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ACID-BASE METABOLISM AND THE PROTON CONDITION

C. D. Russell

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PREFACE

This Memorandum forms part of a continuing program at RAND in the application of mathematical methods to biomedical problems. It resolves some questions of definition which arise when one seeks to represent medical acid-base concepts in mathematical terms, and will be of interest mainly to biochemists and others who study medical acid-base problems from a theoretical standpoint. This work was supported in part by the National Institutes of Health under Grant No. 5-T5-GM-1631-08 to the Tulane University School of Medicine.

The author is a consultant to The RAND Corporation. A former research chemist, he is now an M.D. candidate at the Tulane University School of Medicine, New Orleans.

SUMMARY

This Memorandum applies the proton condition of inorganic chemistry to the problem of describing the net acid content in a biological system. A "proton content" is defined which expresses the net acid content in terms of the detailed chemical composition of the system. This approach is compared with previous approaches to the quantitative description of acid-base metabolism and then applied to the interpretation of experimental metabolic balances.

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CONTENTS

I. INTRODUCTION

Acid-base metabolism has attracted a wide variety of conceptual approaches based on an equally wide variety of experimental procedures. The resulting semantic confusion [1] has hindered progress and led to unnecessary controversy. This Memorandum may add to the confusion, for it explores concepts of acid-base metabolism from still another viewpoint: mathematical definitions in terms of a "proton content" which is closely related to the proton condition of inorganic chemistry.* On the other hand, this new approach makes possible the definition of older concepts in unambiguous terms, clarifying their relationships to each other and to the molecular constitution of the system studied.

The analysis of acid-base disturbances in biological systems leads naturally to the concepts of ingestion, excretion, generation, consumption, and accumulation of acid or base; but these concepts are not easily formulated in precise quantitative terms. The proton condition is particularly useful in describing quantitatively the net Brønsted acid content of mixed inorganic buffers, and

*For example, see Ref. 2, p. 454.

this suggests that an analogous formalism might profitably be applied to biological systems. In this Memorandum, the proton-condition formalism is applied to two distinct problems in acid-base metabolism: 1) the measurement of the net content of labile, pH-determining protons in a biological system (Sec. II); 2) the interpretation of metabolic input-output balances (Sec. III). Certain previous approaches to these problems are discussed and shown to be special cases of the present unifying approach.

The proton condition can most easily be explained in terms of an example. (See the Appendix for more formal treatment.) Consider a solution prepared by dissolving n_{HAc} moles of acetic acid in enough water to make 1 liter of solution. This solution is made up of two homologous series of Brønsted acids and bases. The species H_3O^+, H_2O, and OH^- constitute one homologous series (assigned the index i=1 in the following discussion) and the species HAc and Ac^- constitute the other (assigned the index i=2). Arbitrarily choose one reference species from each set, e.g., H_2O and HAc. Assign to each species a <u>protonation index</u>, p_{ij}, equal to the difference between the number of protons it bears and the number borne by the reference species for the series to which it belongs. Thus the

protonation indexes, given the above reference states, are:

(1)

for H_3O^+,	$p_{11} = +1$	for HAc,	$p_{21} = 0$
for H_2O,	$p_{12} = 0$	for Ac^-,	$p_{22} = -1$
for OH^-,	$p_{13} = -1$		

This table also defines the two-numeral subscript ij used to identify each species. Let $[A_{ij}]$ represent the true molar concentration of each species; and let c_{ij} represent the "apparent," "formal," or "analytical" concentrations of the primary "components." The word "component" is used here in the same sense as in the phase rule of Gibbs, so that the primary components in this example are HAc and H_2O. (These also happen to be the reference states in this case, but in general they need not be.) The proton condition then states that, provided the volume of the system remains constant and only proton-transfer reactions occur,

$$(2) \qquad \sum_{i=1}^{2} \sum_{j=1}^{J_i} p_{ij}[A_{ij}] = \sum_{i=1}^{2} \sum_{j=1}^{J_i} p_{ij}\, c_{ij}$$

where the summation limit J_i is equal to the number of distinct species in series i. In the example chosen, Eq. (2) becomes

$$+1[H_3O^+] + 0[H_2O] -1[OH^-] + 0[HAc] -1[Ac^-]$$

$$= 0c_{H_2O} + 0c_{HAc} \ ;$$

or

$$[H_3O^+] - [OH^-] - [Ac^-] = 0 \ . \tag{3}$$

In this case, the proton condition happens to lead to the electroneutrality equation; but it need not, depending on the choice of reference states.

The proton condition as stated above applies only when the sole reactions involving members of the various homologous series (other than those members which are reference states) are proton transfer reactions. To clarify this, consider the reaction:

$$Cl_2 + 2H_2O \rightleftharpoons H_3O^+ + Cl^- + HOCl \ . \tag{4}$$

Here H_3O^+ and HOCl, members of homologous acid-base series, are formed from Cl_2, which is not a member of a homologous series. Unless H_3O^+ and HOCl are chosen as reference states, the above proton condition will not hold for systems in which this reaction occurs. Such a situation can be treated in either of two ways. One is to include Cl_2 in the proton condition, assigning to it a protonation index equal to the number of protons consumed in forming Cl_2 from reference members of the various homologous series. This can be useful in a simple system where Cl_2 is formed only by a single reaction. If Cl_2 can be formed by more than one route, however, the above rule leads to a unique protonation index only when the reference state for each homologous series is correctly chosen. Such an approach is explored below (Sec. III).

An alternative approach is to regard the left-hand side of Eq. (2) as a measure of labile hydrogen in the system, which can vary if reactions occur which are not simple proton transfers. Terming this quantity the proton content, P,

$$P = \sum_{i=1}^{I} \sum_{j=1}^{J_i} p_{ij}[A_{ij}] \tag{5}$$

where I represents the number of different homologous series. The "proton condition" is then expressed by the statement: P is constant. P represents simply the net number of labile protons in the system less those contributed by the reference species when regarded as Gibbsian "components." The proton content so defined is invariant under proton-transfer reactions and under the addition or removal of reference substances, but reactions such as Eq. (4) serve as sources and sinks.

This dichotomy between proton-transfer reactions and reactions generating labile hydrogen is useful in the analysis of biological systems, for proton-transfer reactions tend to be rapid and determine the pH of a solution at any instant, while the source reactions are slower and determine the variations of pH with time. The proton content can thus be regarded as a primary variable determining the pH of a solution at any given time, and the metabolic processes tending to change the pH can be regarded as sources and sinks in a conservation equation for P. This is the approach in Sec. II following.

II. LABILE PROTON CONTENT IN A BIOLOGICAL SYSTEM

SINGLE PHASE BIOLOGICAL SYSTEMS

First, we should outline the conceptual framework of the following analysis. We deal with the chemical "anatomy" of a biological system; the state of the system at a given time is specified when the chemical composition at each spatial point in the system is specified. Mathematically, the system is approximated by a finite set of homogeneous fluid compartments, and its state at a given time is represented by specifying the composition of each compartment. Generally, the state of the system is a function of time; if the state does not change with time, the system is, by definition, in a steady state. A proton content based on Eq. (5) is defined below for such biological systems. The definition involves the choice of suitable reference states and the modification of the above formalism to cover the case of biological macromolecules. The following definition seeks to endow the ensuing proton function with properties analogous to those of the "base excess" of Astrup, et al. [3-5] (discussed below).

The most convenient choice of reference state for a constituent of a biological system depends on the nature

of the constituent. For this reason, the subsequent discussion covers separately the inorganic or micromolecular constituents, the macromolecular constituents, the chemically undetermined constituents, and the carbonic acid series. The net proton content is then formulated in terms of these reference states.

Reference States for Inorganic and Micromolecular Constituents. In inorganic chemistry, the reference states for the proton condition are usually chosen to be the components from which the solution was prepared, since the quantity of each component is thereby eliminated from the final equation. In a biological system, on the other hand, it is more convenient to choose for each reference state not a single species, but a mixture of species belonging to the same homologous series, in proportions corresponding to their equilibrium proportions at a given pH. The proton condition can be written in terms of such reference states, but to do so requires the introduction of non-integral protonation indexes.

Consider the maximally protonated member H_nA^{+m} of some homologous series for which the protonated species existing in solution are known and small in number. Such a series will generally be inorganic or micromolecular.

Let

$$p_{i1} = \nu_i \text{ for } H_nA^{+m} ,$$

$$p_{i2} = \nu_i - 1 \text{ for } H_{n-1}A^{m-1} ,$$

$$\vdots$$

$$p_{ij} = \nu_i - j+1 \text{ for } H_{\nu_i-j+1}A^{m-j+1} ,$$

where ν_i is arbitrary and is not necessarily an integer. For any arbitrary ν_i, the proton content defined by Eq. (5) is then conserved under proton-transfer reactions. To select as reference state for this homologous series the mixture of species corresponding to a given reference pH, pH_0, proceed as follows. Let f_{ij} be defined by the equation

$$f_{ij} = \frac{[A_{ij}]_o}{\sum_{j=1}^{J_i}[A_{ij}]_o} , \tag{6}$$

in which $[A_{ij}]_o$ represents the concentration of species A_{ij} at $pH = pH_0$, as the fraction of the total moles of components of the series which is in the form of species

A_{ij} at pH = pH_0. Then let the reference state for species i be defined by the equation

$$(7) \qquad \sum_{j=1}^{J_i} p_{ij} f_{ij} = \sum_{j=1}^{J_i} (\nu_i - j+1) f_{ij} = 0 \ .$$

The value of ν_i obtained by solving this equation determines the protonation index $p_{ij} = \nu_i - j+1$ for each species in the series. The corresponding proton content is

$$(8) \qquad P_{inorg} = \sum_{i=1}^{I} \sum_{j=1}^{J_i} p_{ij} [A_{ij}] \ .$$

Neglecting activity effects, this choice of reference state for series i makes the proton function P independent of the amount of component i, in its reference state, which is added to the system. Again, if activity coefficients be neglected, the addition of a constituent to a system at the reference pH will not affect the pH, provided the constituent is added in the form of its reference state; i.e., in the form of a buffer mixture having a pH equal to the chosen reference pH, pH_0. Generally, it is most convenient to choose as reference pH the physiologically normal pH of the system, although this is not essential to the formalism.

One could allow for activity effects in the definition of the reference state by specifying that the concentrations appearing in Eq. (6) be the normal physiologic values.

Reference States for Macromolecular Components. The acid-base behavior of complex biological molecules is hard to interpret in terms of distinct molecular species varying in the site and extent of protonation. It is more convenient to speak of the charge function,

$$z_k = Z_k(\mathrm{pH}) \quad , \tag{9}$$

describing the mean net electrical charge z_k on the molecule k as a function of pH. The analog of p_{ij} is then

$$p_k = z_k - \mathfrak{I}_k \quad , \tag{10}$$

where the constant $\mathfrak{I}_k$ may be chosen arbitrarily. Much as before, $\mathfrak{I}_k$ may be set equal to the value of z_k at some reference pH, pH_0:

$$\mathfrak{I}_k = Z_k(\mathrm{pH}_0) \quad . \tag{11}$$

If pH_0 is the physiologically normal pH of the system, this corresponds to choosing a physiologically neutral preparation of the macromolecular component as a reference state. The proton content P_{macro} for the macromolecular components of a system then becomes

$$(12) \qquad P_{macro} = \sum_{k=1}^{K} p_k[A_k] = \sum_{k=1}^{K} (z_k - \mathfrak{I}_k)[A_k]$$

where K is the number of macromolecular components and $[A_k]$ is the molar concentration of component k. Each index k may be associated either with a single macromolecular species or with a constant-coefficient linear combination of different species; e.g., it can refer to a single protein or to a mixture of proteins occurring in fixed proportions to each other.

Reference States for Chemically Unidentified Components. The unidentified components of a biological fluid can be grouped together and treated in the same way as a single macromolecular component, if it is assumed that they occur in constant proportions to each other. The net charge z'_u in faradays per gram of unidentified solids can be described

by the charge function $Z'_u(pH)$ determined experimentally by titration of the unidentified material. (Since the weight of a given molar quantity of protein will depend somewhat on the pH and the nature of the counter-ion present, the weight of the undetermined components should in principle be based on a reference state in which these variables are specified.) If the concentration c_u of the unidentified components is expressed in grams per liter, then the proton content P_u for the unidentified components may be written

$$(13) \qquad P_u = (z'_u - \mathfrak{Z}'_u)c_u \ .$$

The constant $\mathfrak{Z}'_u$ could be chosen arbitrarily, but is most conveniently set equal to the value of z at the reference pH,

$$(14) \qquad \mathfrak{Z}'_u = Z'_u(pH_0) \ .$$

Reference States for the Carbonic Acid Series. In general, it proves most convenient to define the reference state for carbonic acid as H_2CO_3. This choice makes the proton content invariant upon gain or loss of carbon

dioxide from the system, but it leaves the proton content dependent on the bicarbonate concentration, since the protonation index for HCO_3^- is then -1. The proton content for the carbonic acid series is therefore

$$P = - [HCO_3^-] - 2[CO_3^{-2}] ,$$

where the last term can be neglected at physiological pH. To give the proton content the value zero under basal physiologic conditions, it is desirable to add the physiologically normal bicarbonate concentration $[HCO_3^-]_o$, so that

$$P_{carb} = [HCO_3^-]_o - [HCO_3^-] . \qquad (15)$$

The Net Proton Function for a Single-Phase System.

The net proton content for the system as a whole can now be written:

$$P = P_{inorg} + P_{macro} + P_u + P_{carb} . \qquad (16)$$

More completely,

$$(17)\quad P = \sum_{i=1}^{I} \sum_{j=1}^{J_i} (\nu_i - j+1)[A_{ij}] + \sum_{k=1}^{K} (z_k - \mathfrak{I}_k)[A_k]$$

$$+ (z'_u - \mathfrak{I}'_u)c_u + [HCO_3^-]_o - [HCO_3^-] .$$

In this final expression for the proton content of a single-phase biological fluid, the first term represents the known inorganic and micromolecular species exclusive of HCO_3^- and CO_2; the second term, the known macromolecular components; the third term, the undetermined components (assumed present in fixed proportions to each other); and the remaining terms, the carbonic acid series.

MULTIPHASE BIOLOGICAL SYSTEMS

Biological systems are in general multiphase systems. We assume that the system can be adequately approximated by a collection of a small number of distinct phases; in the simplest case, an intracellular and an extracellular. It is tempting to define the proton content for a multi-phase system as the sum of proton contents for the individual phases, each defined as above. This definition is unsatisfactory, however, for the proton content so

defined is not invariant under transfers of species from one phase to another. If the proton content for each phase is chosen to represent deviations from the physiologically normal state, then the protonation index for a given species varies from one phase to another corresponding to the normal pH difference between phases. Even in the absence of any reaction, if a species is transferred from one phase to another in which it has a different protonation index, the value of the proton content will change. To make the proton content invariant under transfer between compartments, one must use the same reference states for all phases. Apart from this restriction, the reference states may be chosen arbitrarily. The reference states described next yield a proton function which is invariant upon transfers of species between phases, proton-transfer reactions, and the addition of components in their reference states.

In the subsequent discussion, it is assumed that a normal physiological state for the system has been defined, which specifies for each phase r a normal value $pH_0^{(r)}$ for the pH and a normal value $[A_{ij}]_o^{(r)}$ for the concentration of each species A_{ij}. The volume of phase r will be represented by $V^{(r)}$; and the total volume V of the R-phase system,

$$V = \sum_{r=1}^{R} V^{(r)} \quad , \quad \text{will be assumed constant.}$$

Reference States for Inorganic and Micromolecular Components. In place of Eq. (6), which applies to a single-phase system, let

$$(18) \qquad f_{ij} = \frac{\sum_{r=1}^{R} [A_{ij}]_0^{(r)} V^{(r)}}{\sum_{r=1}^{R} \sum_{j=1}^{J_i} [A_{ij}]_0^{(r)} V^{(r)}}$$

for a multiphase system. As for a single-phase system, p_{ij} is obtained from Eq. (7). P_{inorg} is then obtained from the analog of Eq. (8),

$$(19) \qquad P_{inorg} = \sum_{r=1}^{R} \sum_{i=1}^{I} \sum_{j=1}^{J_i} p_{ij} [A_{ij}]^{(r)} V^{(r)} \quad .$$

Reference States for Macromolecular Components. In place of Eq. (11), for a multiphase system let

$$(20) \qquad \mathfrak{I}_k = \frac{\sum_{r=1}^{R} \mathfrak{I}_k^{(r)} [A_k]_0^{(r)} V^{(r)}}{\sum_{r=1}^{R} [A_k]_0^{(r)} V^{(r)}}$$

where

$$(21) \qquad \mathfrak{I}_k^{(r)} = Z_k(pH_0^{(r)}) \ .$$

As before, p_k is obtained from Eq. (10), and P_{macro} is obtained from the analog of Eq. (12):

$$(22) \qquad P_{macro} = \sum_{r=1}^{R} \sum_{k=1}^{K} p_k^{(r)} [A_k]^{(r)} \ V^{(r)} = \sum_{r=1}^{R} \sum_{k=1}^{K} (z_k^{(r)} - \mathfrak{I}_k)[A_k]^{(r)} \ V^{(r)} \ .$$

Reference States for Unidentified Components. In general, the unidentified components will have a different composition for each phase, so that each phase must be considered separately. Furthermore, they will not in general cross a phase boundary in unison, but as individual constituents, so that it is impossible to describe the effect of interphase transfer on the proton function solely in terms of the grams of unidentified components present in each phase. Consequently, in order to use the proton function to describe the complete multiphase system, all components which cross phase boundaries must be known, and they cannot be included among the unidentified

components. With the two restrictions that the unidentified components do not cross phase boundaries, and that their composition (but not necessarily their quantity) is constant for any given phase, one can write

$$P_u = \sum_{r=1}^{R} (z'_u - \mathcal{I}'^{(r)}_u) c_u^{(r)} V^{(r)} \tag{23}$$

where the constants $\mathcal{I}'^{(r)}_u$

$$\mathcal{I}'^{(r)}_u = Z'^{(r)}(pH_0^{(r)}) \tag{24}$$

are determined by the appropriate charge function $Z'^{(r)}$ and reference pH, $pH_0^{(r)}$, for each phase.

Reference States for the Carbonic Acid Series. For a multiphase system, still taking H_2CO_3 as the reference state,

$$P_{carb} = \sum_{r=1}^{R} ([HCO_3^-]_0^{(r)} - [HCO_3^-]^{(r)}) V^{(r)} . \tag{25}$$

Net Proton Function for a Multiphase System. The foregoing definitions correspond to taking as reference state for any given component a mixture of samples of that

component from each compartment, each at the physiologically normal pH of that compartment and in an amount proportional to the content of the given component in that compartment. With such choice of reference states, the proton content of the multiphase system as a whole can be defined by Eq. (16) as the sum of P_{inorg}, P_{macro}, P_u, and P_{carb}. The calculations of each of these terms has been described separately (Eqs. 19, 22, 23, and 25).

CONCEPTS OF ACID OR BASE CONTENT IN BIOLOGICAL SYSTEMS

The analysis of acid-base pathophysiology leads naturally to the concept of an excess or deficit of acid or base, and several distinct formulations of this fundamental concept have appeared in the literature. To describe the non-volatile base content of blood, Singer and Hastings [6] introduced the concept of "buffer base"; and Astrup, Jorgensen, Siggaard-Andersen and Engel [3], introduced the concept of "base excess." Others, such as Welt [7], or Schwartz and Relman [8], prefer to use the serum bicarbonate ion concentration directly as an index of the base content of the blood. The base content of the body as a whole, as distinct from that of the blood, is termed the "whole-body base excess" by the Danish school (Mellemgård and Astrup [4]; Siggaard-Andersen [9]);

and is conceived by others, such as Schwartz and Relman [8], in terms of therapeutic requirements of acid or base.

Several of these concepts--the buffer base, the base excess of the blood, and the whole-body base excess--can readily be formulated (as shown below) in terms of the proton content. Such a formulation permits these quantities to be defined in terms of the detailed chemical changes occurring within the system. The use of serum bicarbonate as a measure of base content is clinically important but is not discussed here, since the proton condition seems to shed little light on this subject. (The relationship between the proton content and the serum bicarbonate is given by Eqs. (16) and (25); the bicarbonate concentration represents only a part of the proton content.) The question of therapeutic requirements of acid or base is beyond the scope of the present discussion, but it is worth noting that the base excess, which (as shown below) is closely related to the proton content, has been used successfully as a guide to therapy [10].

The various indexes of acid or base content represent different ways of quantitating the "metabolic" component of an acid-base disturbance. Most contemporary literature describes biological acid-base disorders in

terms of two independent factors. One factor or variable describes renal and metabolic function, and the other describes respiratory function. The proton content with physiologically normal reference states can be conveniently selected as one of these variables, and the partial pressure of carbon dioxide at some point in the system (in the human, arterial P_{CO_2}) as the other. This is a chemical description rather than a physiologic one. Some researchers classify acid-base disorders on the basis of the chemical disturbance [11], and others classify them on the basis of altered physiology [8]. Since the same factors (renal-metabolic and respiratory) are represented in both systems, this has led to some confusion. Clearly both the chemistry and the physiology are of interest.

Base Excess of the Blood. The base excess has been defined operationally by Siggaard-Andersen [10] as the equivalents of strong acid (or minus the amount of strong base) required to titrate a liter of blood or plasma to a pH of 7.40 at a P_{CO_2} of 40 mm Hg and a temperature of 38°C. This definition tacitly assumes that any two strong bases are equivalent in their effect on the system. This assumption apparently has not been subjected to careful experimental testing; and while perhaps a valuable approximation, it is probably not strictly true.

Consider an alternative definition of base excess: let the base excess be defined as the negative of the proton content, using the reference states specified above. This new definition coincides with Siggaard-Andersen's [9] to the extent that the following assumptions are valid: a) that the response of the system to strong acid or base is independent of the kind of strong acid or base; b) that blood which is not in the normal physiologic state can be returned to the normal state simply by adjusting the partial pressure of CO_2 and by adding strong acid or base.

The sufficiency of these assumptions can be seen from the following corollary of the proton condition. Consider a biological system of constant volume which can be represented by a finite collection of homogeneous phases. Let P(A) be the value of the proton content in state A and let P(N) = 0 be the value of the proton content in the physiologically normal state N. If it is possible to pass from state A to state N by adding or removing n_j moles each of J strong acids HA_j (j=1,...,J) and m_k moles each of K strong bases B_k (k=1,...,K), then

$$P(A) = \sum_{k=1}^{K} m_k - \sum_{j=1}^{J} n_j ,$$

provided only proton-transfer reactions occur. (This is easily proven by the methods given in the Appendix--if A_j^- and B_kH^+ are taken as reference states.)

This corollary states one sense in which the proton content measures the acid content of the system. The restriction to proton-transfer reactions is equivalent to the assumption that no new acid or base is generated by metabolic processes while returning the system to its normal state--an assumption which would presumably be valid for a sufficiently acute change. Note that the operationally defined base excess, in contrast, must fail to measure the base content in the sense of this corollary, to the extent to which assumptions a) and b) above fail.

The Buffer Base of the Blood. Singer and Hastings (1948) originally defined the "buffer base" of blood as follows:

> The anions are grouped into two main divisions. The first of these consists of the fixed acids (A^-), which are predominantly chloride ion (Cl^-), with a much smaller fraction (X^-), which includes sulfate, lactate, and other anions present only in traces in normal blood. The "fixed acids" are so called because their respective concentrations are unaffected by pH changes in the physiological range. The second main division consists of the buffer anions (HCO_3^-) and protein anions (P^-), which are altered with change in CO_2 pressure or pH. In the plasma the non-CO_2 buffer is comprised almost exclusively of the plasma proteins,

> but in the red cells organic phosphates must be reckoned in with the reduced hemoglobin and oxyhemoglobin. It is evident . . . that part of the total base (B+) is equivalent to the fixed acids (A^-), while the remainder is equivalent to the sum of (HCO_3^-) and (P^-). This second fraction is defined as the "buffer base" (Bb+). This may be expressed in two ways:
>
> $$(Bb+) = (HCO_3^-) + (P^-)$$
> $$(Bb+) = (B+) - (A^-)$$ *

In this definition, the "total base (B+)" refers to what is today called total cation. There are several approximations implicit in the above definition, and while they are quite valid and cause little difficulty from a practical standpoint, they are a source of ambiguity when one seeks to pin down the concept of buffer base with mathematical precision. Thus Siggaard-Andersen (1966) has recently commented:

> The concept buffer base means a base which acts as a buffer, i.e. a weak base. However, it depends on the actual pH value which bases act as buffers. . . . Defined in this way the concept loses interest as an indicator of the added strong acid or base, because as the pH changes, new buffer bases appear.†

Siggaard-Andersen [10] went on to propose an operational definition for buffer base, "the titratable base when

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*Ref. 6, pp. 224-226.

†Ref. 9, p. 43.

titrating with strong acid to a pH of 7.093 at a p_{CO_2} of 92.0 mm Hg at 38°, adding an arbitrary constant of 41.7" (p. 51). (This complicated definition is based on the experimental fact that under the conditions specified, normal human blood has a Singer-Hastings buffer base of 41.7 meq/l both in plasma and in erythrocytes.)

The objection of Siggaard-Andersen can be traced back to the statement of Singer and Hastings cited above, that "the fixed acids are so called because their respective concentrations are unaffected by pH changes in the physiological range" (p. 224). This statement does not lend itself to precise analysis, since "physiological range" is undefined and since "unaffected" is better replaced by "negligibly affected" (at least if one wishes, as do Singer and Hastings, to include lactate among the fixed acids).

Following the original definition of Singer and Hastings [6], the buffer base may be defined as the negative of the proton content, with the reference states for those components which act as significant buffers in the pH range of interest taken as their isoelectric states. The reference states for undetermined constituents must also be taken as isoelectric. Known constituents which

do not exert a significant buffer effect in the pH range of interest are not to be included in the expression (Eq. (17)) defining the proton content. This definition accords with the customary experimental method of measuring the buffer base, in which one determines the total normality (or net electrical charge) of the major dissolved inorganic cations and subtracts the total normality (or net electrical charge) of the major dissolved "non-buffer" anions. The difference--the net charge on the organic, unidentified, and inorganic buffer components--corresponds to the number of protons required to bring them all to their isoelectric points. Unlike the previous definitions, this definition of buffer base in terms of the proton function identifies the contribution of individual components to the buffer base. It retains a degree of ambiguity in using the expressions "significant buffers" and "pH range of interest"; but (as discussed above) this ambiguity is inherent in the concept, and is of little practical importance at present. One could eliminate the ambiguity by following the definition of Siggaard-Andersen [9], but other difficulties then arise. For abnormal blood, e.g., from a sick patient, is it still true that under the conditions specified by Siggaard-Andersen [9] the buffer base is identical in plasma and erythrocytes?

Whole-Body Base Excess. Probably all students of acid-base pathophysiology think at some times in terms of net gain or loss of body base stores, but the concept is difficult to quantitate. One approach is to administer a known amount of fixed acid or base to an experimental subject and to measure the response. The amount of base (or minus acid) administered can then be regarded as the "whole-body base excess" (Mellemgård and Astrup [4]; Siggaard-Andersen [12-13]), and correlated with the observed state of the subject. Another subject in the same state might then be presumed to have the same whole-body base excess.

Another approach is to carry out balance studies over some time interval which includes a period when the subject is in the physiologically normal state. One might then seek to relate the state of the subject to the net accumulation of base, with respect to the normal state, over the period of study. However, it has been shown (Goodman, Lemann, Lennon and Relman [14]; Lemann, Lennon, Goodman, Litzow, and Relman [15]) that the net response to such a prolonged disturbance differs from the response to an acute change and varies with the duration of the metabolic insult. Because it can vary when reactions occur which

are not simple proton transfers, the proton content as formulated here is more useful in discussing acute than chronic changes of acid-base status. (A formulation applicable to chronic changes is discussed below.)

The whole-body excess may be defined as the negative of the proton function, just as for the base excess of blood. The above discussion of blood carries over in an obvious manner to the case of the whole body, so it is clear in what ways the base excess so defined will have properties similar to those of the operationally defined whole-body base excess of the Danish researchers. The new definition gives the base excess in terms of the detailed chemical composition of each fluid compartment. It can be applied directly, e.g., to the mathematical model of fluid and electrolyte distribution in the human body described by DeLand and Bradham [16].

Particularly noteworthy is that the whole-body base excess is not necessarily equal to the sum of the base excesses of each compartment because (as discussed above) the former is independent of intercompartmental transfer, while the latter can be influenced by such transfers. However, it is not clear under what circumstances such intercompartmental transfers might be of physiological significance.

III. METABOLIC ACID-BASE BALANCES

The rate of metabolic generation of labile protons has for many years been estimated from the "ash acid" of the diet (Sherman and Gettler [17]). Presuming a diet to contain only K, Na, Mg, C, H, N, O, S, P, and Cl, the ash acid in equivalents may be defined as

$$\text{Ash Acid} = 2m_S + 2m_P + m_{Cl} - m_K - m_{Na} - 2m_{Mg} \tag{26}$$

where m_S, etc., are the number of equivalents of each element ingested. In the steady state, the rate of labile proton generation has been considered equal to the rate at which ash acid is ingested, and this in turn can be equated with the urinary excretion of acid if fecal and other body losses can be neglected.

In recent years some authorities have departed from this traditional approach (Relman, et al. [18,19]; Elkinton [20]). Relman's group has introduced an analysis of the metabolic generation of acid in terms of the classes of reactions which foodstuffs undergo in the human body. This represents a significant advance in our understanding of the detailed metabolic processes

which take place, but it nevertheless does not supplant the older approach. One can be misled on this point by some of the current literature. In speaking of the ash acid approach, Relman, et al. [18]: "This calculation, based simply on the discrepancy between mineral cations and chloride, phosphorus, and sulfur of the diet, has theoretical as well as practical defects and does not in fact correlate well with the rate of acid excretion" (p. 1621). On the contrary, as shown below, the new and old approaches lead to identical predictions and the experimental results of Relman's group can be predicted by the classical method properly applied. The proton condition is useful in showing how this should be done.

In order to apply the proton condition to this problem, it is desirable to choose the reference states in a different manner from that shown above in Sec. II. To avoid confusion, the reader should note carefully the differences between the subsequent formalism and the formalism of Sec. II. It is assumed for the sake of simplicity that the biological organism and its diet involve only the elements K, Na, Mg, C, H, N, O, S, P, and Cl; this assumption is by no means essential to the method.

Select as reference states the following chemical species: K^+, Na^+, Mg^{+2}, CO_2, O_2, H_2O, NH_3 (or equivalently $(NH_2)_2CO$), SO_4^{-2}, Cl^-, and $H_{1.2}PO_4^{-1.8}$. (The reference state for phosphorus is the phosphate ion at pH 7.4 and 37°C as discussed in Sec. II.) The absolute protonation index p_{abs_i} for any given chemical species A_i can then be defined as the number of extra protons required in forming the given species from the chosen reference states; i.e.,

$$(27) \qquad p_{abs_i} = z_i + 2n_{S_i} + 1.8\, n_{P_i} + n_{Cl_i} - n_{K_i} - n_{Na_i} - 2n_{Mg_i}$$

where z_i is the net electrical charge on species A_i and the n_{S_i}, etc., are the number of atoms of each element in a molecule of the species. For chemically unidentified constituents, the analogous definition of p_{abs} on a per gram basis is obvious. The proton function written in terms of these reference states,

$$(28) \qquad P = \sum_{r=1}^{R} \sum_{i=1}^{I} [A_i]^{(r)}\, p_{abs_i}\, V^{(r)}$$

is then conserved regardless of whatever reactions or transfers may occur within the system, for it simply reflects the conservation of each element and of net electrical charge.

An equivalent expression for the proton content in terms of the net molar content m of each element by the system is

$$(29) \qquad P = 2m_S + 1.8m_P + m_{Cl} - m_{Na} - m_K - 2m_{Mg} \ .$$

This is almost identical with Eq. (26) defining the classical ash acid; the sole difference lies in the selection of $H_{1.2}PO_4^{-1.8}$ as the reference state for phosphate instead of HPO_4^{-2}. Note that in this equation not only is the proton content conserved, but each term on the right is conserved individually. The choice of coefficients for each element in Eqs. (27) and (29) is arbitrary; the choice does not affect the conservation property of the proton content. They have been assigned the stated values for convenience in the following respect: this choice makes reference states of the chief species excreted from the mammalian body, assuming a urinary pH of 7.4 or assuming titration of the urine to

pH 7.4 in measuring the acid excretion. Since the proton content is invariant under the addition or removal of a reference substance, this choice simplifies greatly the application of the proton content to the net metabolic balance.

Now consider the application of the proton content concept to experimental metabolic balances in human beings. The choice of reference states according to Eq. (27) implies absolute conservation of the proton content P, so that P can change only by ingestion or excretion. Changes in P are given explicitly by the following expression:

$$\Delta P_{system} = P_{ingested} - P_{fecal} - P_{urine} \tag{30}$$

(if minor losses such as perspiration are neglected and if no pathological losses such as vomiting take place). In the steady state, by definition, $\Delta P_{system} = 0$ so that for any given time interval

$$P_{ingested} = P_{fecal} + P_{urine} \tag{31}$$

Here $P_{ingested}$, P_{fecal}, and P_{urine} are all to be calculated by Eq. (28) or (29). In accordance with Eq. (28), the

proton content of the urine may be expressed in terms of the principal urinary constituents:

$$(32) \qquad P_{urine} = ([NH_4^+] - [HCO_3^-] - 0.2[HPO_4^{-2}] + 0.8[H_2PO_4^-] + P_{org})\ V_{urine}$$

where V_{urine} is the volume of urine excreted in the specified time interval, and P_{org} is the proton content of the unidentified organic constituents per liter of urine. The principal contributor to P_{org} will generally be mono-negative organic anions containing only C, H, N, and O, for which the protonation index is -1, and the approximation can be made:

$$(33) \qquad P_{org} = -\ [\text{organic anions}]$$

where the brackets denote concentration in moles/l. If the term P_{fecal} in Eq. (31) be neglected, then Eq. (31) may be combined with Eq. (32) and (33) to yield

$$(34) \qquad P_{ingested} = ([NH_4^+] - [HCO_3^-] - 0.2[HPO_4^{-2}] + 0.8[H_2PO_4^-] - [\text{organic anions}])\ V_{urine}\ .$$

Relman and co-workers [18,21] have carried out extensive metabolic studies using a standard diet composed almost exclusively of C, H, N, O, S, and P. For this diet, $P_{ingested}$ becomes simply

$$P_{ingested} = 1.8m_P + 2m_S \tag{35}$$

according to Eq. (29). Thus, Eq. (34) becomes

$$1.8m_P + 2m_S = ([NH_4^+] - [HCO_3^-] - 0.2[HPO_4^{-2}] + 0.8[H_2PO_4^-] - [\text{organic anions}])\, V_{urine} . \tag{36}$$

This agrees completely with the experimental findings, which were expressed in the somewhat different form

$$1.8m_P + 2m_S + [\text{"organic acids"}]\, V_{urine} = ([NH_4^+] - [HCO_3^-] + [\text{titratable acidity}])\, V_{urine} . \tag{37}$$

Relman [18] interprets the left-hand side of this equation as the rate of acid production and the right-hand side as the rate of acid excretion. Equations (36) and (37) are

essentially identical, since the experimental method used to determine "organic acids" actually determined principally the organic anions excreted in the urine, and since the term $(-0.2[HPO_4^{-2}] + 0.8[H_2PO_4^{-}])\ V_{urine}$ in Eq. (36) represents the amount of acid required to titrate the phosphate buffers to pH 7.4 in the determination of titratable acidity. Thus, the results of Relman, et al. [18] can be derived from an exact statement of the proton function in a form which closely parallels the classical approach. The advantage of the mathematical formalism espoused here is that its use pinpoints the assumptions made and enables one to handle unusual cases--vomiting, unusual fecal losses, unusual diets, etc.--by the simple inclusion of terms which are normally neglected.

Now consider the linear transformation to a new set of J linearly independent basis vectors $C'^{(i)}$:

$$C'^{(i)} = \sum_{j=1}^{J} m_{ji} \, C^{(j)} \quad . \tag{A3}$$

It is easily shown that

$$S = \sum_{i=1}^{J} s'_i \, C'^{(i)} \tag{A4}$$

where

$$s'_i = \sum_{k=1}^{K} \sum_{r=1}^{R} n'_{ik} \, V^{(r)} \, [A_k^{(r)}] \quad . \tag{A5}$$

The matrix of coefficients (n'_{ik}) is obtained by pre-multiplying the matrix (n_{jk}) by the inverse of the transformation matrix (m_{ji}). Thus the quantities s'_i have the same conservation property as the original s_j and can be interpreted as the "content" of $C'^{(i)}$ in the system.

Assume now that the system contains hydrogen, and let $C'^{(1)} = H^+$. Then for $2 \leq i \leq J$, $C'^{(i)}$ is designated a "reference state." Since s'_1 can be interpreted as the "content" of H^+, it will henceforth be termed the

proton content $\underline{P}$. The coefficients n'_{ik} will be termed protonation indexes, p_k. Thus

$$\underline{P} = \sum_{k=1}^{K} \sum_{r=1}^{R} p_k \, V^{(r)} \, [A_k^{(r)}] \, . \tag{A6}$$

The basic properties of proton content $\underline{P}$ are its invariance under the permitted reactions, which follows from the conservation of s'_i, and its invariance under the addition of a reference state, which follows from the linear independence of the $C'^{(i)}$. A change in reference states is clearly equivalent to a change of basis in the "composition space" of vectors S which preserves H^+ as a basis vector.

In Sec. II above, the $C^{(j)}$ were chosen as the maximally protonated member of each homologous series or as arbitrarily protonated macromolecules. These are conserved in the sense of Eq. (A2) provided that only proton-transfer reactions occur. In Sec. III, the $C^{(j)}$ were chosen as the chemical elements; these are conserved under all circumstances. The restriction to systems of constant volume can of course be lifted if the concentrations are defined on a per weight basis instead of the more convenient per volume basis.

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