}mole^{-1}$)
Isoproponol	1.449	0.37156	-4.3
Ethanol	1.315	0.273	-2.6
Glycerol	1.30	0.262	-2.6
Glycol	1.242	0.218	-1.8
Methanol	1.205	0.184	-1.4
Acetamide	1.156	0.144	-0.8
Formamide	1.064	0.062	+0.5
Urea	0.926	-0.07688	+2.7
Thiourea	0.746	-0.293	+4.0
Sulfanilamide	0.588	-0.53103	+7.0

[a]The intracellular activity coefficients of the substances, f_2, are computed by assuming that the activity coefficients in the dilute extracellular liquid is one.

[b]The regression equation for estimating $\ln f_2$ in terms of $\partial D/\partial m_i$ is $\ln f_{2(i)} = 0.06136 - 0.08032\ [\partial D/\partial m_i - 0.07]$; correlation coefficient, $r = 0.992$; standard error, $SE = 0.0053$; Student "t" - test, $t = 22.22$, $P < 0.001$.

1. 3. 2. Concentration Gradients of Organic Substances Between Erythrocytes and Aqueous Media (Ringer–Locke's Solution or Serum). A study of mammalian erythrocytes by Parpart and Shull [66] is of greater pertinence to animal physiology than the previous examples. These investigators measured the equilibrium distribution of ethylene glycol, glycerol, urea and water between erythrocytes and suspending media (Ringer–Locke and serum gave similar results). They used bloods from a large number of animals of different species: beef, dog, man, and sheep. They assumed that the activity coefficients of the organic substances were the same within the erythrocytes and in the media and that differences in concentrations, in and out, were due to differences in "solvent power" or "osmotic activity" of the cellular water.*

It is of interest to determine if the concentration gradients between cell water and media of ethylene glycol, glycerol and urea are related to the dielectric increment of these species in water. For this purpose the values of gradients for beef blood, computed from Parpart and Shull [66], are plotted against the dielectric increments of these species as computed from Akerlof [70]. The results are shown in Figure 1. The dielectric increments for these

---

*The concept that bound or otherwise sequestered intracellular water is a cause of concentration gradients is still current among physiologists (e.g., [67] and [68]), although modern physicochemical analysis appears to deny the validity of this idea [69].

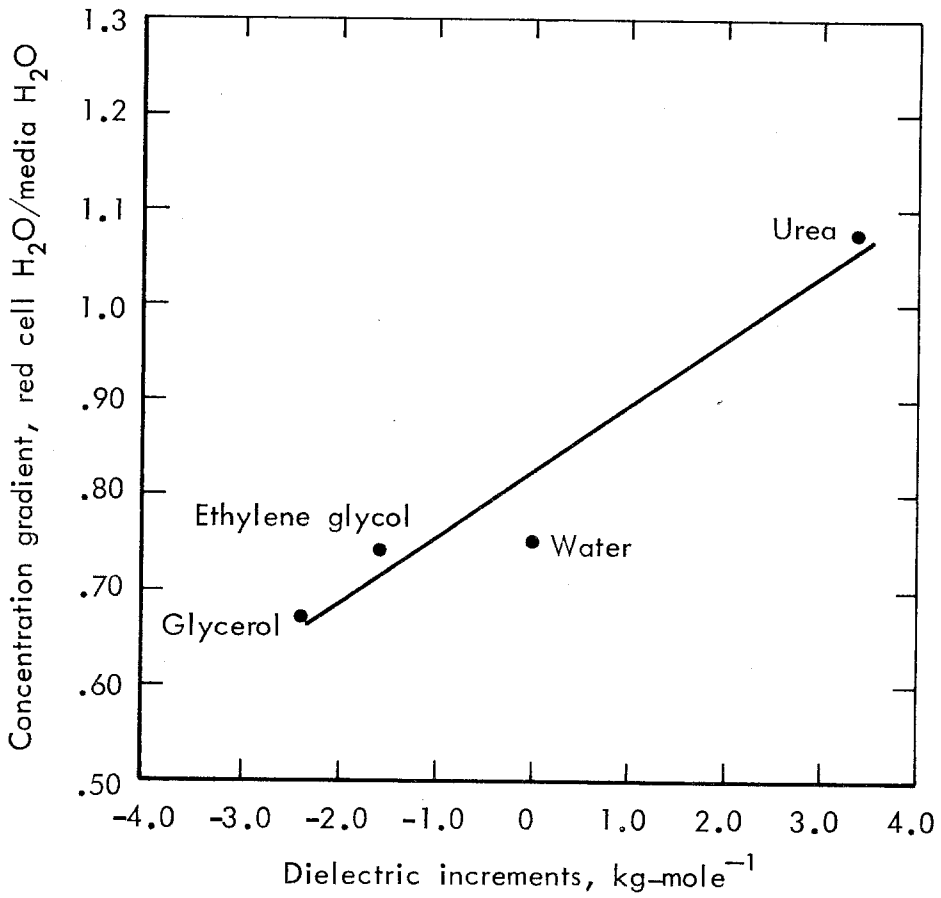

Fig. 1—Relation between concentration gradients and dielectric increments, beef red cells and media

substances as determined by Lindenberg and Zuili [8] are similar and show the same excellent agreement with concentration gradients. Unfortunately, too few substances are measured and reported to say that the relation shown in Figure 1 is conclusive proof that the interaction of dielectric increment and bulk electrostatic properties causes the gradients. On the other hand, these data do not deny the hypothesis, but rather add weight to the evidence for it presented in previous and following sections. The values for beef blood were selected because they represent the averages of analyses of the gradients for thirty–three animals, the most for any species reported.

1. 3. 3. Gradients of Organic Substances Between Plasma and Urine in Humans in Relation to Dielectric Increments. For certain purposes it is convenient and appropriate to consider urine and plasma to be two phases separated from each other by a phase boundary [10, 11]. This conceptual boundary combines the properties attributed to the several real membranes involved in the separation of plasma and urine and allows us to consider the characteristics of concentration gradients of a number of chemical substances as they exist in both plasma and urine and as they may relate to the electrostatic characteristics of the substances and of these fluids. To this end, we have collected data from the literature relating to the compositional gradients of substances for which there are also data on dielectric increments, $\partial D/\partial m_i$. The relatively

few such substances for which the two sorts of information could be located are listed in Table 4. Figure 2 shows $\ln g_{u/p}$, or $\ln f_p/f_u$ plotted versus the dielectric increment and the computed best fit line. In spite of the uncertainty imposed by some of the original data, the relation between the "resting" urine-plasma concentration gradients of the listed substances and the dielectric increments of these substances is surprisingly good. The correlation coefficient between $\ln g_{u/p}$ and $\partial D/\partial m_i$ is -0.9061.

The negative sign of this coefficient indicates that as the dielectric increment becomes less positive the concentration of the substance becomes higher in urine with respect to plasma. Inasmuch as positive dielectric increments are associated with more polar compounds and negative dielectric increments with less polar compounds, the resulting gradients indicate that the body tends to differentially excrete less polar substances (e. g., mannitol) and conserve the more polar substances (e. g., amino acids).

As mentioned earlier, the presently available data indicate that the dielectric increment for glucose (calculated between 0.0 and 5 gm percent) is -24.3 kg-mole^{-1}. This value and the $g_{u/p}$ for glucose at normal human plasma concentration levels are not compatible with the above hypothesis. This incompatibility plus the "threshold" excretion characteristics of glucose indicates that, as is the case for saccharin, the dielectric increment for glucose

Table 4

URINE-PLASMA CONCENTRATION GRADIENTS AND DIELECTRIC INCREMENTS FOR SOME ORGANIC SUBSTANCES

Substance	Concentration Gradient, Urine-Plasma,[a] $g_{u/p}$	$\ln g_{u/p}$ [a] ($\ln f_p/f_u$)	Dielectric Increment (kg $mole^{-1}$)	Molecular Weight (g-$mole^{-1}$)
Arginine	1.49	0.3988	62[c]	174.2
Ornithine	1.04	0.03922	51[c]	132.6
Taurine	0.82	-0.19845	41[c]	125.14
Creatine	8.88	2.1838	32.2[c]	149.15
Glycine	21.1	3.0493	37.3[c]	75.07
Glutamic Acid	31.4	3.4468	26[c]	147.13
Sulfanilamide	45.	3.8067[b]	7[f]	172.2
Urea	53.03	3.90708	3.4[c]	60.06
Mannitol	135.	4.9053[b]	-2.62[d]	182.17
Sucrose	134.	4.8978[b]	-7.22[e]	342.3

[a]Unless otherwise indicated, concentration data for urine and plasma are from Ref. [12].

[b]Concentration gradients for these species were derived from clearance data at low urine flow from Ref. [13].

[c]Dielectric increment from Refs. [14, 32].

[d]From Ref. [1].

[e]From Ref. [7].

[f]From Ref. [9].

[g]The regression equation estimating $\ln g_{u/p}$ ($\ln f_p/f_u$) from $\partial D/\partial m_i$ is $\ln g_i = 2.650 - 0.074\ (\partial D/\partial m_i - 25.006)$; correlation coefficient, $r = -0.9061$; standard error, SE = 0.063; Student "t" - test, $t = 6.06$, $p < 0.001$.

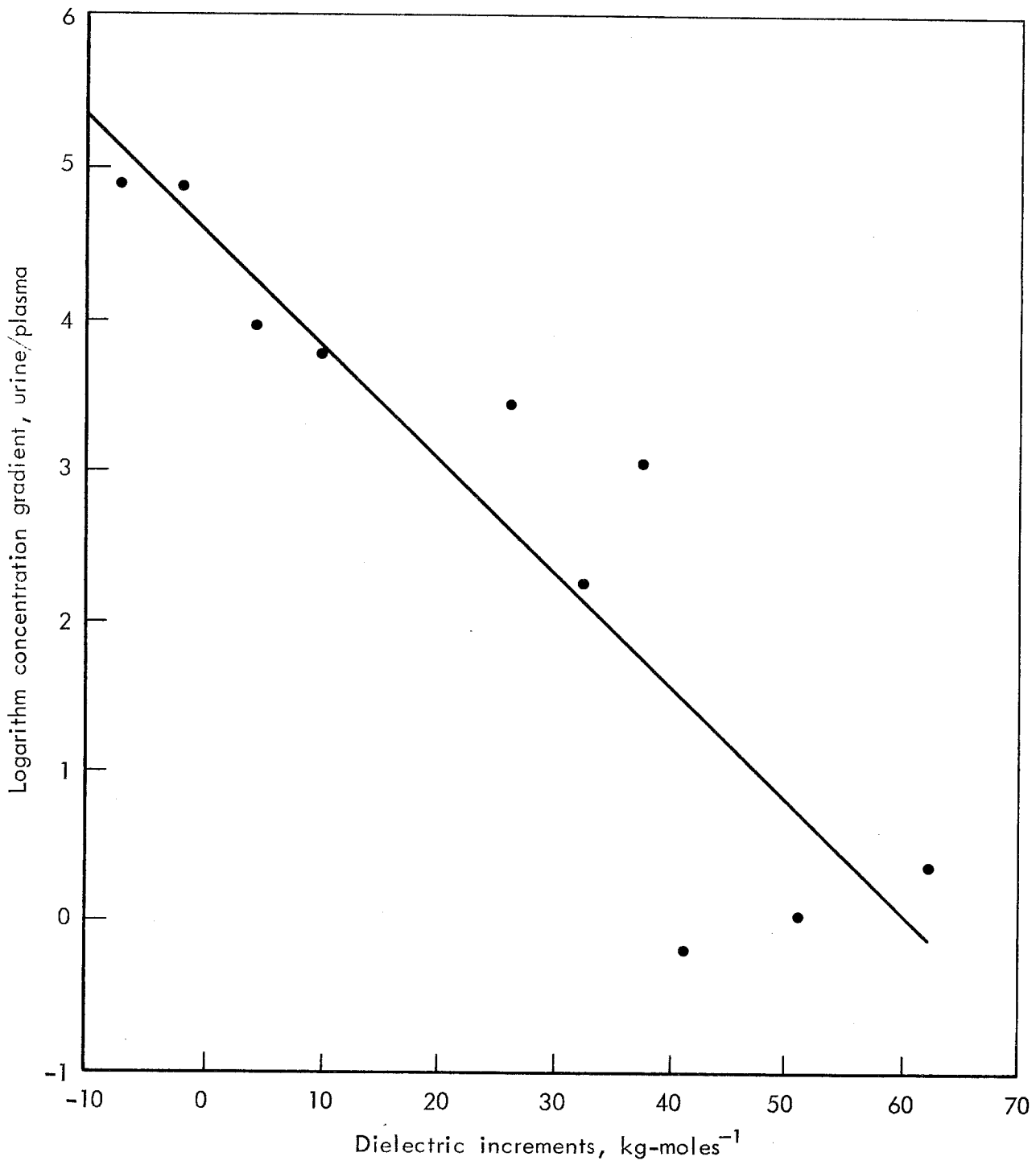

Fig. 2—Relation between logarithm of concentration gradients for a variety of organic substances between urine and plasma and dielectric increments, $\frac{\partial D}{\partial m_i}$

is highly dependent on concentration and that at the normal plasma level of 0.1 gm percent the dielectric increment is likely to be much less negative. The similar "threshold" excretion characteristics of creatine [13] and its $g_{u/p}$ at elevated plasma levels make one suspect that its relation between concentration and dielectric increment at low, physiological concentration levels is highly nonlinear.

1.3.4. Lack of Relation of Gradients to Other Properties of Organic Substances. It has been suggested that gradients of organic substances occurring between body fluids separated by membranes are strongly influenced by the relative lipoid/water solubility of the substance. The conventional rationale is that since substances with high lipoid solubility can more readily penetrate the lipoid substance of the membrane, they are not greatly hindered in passing from one compartment to another. In this connection, it has frequently been suggested that the renal reabsorption of these organic compounds, which ultimately affects their clearance, is determined by their lipoid/water solubility* ratios (see, for example, Ref. [15]).

In the case of some thirteen organic compounds for which we could locate data for both renal clearance and lipoid solubility,

*The lipoid/water solubility ratio is usually approximated by measuring the diethyl ether/water or olive oil/water solubility ratios [16], [17].

there was no meaningful statistical correlation between the two phenomena (correlation coefficient, –0.075).

In the case of seven organic substances for which direct data on urine–plasma concentration gradients and lipoid solubility could be located, there was no apparent relation between these gradients and the relative lipoid solubility (ether–water concentration ratios) of these substances (correlation coefficient, –0.30 for $g_{u/p}$; –0.35 for $\ell n\ g_{u/p}$.

Additionally, to demonstrate that the relation between urine–plasma concentration gradients and the dielectric increments of organic substances is not a ubiquitous relation between these gradients and just any other physical property of the molecule, we examined the relation with the molecular weights listed in Table 4. Here the correlation coefficient between $\ell n\ g_{u/p}$ and molecular weight is 0.32, which, for the size of the sample, indicates that no relation exists.

2. ANTAGONISTIC AND AUGMENTATIVE INTERACTIONS OF CHEMICAL SUBSTANCES IN BIOLOGICAL SYSTEMS AS RELATED TO THEIR RESPECTIVE EFFECTS ON THE BULK CHARACTERISTICS OF THE SEVERAL FLUIDS INVOLVED

For a number of years biologists and physiologists have noted and studied the effects of certain added species on the concentration gradients of other species occurring across natural membranes. Certain added substances increase the gradients of classes of chemicals and decrease or leave unaffected the gradients of others. These effects have been studied mainly in relation to renal clearance [13, 18–20. 23],* in toad bladder [21] and in kidney slice experiments [22]. However, similar interactive effects are noted in connection with bile formation and gall bladder function [23, 24], between muscle and its media [25], between cerebrospinal fluid and aqueous humor and blood [26, 27, 59], and across the placenta [28].

In renal physiology, as mentioned, these mutual effects on concentration gradients have been investigated in terms of the relative influence on the "clearance" of various substances upon the addition to the blood stream of other substances [13, 29]. "Clearance" is defined as

$$\hat{x}_j / \hat{x}'_j \cdot V = C ,$$

*These references and those just following are selected as representative of the literature or are of a review nature and are not intended to be all–inclusive.

where $\hat{x}_j$ is the concentration of the j-th species in urine, $\hat{x}'_j$ its concentration in plasma, and V the volume flow of urine. Clearance, C, divided by urine flow, V, therefore yields the urine-plasma concentration gradient for the species in question.

With the present emphasis on kinetics, current attempts to explain the phenomena in question are usually couched in terms of changes in one-way absorption or secretion of the various substances in the kidney tubules. In toad bladder, kidney slice, intestinal wall and gall bladder experiments, the interactions are usually examined in terms of the mutual effects on one-way fluxes of the various substances under examination.

The types of substances studied include the pentoses, hexoses, and higher sugars; nitrogenous compounds, including amines, amides, and amino acids; organic dyes; and other classes of compounds that importantly alter fluxes or that have been found empirically to have diuretic or antidiuretic effects. The most widely accepted hypothesis as to the mode by which these compounds interfere with others is that there are membrane carriers which transport these substances, and that certain of the substances differentially compete for the carrier substrates and thus compete for transport across the membrane. Because various substances seem to compete with some but not with other substances, it has been necessary to postulate a number of different carrier mechanisms

for various classes of substances. Other differentiations among the classes of competing and noncompeting compounds are based on their relative water–lipoid solubility or their general chemical structure [30]. The implication of the latter classification is that only compounds having certain structures can combine with the substrates of the various carrier systems. The present explanations for these competitive–noncompetitive transport interactions and their influences on the steady–state concentration gradients leave much to be desired. For example, few if any of the many carrier substrates postulated to exist in various membranes have yet been identified in the laboratory.

The present exercise is a preliminary attempt to examine the speculation that the alterations in gradients or fluxes that occur when certain substances are added to biological systems result from changes in the bulk characteristics, especially the electrostatic characteristics, of the biological fluids on either or both sides of membranes. This process differentially alters the activity coefficients of all of the other species present and causes a redistribution of these species between the several fluids. We propose that just as for the simpler systems described earlier, the main mode of action on the electrostatic state of the fluids arises through the effects of the added substances on the dielectric constant of the fluids. Thus, compounds that have a high or low

dielectric increment should be expected to have a large influence in altering concentration gradients and in causing a redistribution of species between fluids.

2.1. A Model of Species Interaction Derived from Chemical Thermodynamics

To test the hypothesis of interaction described above, it will first be useful to derive an expression for the gradients of uncharged species between two fluids of different bulk characteristics. This model can then be checked against appropriate experiments to determine how well it predicts the interactive effects of species on one another.

Consider a two–phase, aqueous, electrolytic system containing similar uncharged substances, $X_1, \ldots, X_j, \ldots, X_J$ in both phases. Let $S_1, \ldots, S_i, \ldots, S_I$ be equilibrium states in which the system is placed as the result of various stresses. Let $g_{j,i}$ be the ratio, $\hat{x}^{I}_{j,i}/\hat{x}^{II}_{j,i}$, of the mole fractions of the species X_j when the system is in the state S_i. Let D^i_I and D^i_{II} be the dielectric constants of phases I and II when the system is in state S_i. Let

$$\lambda_i = \left(\frac{1}{D^{II}_i} - \frac{1}{D^{II}_1} - \frac{1}{D^{I}_i} + \frac{1}{D^{I}_1} \right) . \tag{9}$$

Define A_j to be the quantity A of equation (2) of Sec. 1.1, associated with the species X_j. It is important to observe that A_j does not

depend on the state of the system; that is, we are assuming that A_j is independent of composition. Let $\mu^{I}_{j,i}$ and $\mu^{II}_{j,i}$ be the chemical potentials of X_j in phases I and II respectively, when the system is in the state S_i.

Let us write:

$$\frac{1}{RT}\,\mu^{I}_{j,i} = c^{I}_{j} + A_j\left(\frac{1}{D^{I}_{i}} - \frac{1}{D^{I}_{1}}\right) + \ln \hat{x}^{I}_{j,i} \tag{10}$$

and

$$\frac{1}{RT}\,\mu^{II}_{j,i} = c^{II}_{j} + A_j\left(\frac{1}{D^{II}_{i}} - \frac{1}{D^{II}_{1}}\right) + \ln \hat{x}^{II}_{j,i}\,. \tag{11}$$

Here we have used the result from Edsall and Wyman, quoted in Sec. 1.1, that $RTA_j\left(\frac{1}{D^{I}_{i}} - \frac{1}{D^{I}_{1}}\right)$ and $RTA_j\left(\frac{1}{D^{II}_{i}} - \frac{1}{D^{II}_{1}}\right)$ are the electrostatic components of the chemical potential of X_j in phases I and II respectively when the system is in state S_i. We have also assumed that the system is "ideal" except for the electrostatic components and thus we have assumed that the quantities c^{I}_{j} and c^{II}_{j} are independent of composition and hence do not depend on the state S_i.

At equilibrium*

$$\mu^{I}_{j,i} = \mu^{II}_{j,i}\,. \tag{12}$$

---

*See equation 14.11, p. 100, ref. [74].

If we combine equations (9) through (12) and the definition of $g_{j,i}$, we obtain

$$\ell n\, g_{j,i} = c_j + A_j \lambda_i \tag{13}$$

where $$c_j = c_j^{II} - c_j^{I} .$$

2. 2. Evaluating the Model of Chemical Interaction

The most complete data for evaluating the model represented by (13) above was found in a report of a series of experiments by Pitts [31] in which he examined changes in clearances of xylose, sucrose, creatine, and creatinine as solutions of these substances and water were administered by mouth or given subcutaneously to dogs or human subjects. Pitts' original data are too extensive to warrant reproduction here; the interested reader is referred to the original paper [31]. From these data, $g_{u/p}$ and $\ell n\, g_{u/p}$ were computed using the clearance and urine flow.

The model represented by (13) was fitted by least squares techniques to these data derived from Pitts. The results of this least squares computation is shown in Table 5. As can be seen, the results supply a weak confirmation of the model. We grant that other models could conceivably produce the same functional relationship, (13), among the gradients.

* * * * * * *

Table 5

ROOT MEAN SQUARE FITTING ERRORS BETWEEN EQUATION (13) DERIVED FROM ELECTROSTATIC SOLUTION THEORY AND VARYING CONCENTRATION GRADIENTS, URINE/PLASMA, FOR XYLOSE, CREATINE, CREATININE AND SUCROSE, DERIVED FROM PITTS [31]

Experiment No.	Subject	No. of Chemical Species J	No. of States, I	No. of Effective Parameters, 2J + I − 2	No. of Observations, IJ	Root Mean Square Fitting Error
107	Dog 10	3[c]	6[d]	10	18	0.007
130	Dog 22[b]	3	6	10	18	0.010
133	Dog 22	3	9	13	27	0.020
123	Dog 20	3	5	9	15	0.030
126	Dog 21	3	5	9	15	0.020
103[a]	Dog 12	3	6	10	18	0.020
105[a]	Dog 17	3	6	10	18	0.020
127	Human R. F. P.	3	4	8	12	0.020
128	Human R. F. P.	3	4	8	12	0.003
131	Human M. B.	3	4	8	12	0.005
132	Human M. B.	3	4	8	12	0.005

[a]Experiments 103 and 105 involved the administration and measurement of creatine, creatinine and sucrose. All of the other experiments involved xylose, creatine and creatinine.

[b]States with missing data were not used in fitting.

[c]Concentration gradients, urine/plasma, were computed from values of clearances and urine flows.

[d]States are the various time periods after administration of the chemical stresses at which samples of urine and plasma were taken for analysis.

However sparse the data suitable for evaluating the hypothesis that the bulk electrostatic properties of body fluids are important in establishing and controlling concentration gradients, we believe that the several cases presented, ranging from synthetic clathrates to mammalian renal function, support and certainly do not deny the hypothesis. We believe the membrane also serves important purposes in physiological regulation, and do not intend by our emphasis on fluid properties to deny the importance of membranes in this function.

3. THE ROLE OF THE MEMBRANE

Advocates of the "phase" hypothesis of biological permeability in the Soviet Union originally tended to deny that the membrane played any role in the kind of phenomena we have described. Rather, they held that the bulk characteristics of body fluids were the controlling determinants. This attitude undoubtedly reflected overcompensation for the otherwise widely-held impression that membrane properties could explain almost everything in biology. The current philosophy of the Soviet "phase theorists" is the eminently reasonable one that a combination of the classical membrane theories with the "phase" hypothesis provides the basis for a much more realistic explanation of the facts [6, 73].

It would be unrealistic to deny that membranes separate many types of body fluids from others, and thus prevent large molecules, like proteins, from migrating between these fluids or between certain body compartments. This characteristic plus membrane permeability to smaller charged species and the ionization of the impermeable macromolecules provides a firm basis for explaining the Gibbs-Donnan gradients of charged species that occur between compartments in nature.

If the membrane contains small, molecular-sized channels, sites which can differentially bind Na^+, K^+, H^+ and a source of H^+ as from metabolic processes, then, as elegantly demonstrated

by Dantzig and Pace [33, 34], one may derive a statistical basis for explaining the "sodium pump" mechanism. These relatively simple requirements, that can easily be provided in synthetic membranes, do not stretch one's credulity in respect to membrane structure as do some of the other hypotheses of membrane functions.

Even for the cases of structurally more complex membranes (e. g., isolated anuran skins), a combination of membrane-associated and fluid-associated properties appears to provide a better basis for explaining certain behavior than does the conventional emphasis on membrane alone. For example, the high flux of water through these membranes and the large alteration of this flux with additions of antidiuretic hormone to the bathing media has been attributed to the bulk flow of water through molecular-sized pores and the change of size of the pores with the addition of hormone [35]. The membrane cells have been observed to swell and to shrink as levels of hormone and other substances in the media are altered, thus supposedly changing the effective diameters of the flow channels [36]. It seems reasonable to believe that the alterations in cellular size in these circumstances could result from alterations in the activity coefficients of the various chemical species and in the bulk characteristics of the intracellular, extracellular or of both fluids, resulting in a net movement of substance to or from the membrane cells.*

---

*In some cases, changing hormone levels may alter the permeability of the intercellular cement of membranes [60-65, 75].

Studies of the isolated toad bladder show that this complex membrane also demonstrates the same type of flux behavior as described above [21]. Orloff and Handler ([37] page 260), however, point out that the inequality of the logarithms of the flux and activity ratios for water and the phenomenon of solvent drag* cannot be accepted unquestioningly as evidence of varying sized pores and nondiffusional flow. For example, Sidel and Hoffman [39] observed entirely similar behavior in a nonliving system made from a homogeneous liquid membrane separating two sodium chloride solutions having different concentrations. Orloff and Handler [37] propose that vasopressin (and certain other substances) imposes its influence on membrane permeability by initiating a series of biochemical changes, largely enzymatic in nature, within the membrane cellular medium. We suggest that hormones can influence these reactions by altering the bulk electrostatic properties of the medium. Under *in vitro* conditions, it is known that changes in medium dielectric constant can exert a major influence in altering both the rate of enzymatic reactions and their equilibrium state [40-42]. It seems reasonable to expect that the same electrostatic effect may pertain for *in vivo* situations. The manner in which hormones could alter the electrostatic properties of the medium will be discussed in the next section.

---

*Solvent drag is defined as a flux of a particular solute in the direction of net water flow that is greater than the flux of the same solute in the opposite direction [38].

4. A POSSIBLE MEANS BY WHICH HORMONES MAY ALTER PERMEABILITY AND CONCENTRATION GRADIENTS

From the brief discussion above it is apparent that a combination of relatively simple membrane properties and alterations in the physicochemical properties of chemical substances and of body fluids can provide an attractive basis for explaining many physiological phenomena. As mentioned earlier, however, it is not conventional, at least among western physiologists, to attribute importance to other than the membrane in connection with these phenomena. The complexity assigned to the membrane functions is illustrated by the following statement:

> The columnar absorptive cell of the small intestine must transport from the lumen of the intestine all of the nutrients required for the survival of all of the cells of the animal body. The variety and complexity of transport processes in this cell is quite staggering. One could name 25 transport systems from available data, and there may well be an equal number more. The plasma membrane of this versatile cell possesses the potential for virtually every type of transport mechanism known. [43]

Most of these multitudes of postulated membrane transport processes involve supposed carriers (few if any of which have been positively identified), even for synthetic substances that did not exist as the animal evolved and are purported to be transported by carriers against concentration gradients. We find it more intellectually satisfying (if not yet proved) to consider that many of these substances move from one body compartment to another as dictated

by their relative electrochemical potentials in the two compartments. We find it not at all surprising that substances move in a direction toward a higher concentration; as demonstrated earlier, concentration can be an unreliable indicator of activity in complex electrolytic solutions. In nonliving systems, in fact, such a movement is taken as a demonstration that the activity of the substance is higher in the phase with the lesser concentration.

Then, too, the measurement of the activity of substances in biological fluids is a task of great difficulty, bordering on the fringe of present technical feasibility. Consider the changes that may occur in the activity or activity coefficient of water in plasma and urine during normal nondiuretic conditions and during dilution diuresis. In order to illustrate the magnitude of the change, we may assume that urine and plasma are in equilibirum under both conditions. The activity coefficient of water need change by only 0.8 percent to provide the change in urine concentration that occurs between a normal resting state and in dilution diuresis (e.g., in going from a molality of, say, 0.9 moles kg^{-1} to 0.33 moles kg^{-1}). This is equivalent to a change in partial pressure of water vapor in the fluids of only about 0.4 mm Hg between the two states.

The fact that alterations in concentration levels of hormones cause changes in both the fluxes and steady–state gradients of a wide variety of charged and uncharged chemical substances in

biological systems seems well authenticated and not questioned. Alterations in hormonal levels also selectively influence intra-cellular reactions, especially those involving enzymes [44]. As is usual for explanations of intercompartmental flows, the hormones are postulated to influence the cell membranes by (1) affecting the carrier reaction mechanisms, and (2) affecting physical barriers to permeation through alteration of the membrane structure. Knowledge of the modes of action of different hormones on intra-cellular reactions appears to be in an early, speculative, state. It is generally agreed that very tiny amounts of different hormones can exert large and often opposite effects on biological systems. This extreme potency of hormones has dissuaded explanations of their actions based on gross chemical effects.

The only general hypothesis encompassing hormonal action that has come to our attention is that put forward by Ling [45, 46]. This hypothesis, which Ling calls the "association-induction" theory, postulates that intracellular solutes are strongly associated with protein complexes that are joined in a three-dimensional lattice-work, thus providing a fixed-charge system. By "induction," various counter ions influence the fixed charge arrangement and binding energies of the protein lattice. Because hormones are known to be strongly absorbed onto proteins, the association-induction hypothesis predicts that they (hormones) will alter the

charge distribution and binding energies of the protein lattice, thereby changing the distribution of ionic and other polar species between the cell and the exterior medium ([45] page 458).

Ling denies the applicability of modern solution theory to the cellular medium. This denial is importantly based on weaknesses of the Linderstrom–Lang theory for characterizing solutions containing proteins. We would point out that in more recent years improvements have been made over the old L–L theory in models of solutions containing proteins. Secondly, no differences in reaction kinetics or in equilibrium states have been noted in many situations involving reactions of proteins that occur in the cell and in equivalently concentrated solutions. Hemoglobin solutions and the red cell are perhaps the most carefully studied examples [47, 48]. Thirdly, many fluids whose properties may be altered by changing levels of hormones are not intracellular fluids. Examples are blood plasma, cerebrospinal fluid, aqueous humor, bile, etc. Thus, for reasons like those given above, we believe that Ling's proposal to discard the not inconsiderable physicochemical theory relating to solutions containing macromolecules is premature.

We are impressed, however, by the implications of the strong chemical attraction of hormones for a wide variety of proteins. In addition to the references to this phenomenon given by Ling ([45] p. 458), we add that of Talalay et al. ([49] p. 881)

for the specific cases of the steroid hormones. Talalay et al. propose that the physiologically effective result of hormone-protein association is transhydrogenation. More importantly, we believe, Talalay et al. propose a selective attraction of the hormones for particular proteins that are tissue specific. Thus, various tissues would respond in different degree, or not at all, to changes in the level of hormones, depending upon their content of the specific associative proteins (hydroxysteroid dehydrogenases in this case).

These associative characteristics of hormones and proteins are not necessarily limited to enzymatic proteins ([50], p. 215). The change in hormonal concentration can elicit a large number of different biological actions. Tata ([51], p. 92) raises the important question as to "whether this multiplicity of action [of thyroid hormones] results from different and independent interactions with various cellular sites or whether it represents multiple secondary manifestations of a fundamental biochemical effect." He concludes that "recent work favors the second possibility at least for the major physiological actions." We propose that this fundamental biochemical effect of hormones is realized through (1) their influence in altering the polar nature of specific proteins and (2) the resulting change in the bulk electrostatic characteristics of the various physiological fluids containing them. This influence of hormones on proteins may be accomplished through strong chemical association or actual chemical

binding. The ensuing effects on the fluid bulk characteristics can result from changes in the affected protein structural geometry and/or binding site energies which then exert influences on the polarity of the molecule.

4.1. Justification for the Protein-Interactive, Bulk-Fluid Mode of Hormonal Influence

Far more questions are raised by this hypothesis of hormonal action than the available data can answer. In attempting to justify consideration of the hypothesis, we assume that our previous examples of the influence of the bulk electrostatic characteristics upon concentration gradients of chemical substances between physiological compartments have been sufficiently convincing so that our present task is only to examine the possibilities for hormonal alteration of these bulk electrostatic characteristics. If so, one of the first questions that arises is whether the hypothesis corresponds with the findings that very small amounts of hormones can have large effects on concentration gradients and on the amounts of substances in various body compartments. We find in the literature no reports in which the dielectric constants of body fluids are measured under conditions such that the influence of proteins are apparent. The several dielectric studies of blood serum have been made at very high frequencies, to avoid measurement losses due to conduction so that anomalous dispersion of the protein molecules occurs and

the measured dielectric constant reflects mostly the properties of the solvent at high frequencies and not those of the total fluid at *in vivo* conditions.*

The dielectric constant of blood plasma and the possible contributions of plasma proteins to this value may be computed, however, if certain simplifying assumptions are made. These assumptions are indicated in Table 6. where the basis for this computation is given.

The rather remarkable result of the computations shown in Table 6 is that, within the limitations of the assumptions, the plasma proteins appear to have the potential for establishing the dielectric constant of plasma at a very high value with respect to water or with respect to other body fluids that do not contain much protein. Noticing from Table 6 that electrolytes (represented by NaCl) and other nonelectrolytes (represented by urea) are not of great importance in establishing the dielectric constant of the fluid, we compute, for example, that the dielectric constant of urine is probably close to that for water.

Good evidence exists that the antidiuretic hormone circulates in the blood bound to plasma protein [54]. More specifically, the hormone appears to circulate as a definite protein–peptide complex

---

*Because of the very large effect of the suspended red cells on the dielectric constant of whole blood, reported values of the dielectric constant of whole blood are useless for establishing a value for plasma.

Table 6

CALCULATION OF THE PROTEIN CONTRIBUTION TO THE DIELECTRIC CONSTANT OF BLOOD PLASMA[a]

Substance	Molecular Weight, gm	Amt. per kg Plasma	Mole Fractions, N_2	Dielectric Increments[b] $\partial D/\partial g$	$\partial D/\partial m$	$\partial D/\partial N_2$
Serum Albumin	69,000	13.1 g	3.42×10^{-6}	0.17	11,730	6.511×10^{5}
Mercaptalbumins	69,000	26.2 g	6.85×10^{-6}	0.87	60,000	3.33×10^{6}
Globulins (all) Fibrinogen	180,000 avg.	26.2 g	2.62×10^{-6}	1.08	194,000	1.077×10^{7}
β Globulins	180,000 avg.	10 g	1.00×10^{-6}	4.10	737,200	4.09×10^{7} [c]
Globulins (rest) Fibrinogen	180,000 avg.	16.2 g	1.62×10^{-6}	1.08	194,000	1.077×10^{7}
Urea	60.06	.005 moles	1.02×10^{-4}	——	3.4	1.887×10^{2}
Na^+	22.99	0.16 moles	2.87×10^{-3}	——	−5.5	-3.053×10^{2}
Cl^-	35.46	0.16 moles	2.87×10^{-3}	——	−5.5	-3.053×10^{2}

$\Delta = \partial D/\partial N_2$; $\beta = -\Delta/D_0^2$; $D = D^0 + \Sigma\beta_i N_2^i$; $\log f_1' - \log f_1 = -A(1/D_2 - 1/D_1)(N_3/(1 - N_3))$[d]; $D_1 = 231$, $D_2 = 622$; $f_1'/f_1 = 1.0022$.

[a]Data are from [3, 52, 53].

[b]Dielectric data are for 25°C as no values are reported for 37°C. The dielectric increments are assumed to have the same values in plasma as in water in which they were determined.

[c]The values within the dotted lines are used to compute D_2, the dielectric constant of plasma at elevated ADH levels. When combined with ADH, the β globulins are assumed to demonstrate an increase in their dielectric increments by a factor of 3.8, the same increase demonstrated by hemoglobin when combined with oxygen [56].

[d]These formulas, used to compute the dielectric constants of plasma under two conditions and the possible change in the activity coefficient for water, are derived from equations of [3] as used and defined in the first section of this paper.

with the β globulins [55]. The question now is, if when combining with the β globulins the hormone alters the dielectric increment of the β globulins, could this alteration significantly change the dielectric constant of plasma? There appear to be about 10 gm kg^{-1} of β globulins in normal plasma ([53] pp. 554–555). If it is assumed that the dielectric increment of these porteins (not complexed with ADH) in plasma is the same as that measured in water for purified pseudoglobulins [57], then, as indicated in Table 6, the computed dielectric constant of the plasma is 231. Upon complexing with ADH, the dielectric increment of these β globulins is assumed to increase by the same factor (3.8) as does hemoglobin in complexing with oxygen [56].* As a result of this combination we compute an increase in the dielectric constant of plasma to 622. Using the simplified equation of Edsall and Wyman [3] as described in the early part of this paper, we compute a 0.22 percent increase in f_1, the activity coefficient of water, as a result of this change in dielectric properties. This alteration in activity coefficient of water is of the same order of magnitude as the 0.8 percent increase we previously computed as required to change the osmolarity of

---

*Hemoglobin is the only protein for which we could find any data on the effect of chemical combination on the dielectric increment. The assumed value of 4.1 as $\partial D/\partial g$ for the complexed globulin–hormone is small compared to values ranging up to 2200 as the dielectric increment on the same scale, for macromolecules whose shape may be other than globular [58].

urine from that of a hydropenic state to that of frank dilution diureses. In spite of the many assumptions made in these computations, it is somewhat encouraging to note that it appears feasible for alterations in small amounts of hormone, operating by changing electrostatic properties of fluids, to exert a large effect on the system.

Alterations in the degree of complexing of ADH, operating through its effects on the bulk electrostatic properties of plasma as described, could also affect the distribution between plasma and tubular fluid of substances other than water. Thus at least some of the changes in clearances and rates of excretion observed between the diuretic and nondiuretic states may result from this phenomenon.

Even more generally, this mode of action for hormones could provide a rich basis for explaining some hormonal phenomena that otherwise appear rather mysterious. The localized action of specific hormones can be explained by their selectivity in complexing with macromolecules that are segregated in specific inter– or intra–cellular compartments. Mutually antagonistic effects of certain hormones could result from competition for complexing sites; differing solution effects would depend upon which hormone complexed more completely. The similar effects of certain hormones (differing only in degree) could result from complexing with the same or other

sites that result in comparable fluid–property alterations. Changes in the extent or velocity of enzymatic reactions with hormonal levels can be the reflection of changed electrostatic fluid properties on these biochemical reactions.

Modern solution theory and practice leave little doubt that the fluid properties discussed here influence reaction rates and the distribution of chemical substances between phases. The important uncertainty is whether in biological systems these fluid phenomena exert significant, quantitative effects. This survey, admittedly imperfect for practical and theoretical reasons, has convinced the authors that these fluid phenomena may be quantitatively important in physiological regulation.

REFERENCES

1. Washburn, E.W. (ed.) International Critical Tables, McGraw-Hill Book Co. Inc., New York, 1929.
2. Harned, H. S. and B. B. Owen, The Physical Chemistry of Electrolytic Solutions, 3rd ed., Reinhold Publishing Corp., New York, 1958.
3. Edsall, J. T. and J. Wyman, Biophysical Chemistry, Vol. 1, Academic Press, Inc., New York, 1958.
4. Schwan, Herman P., "Electrical Properties of Tissue and Cell Suspensions," Advances in Biology, Medicine and Physiology, Vol. 5, Academic Press, New York, 1957, pp. 147-209.
5. Troshin, A. S., "The Distribution of Substances between a Cell and a Medium," Biulletin experimental noi biologii i meditsiny, 31, 1951, 180-185.
6. Troshin, A. S., "Theories of Permeability," Rukovodstvo Po Tsitologii, Vol. I (Manual of Cytology), Nauka, 1965, pp. 537-556.
7. Malmberg, C. G. and A. A. Maryott, "Dielectric Constants of Aqueous Solutions of Dextrose and Sucrose," Jnl. Res. Natl. Bur. Stds., 45, 1950, pp. 299-303.
8. Lindenberg, B. A. and S. Zuili, "Variation in the Solvent Power of Cellular Water as a Function of the Dielectric Increment of Solute Molecules," Comp. rend., 234, 1952, pp. 2573-2575.
9. Lindenberg, A. B. and M. Polonovski, "The Influence of Dielectric Polarity on the Intracellular Concentration of Diffusible Organic Substances," J. Physiol.,(Paris), 46, 1954, pp. 435-436.
10. De Haven, J. C. and N. Z. Shapiro, Intrinsic Control of Body Fluid and Electrolyte Distribution and Urine Formation, The RAND Corporation, RM-4609-PR, July 1965.
11. De Haven, J. C. and N. Z. Shapiro, "On the Control of Urine Formation," Supplementum ad Nephron, 4, 1967, pp. 1-63.
12. Dittmer, D. S. (ed.) Blood and Other Body Fluids, Federation of American Societies for Experimental Biology, Washington, D.C., 1961.
13. Smith, H. W., The Kidney, Oxford University Press, New York, 1958.
14. Wyman, J., Jr., "The Dielectric Constant of Solutions of Dipolar Ions," Chem. Revs., 19, 1936, p. 213.

15. Nicholes, H. J. and R. C. Herrin, "The Tubular Reabsorption of Urea, Thiourea and Derivatives of Thiourea in the Dog Kidney," Am. Jnl. Physiol., 135, 1941, pp. 113-123.

16. Collander, R., "Die Verteilung organischer Verbindungen zwischen Aether und Wasser," Acta. Chem. Scand., 3, 1949, pp. 717-747.

17. Macy, R., "Partition Coefficients of Fifty Compounds between Olive Oil and Water at 20° C," Jnl. Ind. Hyg. Toxicol., 30, 1948, pp. 140-143.

18. Peters, L., "Renal Tubular Excretion of Organic Bases," Pharm. Revs., 12, 1960, pp. 1-35.

19. Webber, W. A., J. L. Brown and R. F. Pitts, "Interaction of Amino Acids in Renal Tubular Transport," Am. Jnl. Physiol., 200, 1961, pp. 380-386.

20. Sperber, I., "Competitive Inhibition on Specificity of Renal Tubular Transport Mechanisms," Arch. Int. Pharmacodyn., XCVII, No. 2, 1954, pp. 221-231.

21. Leaf, A. and R. M. Hays, "Permeability of the Isolated Toad Bladder to Solutes and Its Modification by Vasopressin," Jnl. Gen. Physiol., 45, 1962, pp. 921-932.

22. Rosenberg, L. E., S. J. Downing and S. Segal, "Competitive Inhibition of Dibasic Amino Acid Transport in Rat Kidney," J. Bio. Chem., 237, 1962, pp. 2265-2270.

23. Sperber, I., "Secretion of Organic Anions in the Formation of Urine and Bile," Pharm. Rev., 11, 1959, pp. 109-134.

24. Diamond, J. M., "The Reabsorptive Function of the Gall Bladder," J. Physiol., 161, 1962, pp. 442-473.

25. Wool, I. G., "Relation of Effects of Insulin on Amino Acid Transport on Protein Synthesis," Fed. Proc., 24, 1965, pp. 1060-1070.

26. Davson, H. and M. Pollay, "Influence of Various Drugs on Transport of 131 I and PAH Across the Cerebrospinal Fluid-Blood Barrier," J. Physiol, 167, 1963, pp. 239-246.

27. Ross, E. J., "Insulin-Like Effect of Anterior Pituitary Extract in Accelerating the Transfer of Glucose Across the Blood Aqueous Humor Barrier," J. Physiol., 127, 1955, pp. 247-257.

28. Hytten, F. E. and I. Leitch, The Physiology of Human Pregnancy, F. A. Davis Company, Philadelphia, 1963.

29. Pitts, R. F., Physiology of the Kidney and Body Fluids, Year Book Medical Publishers, Chicago, 1964, pp. 75-86, 118-124.

30. Despopoulos, A., "A Definition of Substrate Specificity in Renal Transport of Organic Anions," J. Theoret. Biol., 8, 1965, pp. 163-192.

31. Pitts, R. F., "The Clearance of Creatine in Dog and Man," Am. Jnl. Physiol., 109, 1934, pp. 532-541.

32. Greenstein, J. P. and J. Wyman, Jr., "Further Studies on Dielectric Constant and Electrostrition of Amino Acids and Peptides," J.A.C.S., 58, 1936, pp. 463-465.

33. Dantzig, G. B., "New Mathematical Methods in the Life Sciences," Amer. Math Monthly, 71, 1964, pp. 4-15.

34. Dantzig, G. B. and N. Pace, Molecular-Sized Channels and Flows Against the Gradient, Research Report 31, Operations Research Center, University of California, Berkeley, October 1962.

35. Koefoed-Johnsen, V. and H. H. Ussing, "The Contributions of Diffusion and Flow to the Passage of D_2O Through Living Membranes," Acta. Physiol. Scand., 28, 1953, pp. 60-76.

36. Whittembury, G., "Action of Antidiuretic Hormone on the Equivalent Pore Radius at Both Surfaces of the Epithelium of the Isolated Toad Skin," J. Gen. Physiol, 46, 1962, pp. 117-129.

37. Orloff, J. and J. S. Handler,"Mechanisms of Action of Antidiuretic Hormones on Epithelial Structures," in Hoffman, J. F. (ed.) The Cellular Functions of Membrane Transport, Prentice-Hall, Inc., Englewood Cliffs, N.J., 1964.

38. Anderson, B. and H. H. Ussing, "Solvent Drag on Non-Electrolytes During Osmotic Flow Through Isolated Toad Skin and Its Response to Antidiuretic Hormone," Acta. Physiol. Scand., 39, 1957, pp. 228-239.

39. Sidel, V. W. and J. F. Hoffman, "Water Transport Across Membrane Analogues," Fed. Proc., 20, 1961, p. 137.

40. Hiromi, K., "Theory of the Influence of the Dielectric Constant on the Rate of Reaction in Solution with Application to Enzyme Reactions," Bulletin Chem.Soc. of Japan, 33, 1960, pp. 1251-1269.

41. Castañeda-Agullo, M. and L. M. Del Castillo, "The Influence of the Medium Dielectric Strength Upon Trypsin Kinetics," J. Gen. Physiol., 42, 1959, pp. 617-634.

42. Vogelhut, P. O., The Dielectric Properties of Water and Their Role in Enzyme-Substrate Interactions, Institute of Engineering Research, University of California (Berkeley) Series No. 60, Issue No. 476, August 24, 1962.

43. Wilson, T. H., "Structure and Function of the Intestinal Absorptive Cell," in, J. F. Hoffman (ed.), The Cellular Functions of Membrane Transport, Prentice Hall, Inc., Englewood Cliffs, N. J., 1963, p. 215.

44. Litwack, G. and D. Kritchevsky (eds.), Actions of Hormones on Molecular Processes, John Wiley and Sons, Inc., New York, 1964.

45. Ling, G. N., A Physical Theory of the Living State, Blaisdell Publishing Co., New York, 1962.

46. Ling, G. N., "The Membrane Theory and Other Views for Solute Permeability, Distribution, and Transport in Living Cells," Perspectives in Biology and Medicine, IX, Autumn 1965.

47. Roughton, F. J. W., J. W. Legge and P. Nicolson, "The Kinetics of Haemoglobin in Solution and in the Red Blood Corpuscle," in, F. J. W. Roughton and J. C. Kenchew (eds.), Haemoglobin, Interscience Publishers, Inc., New York, 1949, pp. 67-82.

48. Roughton, F. J. W., personal communication, July 1966.

49. Talalay, P., B. Hurlock and H. G. Williams-Ashman, "On a Coenzymatic Function of Estradiol-17β," Proc. Natl. Acad. Sci., 44, 1958, pp. 862-884.

50. Tomkins, G. M. and K. L. Yielding, "Action of Steroid Hormones on Glutamic Dehydrogenase," in, G. Litwack and D. Kritchevsky (eds.), Actions of Hormones on Molecular Processes, John Wiley and Sons, New York, 1964.

51. Tata, J. R., "Biological Action of Thyroid Hormones at the Cellular and Molecular Level," in G. Litwack and D. Kritchevsky (eds.), Actions of Hormones on Molecular Processes, John Wiley and Sons, New York, 1964, pp. 58-131.

52. Hasted, J. B., D. M. Ritson, and C. H. Collie, "Dielectric Properties of Aqueous Ionic Solutions," Jnl of Chem. Phys., 16, No. 1, 1948, pp. 1-21.

53. Diem, K. (ed.) Documenta Geigy, 6th ed., Geigy Pharmaceuticals, Ardsley, New York, 1962.

54. Heller, H., "The Metabolism and Fate of the Neuro-hypophysial Principles," in, H. Heller (ed.), The Neurohypophysis, Academic Press, Inc., New York, 1957, pp. 77-96.

55. Thorn, N. A. and L. Silver, "Chemical Form of Circulating Antidiuretic Hormone in Rats," Jnl. Exp. Med., 105, 1957, pp. 575-583.

56. Takishima, S. and R. Lumry, "Dielectric Properties of Hemoglobin," J.A.C.S., 80, 1958, pp. 4238-4248.

57. Oncley, J. L., "The Investigation of Proteins by Dielectric Measurements," Chem. Rev., 30, 1942, pp. 433-450.

58. Dintzis, H. M., J. L. Oncley and R. M. Fuoss, "Dielectric Increments in Aqueous Solutions of Synthetic Polyelectrolytes," Proc. N.A.S., 40, 1954, pp. 62-70.

59. Pappenheimer, J. R., S. R. Heisey and E. F. Jordan, "Active Transport of Diodrast and Phenolsulfonphthalein from Cerebrospinal Fluid to Blood," Am. J. Physiol., 200, 1961, pp. 1-10.

60. Ginetzinsky, A. G., "Role of Hyaluronidase in the Reabsorption of Water in Renal Tubules: The Mechanism of Action of the Antidiuretic Hormone," Nature, 182, 1958, pp. 1218-1219.

61. Natochin, Y. V., "Hyaluronidase Secreted by the Kidneys in Various Classes of Vertibrates," Byull. Eksptl. Biol. i Med., 48, 1959, pp. 10-14.

62. Dicker, S. E. and M. G. Eggleton, "Hyaluronidase and Antidiuretic Activity in Urine of Man," J. Physiol., 154, 1960, pp. 378-384.

63. Berlyne, G. M., "Urinary Hyaluronidase, A Method of Assay and Investigation of Its Relationship to the Urine Concentrating Mechanism," Clin. Sci., 19, 1960, pp. 619-629.

64. Leaf, A., "Some Actions of Neurohypophyseal Hormones on a Living Membrane," J. Gen Physiol., 43, Suppl. 1, 1960, pp. 175-189.

65. Bentley, P. J., "Hyaluronidase, Corticosteroids and the Action of Neurohyphysial Hormone on the Urinary Bladder of the Frog," J. Endocrin., 24, 1962, pp. 407-413.

66. Parpart, A. K. and J. C. Shull, "Solvent Water in the Normal Mammalian Erythrocyte," J. Cell. Comp. Physiol., 6, 1935, pp. 137-150.

67. Savitz, D., V. W. Sidel and A. K. Solomon, "Osmotic Properties of Human Red Cells," J. Gen. Physiol., 48, 1964, pp. 79-94.

68. Bozler, E., "Distribution of Nonelectrolytes in Muscle," Am. J. Physiol, 200, 1961, pp. 651-655.

69. O'Konski, C. T., "Theory of Ionic Polarization in Polyelectrolytes," J. Phy. Chem., 64, 1960, pp. 605-619.

70. Akerlof, G., "Dielectric Constants of Some Organic Solvent-Water Mixtures at Various Temperatures," J. A. C. S., 54, 1932, pp. 4125-4139.

71. Long, F. A. and W. F. McDevit, "Activity Coefficients of Nonelectrolyte Solutes in Aqueous Salt Solutions," Chem Rev., 51, 1952, pp. 119-169.

72. Hill, T. L., "Osmotic Pressure, Protein Solutions and Active Transport," J. A. C. S., 80, 1958, pp. 2923-2926.

73. Sedunov, B. I. and D. A. Frank-Kamenetskii, "Dielectric Constants of Biological Objects," Soviet Physics Uskekhi, 6, No. 2, 1963, pp. 279-293.

74. Moore, W. J., Physical Chemistry, 3rd ed., Prentice-Hall, Inc., Englewood Cliffs, N.J., 1964.

75. Corbascio, A. N. and L. Dong, "The Mechanism of Action of Antidiuretic Hormone," Investigative Urology, 4, 1966, pp. 267-272.